INVESTIGATING ACTION THRESHOLDS AND ALTERNATIVE MANAGEMENT APPROACHES TO CONTROL COTTON BOLLWORM [*HELICOVERPA ZEA* (BODDIE)] INFESTATIONS

A Dissertation

by

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ABSTRACT

Experiments were conducted to devise economic thresholds for foliar insecticide applications targeting bollworms in cotton. *Bt* cotton technologies including TwinLink™ (TL), TwinLink Plus™ (TLP), Bollgard II® (BG2), Bollgard 3® (BG3), WideStrike® (WS), WideStrike 3® (WS3), and a non-*Bt* (NBT) were evaluated. A 6% fruiting forms injury threshold was selected and compared to preventive treatments utilizing chlorantraniliprole. The 6% fruiting forms injury threshold resulted in a 25 and 75% reduction in insecticide applications relative to preventive sprays for WS and BG2, respectively. Current *H. zea* threshold recommendations for Texas, Mississippi, and South Carolina were assessed. Three *Bt* cotton technologies (non-*Bt*, Bollgard II®, Bollgard® 3) and five thresholds (20% oviposition, 6% fruiting forms injury, $\geq$3% large larvae, preventive sprays, non-treated) were utilized. Within NBT, all treatment thresholds provided greater control of *H. zea*, higher yield, and profitability relative to non-treated NBT. BG2 sprayed based on 20% oviposition or preventively resulted in greater control of *H. zea*, and all treatments resulted in a yield or profitability equivalent to that of the non-treated BG2. Spraying BG3 based on any of the thresholds was not beneficial. In 2020-2021, sorghum was evaluated as a trap crop of *H. zea* and a nursery crop for natural enemies of *H. zea* and *Hear*NPV dissemination into cotton. Treatments including cotton-only, non-treated cotton-sorghum, and *Hear*NPV-treated cotton-sorghum were used. Intercropping cotton with grain sorghum did not result in a consistent increase in *H. zea* control and beneficial arthropods relative to the cotton-only treatment. *Hear*NPV was detected in samples collected from all treatments indicating that the virus is naturally occurring in the locations where the study was conducted. Hence, there was no clear evidence that grain sorghum could serve as a source of *Hear*NPV for nearby cotton.

Laboratory strains of *H. zea* including Benzon susceptible, CRY-RR, CRY-RS, VIP-RR-70, VIP-RR-15, and TRE-RR, and 8 field-collected populations were evaluated (2021-2022) for their susceptibility to *Hear*NPV utilizing diet-overlay bioassays. The Benzon strain was consistently more susceptible to *Hear*NPV than any of the field populations evaluated, and the Cry-RR in 2021. However, the TRE-RR, VIP-RR-70, and VIP-RR-15 strains were as susceptible as the Benzon strain.

DEDICATION

This work is dedicated to God, my family, and my friends. Your support, love, and kindness have made me stronger and more fulfilled than I could have ever imagined.

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I thank God for always guiding me through every step of my life. I am so grateful to my parents and siblings for their unfailing support throughout my life. I would like to thank my wife for her constant assistance and words of encouragement during this journey. I thank my adorable children who endured with love so much time away from me to make this achievement possible.

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This work was supervised by a dissertation committee consisting of Dr. David L. Kerns (advisor), Drs. Suhas Vyavhare, and Megha Parajulee of the Department of Entomology, Dr. Ronnie W. Schnell of the Department of Soil and Crop Sciences, and Dr. Lindsey C. Perkin of the United States Department of Agriculture-Agricultural Research Service.

Field experiments were conducted by the student and collaborators, Drs. Jeremy K. Greene and Jeff Gore, at the Department of Plant and Environmental Sciences at Clemson University and Delta Research and Extension Center at Mississippi State University, respectively. All other work conducted for the dissertation was completed by the student independently.

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1. INTRODUCTION

1.1. Justification

Cotton (*Gossypium hirsutum* (Linnaeus)) is an economically vital crop in the southern U.S. and the most important source of textile fiber worldwide representing approximately 35% of all fibers produced (AgMRC 2022). In 2017, the U.S harvested 11 million acres of cotton valued at $7.3 billion (USDA NASS 2019). The bollworm (*Helicoverpa zea* (Boddie)) is a key pest of cotton. In 2019, yield losses and costs associated with the bollworm/budworm complex were estimated at approximately $117 million (Cook and Threet 2019).

In 2009, among the mid-southern states, and southern and eastern Texas, *Bacillus thuringiensis* (*Bt*) cotton varieties comprised 96% of the acreage, 40% of the cotton acres were treated for *H. zea*, and yield losses attributed to the pest were estimated at 0.6% (Williams 2010). By 2017, for these regions, *Bt* cotton varieties comprised 99% of the acreage, 75% of which were treated for *H. zea*, and yield losses attributed to *H. zea* were estimated at 3.38% (Cook 2018). Thus, despite more acres of more advanced *Bt* cotton technologies and the availability of more effective insecticides, southern cotton growers have experienced a 38% increase in insecticide applications targeting *H. zea* and increase losses approaching 18% within a span of 8 years.

Helicoverpa zea had largely been relegated as an occasional or minor pest since the introduction and widespread adoption of genetically modified cotton expressing *Bt* toxins. However, in recent years, due to practical and field-evolved resistance to some *Bt* toxins, *H. zea* has re-emerged as a major economic pest of cotton in much of the southern U.S. resulting in renewed emphasis on integrated pest management approaches including scouting, implementation action thresholds, and foliar insecticides for *H. zea* management (Tabashnik and Carriere 2015, Dively et al. 2016, Reisig et al. 2018, Reisig et al. 2019, Yang et al. 2019, Kaur et al. 2019).

Since 1996, transgenic crops expressing *Bacillus thuringiensis* (*Bt*) proteins have been widely planted worldwide. Currently, corn (*Zea mays* (L.)), cotton, and soybean (*Glycine max* (L.)) are the major *Bt* crops. In 2015, global commercialization of *Bt* crops exceeded 83.9 million hectares, including 56.8% corn, 28.1% cotton and 15.1% soybean (James 2016). These *Bt* crops are effective in controlling their target insect pests while causing marginal to no harm to non-target organisms (Carpenter 2010, Yu et al. 2011). *Bacillus thuringiensis* crops have offered great benefits including reduced chemical insecticide use and crop yield loss (Carpenter 2010, Hutchison et al. 2010, Yu et al. 2011, Edgerton et al. 2012, Kathage and Oaim 2012, Lu et al. 2012). However, the extensive use of *Bt* crops has placed a strong selection pressure on target pest populations, with the potential consequence of rapid evolution of resistance, threatening these benefits (Gould 198, Huang et al. 2011, Tabashnik et al. 2013). In recent years, field-evolved resistance to *Bt* crops that resulted in control problems has occurred in some target pests, including *H. zea*, stem borer (*Busseola fusca* (Fuller)), fall armyworm (*Spodoptera frugiperda* (J.E. Smith)), pink bollworm (*Pectinophora gossypiella* (Saunders)), western corn rootworm (*Diabrotica virgifera virgifera (LeConte)),* and African stem borer (*Busseola fusca* (Fuller)) (Van Rensburg 2007, Storer et al. 2010, Dhurua and Gujar 2011, Gassman et al. 2011, Farrias et al. 2014, Huang et al. 2014, Dively et al. 2016, Reisig et al. 2018). *Helicoverpa zea* is a major target of cotton and corn expressing *Bt* toxins in North America and there are many similarities between the toxins present in current commercially available cotton varieties and corn hybrids.

Because of the issues surrounding *Bt* resistance, *H. zea*, reliance on insecticide to manage the pest in *Bt* cotton has become common and widespread (Kerns et al. 2018, Cook 2018, Reisig et al. 2019). However, *H. zea* control with insecticide has been inconsistent due to problems associated with insecticide resistance and action threshold timing (Kerns et al 2017, Reisig et al. 2019). With approximately 100% reliance on chlorantraniliprole insecticides for managing *H. zea* in cotton, along with its long residual and high selection pressure, there is great concern that resistance to diamide insecticides may rapidly develop (Adams 2016). Field-evolved resistance to chlorantraniliprole has already been described for numerous pests, including diamondback moth (*Plutella xylostella* (Linnaeus)), tomato pinworm (*Yuta absoluta* (Meyrick)), and beet armyworm (*Spodoptera exigua* (Hübner)) (Wang et al. 2012, Silva et al. 2018, Yeole et al. 2018). Thus, there is a fundamental need to reassess action threshold in *Bt* cotton, validate current recommendations, and to evaluate insecticide alternative approaches to enhance biological control and alternative biopesticides.

1.2. Description and Biology of Cotton

The currently grown cotton varieties, *Gossypium* spp. (Malvales: Malvaceae) comprises four different species: *Gossypium arboretum* L., *Gossypium herbaceum* L., *Gossypium barbadense* L., and *Gossypium hirsutum* L. These cotton species are believed to have evolved following the introduction of a cultivated Old-World species, *Gossipium arboretum* L. (Tripathi et al. 2011, Fang and Percy 2015). *Gossypium arboretum* and *G. herbaceum* are diploids whereas *G. barbadense* and *G. hirsutum* are allotetraploids (Fang and Percy 2015). Cotton is a perennial plant with indeterminate growth and has four different growth stages. The first stage comprises germination, seedling emergence and establishment; the leaf area and canopy development form the second stage; the third stage is the development of fruiting structures; and the fourth stage is fruit maturation (Oosterhuis 2001). Cotton plants produce two types of branches-fruiting branch or sympodia and vegetative branches or monopodia. Vegetative branch has only one meristem which makes it grow straight similarly to the main stem, whereas fruiting branch has multiple meristems causing it to grow in alternating pattern (Albers 1993, Main 2012).

Flowering in cotton commences with the appearance of the initial flower bud called a square. Each square is surrounded by three leaf-like bracts. The first developmental stage of cotton flower is known as pinhead square where it is possible to identify the square; the second and intermediate stage is called match-head square; and the third stage is the pre-bloom or pre-flower stage. In the later stage, a candle shape can be observed (Main 2012). Upon maturity of the cotton reproductive system, approximately twenty-one days after the first square appearance, the flower bloom arises. Initially, the cotton bloom is white and subsequently turns pink. Cotton bolls form after the flowers have been pollinated (Albers 1993, Ritchie et al. 2007).

1.3. Description and Biology of *Helicoverpa zea*

Cotton bollworm (*Helicoverpa zea* (Boddie)) belongs to the order Lepidoptera and the family Noctuidea. *Helicoverpa zea* can be found across North America except for northern Canada and Alaska. This insect has high dispersive capability and usually disperses from southern to northern states and Canada (Capinera 2020, Reay-Jones 2019). *Helicoverpa zea* is able to overwinter, hence, a population of *H. zea* can include both overwintering and immigrant individuals (Delahaut et al. 2004, Capinera 2020). In tropical and subtropical climates, the insect is active all year long. However, it is more prominent during the summer in high latitude (Capinera 2020).

Helicoverpa zea is a polyphagous insect that attacks numerous plant species including corn, sorghum (*Sorghum bicolor* (L.) Moench), cotton, soybean, and tomato (*Solanum lycopersicum* (L.)) (Delahaut et al. 2004, Capinera 2020, Foster 2017, Reay-Jones 2019). In cotton, *H. zea* lays eggs individually on the upper sides or undersides of leaves. The insect has an affinity for young, tender and terminal leaves situated in the upper third of the plant canopy. However, *H. zea* can also lay eggs elsewhere on the plant including blooms, drying petal of blooms or in other plant parts very profound inside the cotton canopy (Vyavhare et al. 2018). *Helicoverpa zea* egg is pearly white or cream when freshly laid and turns light brown just prior hatching. The egg resembles a dome or a flattened sphere with a diameter of 0.5 to 0.6 mm, and a height of 0.5 mm. *Helicoverpa zea* eggs generally hatch in approximately three to four days after being laid (Delahaut et al. 2004, Capinera 2020, Vyavhare et al. 2018). The larvae of *H. zea* are variable in color and can be brown, pale green, pink, red, yellow, brown, or black (Capinera 2020, Vyavhare et al. 2018, Bessin 2019). *Helicoverpa zea* larvae head is usually orange or light brown and has black thoracic plates (Capinera 2020). The larvae are approximately 3.81 cm long when mature (Cook 2004). When they are young, *H. zea* larvae generally feed for a span of two days on tender leaves, leaf buds and small squares located in the plant terminal prior to moving to the lower portion of the plant to feed on larger squares and bolls (Vyavhare et al. 2018). *Helicoverpa zea* forewings are yellowish brown with a small dark spot located centrally. This dark spot is more visible from the underside. The forewings may also have a dark band displayed transversally near the tip. The base of the hind wings is creamy white and ending with a blackish coloration. Similar to the forewings, the hind wings also have a central dark spot (Capinera 2020).

1.4. *Helicoverpa zea* Management Strategies in Cotton

Several management approaches, including genetically modified cotton with *Bt*, synthetic insecticides, biological insecticides, biological control with arthropods, and trap cropping have been adopted for *H. zea* management in cotton.

1.4.1. Genetically Modified Cotton with *Bacillus thuriengiensis*

Genetically modified organisms are organisms that have been altered by inserting one or more genes into them for performance optimization (Phillips 2008). To optimize lepidopteran pest control in cotton production, cotton plants have been altered genetically by inserting genes of a soil-dwelling bacterium, *B. thuringiensis*, allowing production of proteins with insecticidal activity against specific insect pests (Hardee et al. 2001, Stewart 2007, Perez et al. 2015). Currently commercialized *Bt* cotton varieties express either crystal (Cry) proteins, vegetative insecticidal proteins (Vips) or both Cry and Vips combined targeting particular lepidopteran pests including beet army worm (*Spodoptera exigua* (Hubner)), cotton bollworm and tobacco budworm (*Chloridea virescens* (Fabricus)). The first introduced *Bt* cotton (Bollgard®) contained a single *Bt* gene. That single *Bt* gene expressed Cry1Ac (Vyavhare and Kerns 2017). Due to the increasing decline in efficacy of this original *Bt* cotton against cotton bollworm, a second-generation of dual-gene *Bt* cotton expressing two Cry toxins has been developed and introduced (Gore et al. 2003). Recently, for further optimization of *Bt* cotton efficacy against cotton bollworm, a third generation of *Bt* cotton containing three-genes expressing two Cry toxins and one Vip toxin has been introduced (Vyavhare and Kerns 2017).

1.4.1.1. Cry and Vip Proteins Mode of Action

For the *Bt* proteins to have an effect on *H. zea* larvae, it has to be ingested. Upon ingestion of the protein, the insect digestive enzymes activate the toxic form of the proteins.

Subsequently, the proteins cross the peritrophic membrane. The proteins then bind to specific proteins located in the apical membrane of the epithelial midgut cells allowing pore formation to occur. The occurrence of pores in the midgut cells provokes their disintegration; such condition causes leakage of cellular materials to the lumen allowing the proteins to reach the sites necessary for toxicity (Hardee et al. 2001, Chakroun et al. 2016, Syed et al. 2020, Jurat-Fuentes et al. 2021). Although Vip and Cry proteins have close similarities in their mode of action, they have different sequence and binding sites, indicating that cross resistance is less likely to occur (Chakroun et al. 2016, Syed et al. 2020).

1.4.2. *Bt* Resistance

Currently, most of the corn and cotton crops in the southern U.S. are grown in close proximity to one another (Huang et al. 2014). From the initial commercialization of *Bt* corn in 1996, a structured refuge was mandated as blocks or strips of non-*Bt* plants for delaying insect resistance to *Bt* crops in the U.S. (Ostile et al. 1997, US-EPA 2001). However, refuge compliance has been less than desirable in some regions of the country. In North Carolina, refuge compliance has been documented at 40% (Reisig 2017), while in some areas on the midsouth, compliance has been estimated at 10-20%. Thus, there is concern that resistant *H. zea* developed from *Bt* corn are not mating with susceptible moths, but other resistant moths, and subsequently infesting cotton (Von Kanel et al. 2015).

Susceptibility of *H. zea* to *Bt* crops has been variable, and reasoning behind fluctuations in susceptibility has been controversial. Before the introduction of *Bt* corn and cotton in 1996, field collections of *H. zea* demonstrated a diverse response in Cry1Ac and Cry1Ab assays (Luttrell et al. 1999, Siegfried et al. 2000). They showed LC_{50} values for diet incorporated Cry1Ac ranging from 0.02 to 5.97 ug/ml diet. Additionally, the ability of *H. zea* to survive

Bollgard$^{®}$ cotton was found to be similar to non-*Bt* cotton, although fruit injury was reduced (Jackson et al. 2004). These findings, supported by other field efficacy reports with cotton expressing only Cry1Ac, suggested that single-gene expressing cotton did not comply to a "high dose" strategy and, thus, was more susceptible to the development of resistance. Although no resistance was reported in the southern U.S. during the baseline surveys of bollworm prior to commercialization of Cry 1Ac (Luttrell et al. 1999), populations collected from 2002 to 2006 demonstrated elevated resistance ratios. These surveys found over 50 collections exhibiting resistance ratios >10, 14 strains with resistance ratios >100, and two strains with resistance ratios >1000 (Luttrell et al. 2004, Jackson et al. 2004, Ali et al. 2006, Luttrell and Ali 2007). Resistance ratios >10 are likely indicators of genetically based resistance (Tabashnik et al. 2009). Although widespread failure in the field was not reported, control problems associated with *H. zea* resistance to Cry1Ac in the field was well documented (Tabashnik et al. 2008a, Tabashnik and Carrière 2010). *Helicoverpa zea* surviving on cotton expressing Cry1Ac have demonstrated resistance ratios of 22 and 40 (Ali et al 2006), and larval survival on *Bt* relative to non-*Bt* corn leaves was greater for both of these strains than for a susceptible strain (Luttrell et al 2004). This pattern was observed again in 2006 with similar experiments on two additional field-derived resistant strains (Luttrell and Ali 2007, Tabashnik et al. 2008b) and in 2016-18 for Cry1Ac and Cry2Ab2 (Yang et al. 2017, Yang et al. 2018). Similarly, decreased susceptibility accompanied with increased *Bt* corn injury has also been reported for Cry1Ac and Cry1A.105 + Cry2Ab2 *Bt* sweet corn (Dively et al. 2016).

In cotton, there is evidence of practical *H. zea* resistance to pyramided cotton containing Cry1Ac and Cry2Ab (Reisig et al. 2018, Yang et al. 2022). In diet incorporated assays conducted from 2001-2007, although response to Cry2Ab2 was variable, there appeared to be trend for increasing resistance ratios over time, with some resistance ratios approaching 100 (Luttrell and Ali 2009). In 2000 the frequency for major alleles conferring resistance for *H. zea* in North Carolina was considered low, 0.00043 and 0.00039 for Cry1Ac and Cry2Aa, respectively (Burd et al. 2003). Since then, increasing *Bt* resistance allele frequency for Cry1 and Cry2 proteins is suspected (Luttrell and Ali 2009, Tabashnik et al. 2012). Currently, data suggests widespread field-evolved *H. zea* resistance to Cry1Ac, Cry2Ab2, and Cry1F *Bt* toxins (Yang et al. 2018). The addition of Vip3Aa in pyramided cotton has resulted in exceptional bollworm control (Kerns et al. 2019, Rabelo et al. 2020). However, unexpected injury events involving Vip3Aa expressing corn (Yang et al. 2018, Dively et al. 2021) and cotton (Kerns et al. 2015, Brown et al. 2019) have been reported. Recently, a strain of *H. zea* collected from corn expressing Vip3Aa20 in Texas, demonstrated the ability to survive elevated concentrations of Vip3Aa51 in diet overlay assays and on *Bt* cotton tissue expressing Cry1Ac+Cry1F+Vip3Aa19 (Yang et al 2019). Major resistance alleles conferring high levels of Vip3Aa resistance in a field-derived strain of bollworm has been reported in Texas, Arkansas, Louisiana, Mississippi, and Tennessee (Yang et al. 2020, Santiago-González et al. 2023). Vip3Aa resistance allele frequency in *H. zea* was estimated at 0.0155 for Arkansas, Louisiana, Mississippi, and Tennessee indicating that frequency of Vip3Aa resistance in *H. zea* in these states is not uncommon (Santiago-González et al. 2023). Moreover, a 3-year monitoring study across 18 to 30 sites in the U.S. and eastern Canada demonstrated that phenotypic frequency of resistance in *H. zea* to Vip3A is slowly increasing (Dively et al. 2021). Resistance to Cry *Bt* toxins in cotton has rendered the last remaining efficacious *Bt* toxin, Vip3A, virtually a stand-alone toxin (Reisig et al. 2018, Yang et al. 2018). Continued selection pressure in corn expressing Vip3A and lack of implementation of appropriate insect resistance management (IRM) strategy establish great doubt regarding the durability and sustainability of Vip3A efficacy toward *H. zea* (Reisig and Kurtz 2018, Yang et al. 2019, Caprio et al. 2019).

1.4.3. Synthetic Insecticides

Studies have demonstrated the frequent benefit from treating *Bt* cotton with foliar insecticides for *H. zea* (Kerns et al 2017, Kerns et al 2018). In cotton, only those varieties expressing Vip3Aa19 consistently provide good control of *H. zea*, but even those varieties occasionally required remedial insecticide sprays to prevent unacceptable injury (Kerns et a. 2018, Reisig et al. 2019, Little et al. 2019). Nevertheless, the availability of efficacious insecticides for managing *H. zea* in cotton is highly limited.

Current recommended insecticides include: pyrethroids, diamides, spinosyns, methomyl, and indoxacarb. However, the actual utility of these insecticides is greatly limited. Because pyrethroid insecticides are relatively inexpensive, they have been traditionally the first choice of growers for foliar control of *H. zea* in cotton. Prior to *H. zea* resistance to *Bt* Cry toxins, bollworms that survived *Bt* were severely stunted and highly susceptible to pyrethroids (Brown et al. 1998, Brickle et al. 2001). However, control with pyrethroids has become erratic in some regions. Pyrethroid susceptibility monitoring from Virginia to Texas for 2007-2016 demonstrated that a 36.1% increase in *H. zea* survival to a 5 ug/vial diagnostic concentration of cypermethrin (Musser et al. 2017). Consequentially, pyrethroid field-control failures have been common and many state extension services no longer recommend, or have cautioned the use of, pyrethroids for *H. zea* management (Reisig et al. 2019). Additionally, pyrethroids are highly disruptive biologically, eliminating key beneficial arthropods and often resulting in secondary pest outbreaks (Croft and Whalon 1982, Kidd and Rummel 1997). Spinosyns, indoxacarb, and methomyl are rarely used for *H. zea* management in cotton due to effectiveness, cost, toxicity, and/or short residual control relative to diamide insecticides. Diamide nsecticides containing chlorantraniliprole are currently the most utilized insecticides for managing bollworms in cotton. Diamide insecticides offer long residual control, up to 3 weeks, and are relatively safe to most beneficial arthropods (Gentz et al. 2010, Plummer et al. 2018). However, even diamide insecticides have experienced occasional control failures. Most control failures with diamide insecticides are thought to result from poor spray coverage, insufficient use rates, or poor timing. Timing insecticide application toward hatching or small larvae has proven to be most effective. Once the larvae reach second instar, they commonly move deeper into plant canopy and burrow into fruiting structures, which greatly limits insecticides exposure (Reisig et al. 2019).

The greatest deterrent to utilizing diamide insecticides for *H. zea* management in cotton is cost. The cost associated with treating *Bt* cotton for *H. zea* is primarily driven by the high cost of diamide insecticides. In 2017, estimated cost for treating *H. zea* in cotton averaged approximately $18.00 per acre per application (Cook 2018). This cost coupled with low cotton commodity prices have driven desire for less expensive and equally biologically non-disruptive alternative insecticide choices.

1.4.3.1. Mode of Action of Diamide: Chlorantraniliprole

Chlorantraniliprole attacks its target insect through selective activation of the ryanodine receptor (RyR), a calcium release channel located in the endoplasmic reticulum of the insect (Teixeira and Andaloro 2013). Ryanodine binds to the ryanodine receptor causing its activation. The activation of the ryanodine receptor causes the calcium channels to remain open provoking the depletion of the calcium stores. This action leads to the impairment of muscle contraction regulation resulting in paralysis and eventually death of the pest (Cordova 2006, Dinter et al. 2008, Jeanguenat 2012).

1.4.4. Problem Associated with Current Action Threshold

Historically, action thresholds for *Bt* cotton were based on larval counts and damage to reproductive structures to allow time for the *Bt* proteins to work after larvae hatch (Sullivan et al. 1998, Gore and Adamczyk 2004, Vyavhare et al. 2018). Currently, resistance to one or more *Bt* toxins in some bollworm populations has rendered those thresholds ineffective, and many university extension specialists have adopted a threshold based on egg counts in dual-gene *Bt* cotton (Clemson Cooperative Extension 2020, UA-Research & Extension 2019, Reisig and Huseth 2023, LSU AgCenter 2023, MSU-Extension 2021). Because of the uncertainty of *Bt* efficacy, insecticide resistance, spray application timing, impact on beneficial arthropods, and economic benefit, there is a great uncertainty and lack of scientifically vetted data to support reliance on current derived thresholds and treatment recommendations. Regardless, the *H. zea* egg thresholds approach has proven popular among growers and agricultural consultants and has been highly effective. However, the egg threshold has several significant disadvantages: 1) tobacco budworm (*Chloridea virescens* (Fabricius)) eggs (which have no reported incidences of field-evolved *Bt* resistance) cannot be easily distinguished from *H. zea* eggs, 2) no time is allowed for natural predation and mortality of *H. zea* eggs and small larvae, and 3) the *Bt* technologies are not provided any opportunity to demonstrate acceptable efficacy (Reisig et al. 2019). Thus, there is a fundamental need to reassess action thresholds in *Bt* cotton, validate current recommendations, and to evaluate insecticide alternative approaches to enhance biological control and alternative biopesticides.

1.4.5. Trap Crop and Biopesticides (*Hear*NPV)

Helicoverpa armigera nucleopolyhedrovirus (*Hear*NPV) is a viral pesticide that is specific to Heliothines, including *H. zea*. In much of the midsouth in recent years, *Hear*NPV has been widely adopted for the primary soybean pest, *H. zea* (Musser et al. 2016). In many parts of the world *Hear*NPV is widely utilized for control of *H. armigera* in grain sorghum (Roome 1975, Teakle et al. 1985). Recently, *Hear*NPV has also been marketed for *H. zea* in grain sorghum and hemp (*Cannabis sativa* (L.)) (OSU Extension service, Musser et al. 2016, Stewart et al. 2022).

*Hear*NPV is in the viral family Baculoviridae. This virus contains a protein structure, the occlusion body, that aids in protection from environmental conditions (Bilimoria 1986, Bilimoria 1991). *Hear*NPV is sprayed like chemical insecticides and is activated upon larval consumption. The protective structure breaks down upon reaching the midgut, and the viral DNA infects the midgut cells where replication begins. The replicated viral DNA ultimately spread throughout the host causing it to liquefy (O'Reilly et al. 1992, Hunter-Fujita et al. 1998). Once the host larva liquefies and dies, millions of viral particles are released into the environment where horizontal and abiotic transmission can occur (Boucias and Pendland 1998).

In soybean, sprayable *Hear*NPV persistence within the shaded canopy has been shown to last as long as 96 hours, and horizontal and abiotic transmission from natural virus dispersal may exceed 200 ft and persist between 13 and 21 days (Black et al. 2019). In cotton, *Hear*NPV persistence has not been sustained. This lack of persistence is thought to be primarily due to the pH of dew on cotton leaves resulting in virus deactivation as the dew dries (Yearian and Young 1974, Young et al. 1977, McLeod et al. 1997). Although initial *Hear*NPV infection of *H. zea* larvae in cotton is possible, it is unlikely an epizootic event will persist. Thus, the challenge of effectively integrating *Hear*NPV into cotton IPM is to devise a system where an epizootic nursery source of *Hear*NPV can be initiated for persistent horizontal and/or abiotic transmission into cotton.

The manipulation of cropping systems to incorporate trap crops as diversionary hosts or to provide refuges for beneficial organisms that will later colonize susceptible crops has often been suggested but has rarely been successfully implemented (Fitt 1989, Javaid and Joshi 2010). Grain sorghum has been demonstrated to be an effective diversionary trap crop for *H. zea* from cotton (Tillman and Mullinix 2004). In Botswana, the application of *Hear*NPV to control *H. armigera* in cotton was found to be more effective and persistent in cotton when applied to sorghum rather than directly to cotton (Room 1975, Roome and Daoust 1975). In India, applications of *Hear*NPV to *H. armigera* trap crops resulted in 14.2-20.2% reduction in *H. armigera* infestation in nearby cotton (Duraimurugan and Repgupathy 2005). Additionally, predators of *H. armigera* are more abundant on sorghum than cotton (Robinson et al. 1972). Thus, sorghum has the potential to serve as a trap crop for *H. zea* in the US., as a nursery for *H. zea* natural enemies, and as a promoter for *Hear*NVP horizontal and/or abiotic dispersal into nearby cotton.

1.4.6. Biological Control with Arthropods: Predators and Parasitoids

Several arthropods have been reported as *H. zea* natural enemies. These natural enemies include: green lacewing (*Chrysoperla* spp.), brown lacewings (*Hemerobius spp.*), convergent lady beetle (*Hyppodamia convergens* Guérin-Méneville), red-cross beetle (*Collops* spp.), Scymnus beetle (*Scymnus spp.*), seven-spotted lady beetle (*Coccinella septempunctata* Linnaeus), harmonia lady beetle (*Harmonia axyridis* Pallas), pink spotted lady beetle (*Coleomegilla maculate* De Geer), big-eyed bug (*Geocoris* spp.), leafhopper assassin bug (*Zelus spp*.), spined assassin bug (*Sinea diadema* F.), minute pirate bug (*Orius spp.*), damsel bugs (*Nabis spp.*), spined soldier bug (*Podisus maculiventris* Say), fire ants (*Solenopsis spp.*), trichogramma wasps (*Trichogramma spp.*), braconid wasps (*Cotesia marginiventris* Cresson and

Microplitis croceipes Cresson), chelonus wasp (*Chelonus insularis* Cresson), tachinid fly (*Archytas spp.*), jumping spiders (Araneae: Salticidae), lynx spiders (Araneae: Oxyopidae) and crab spider (Araneae: Thomisidae) (Lopez et al. 1976, Knutson and Ruberson 2005, Parajulee et al. 2006, Diaz et al. 2004). These beneficial arthropods can significantly aid in regulating *H. zea* population by maintaining the pest population below economic threshold level (King and Coleman 1989, Knutson and Ruberson 2005). Laboratory and field-cage studies showed that *C. carnea*, *G. punctipes*, *C. maculata*, and *P. maculiventris* can provide substantial control of cotton bollworm/tobacco budworm eggs and/or larvae (Lopez et al. 1976). Another cage study by Van Den Bosh et al. (1969) revealed that *G. pallens*, *N. americoferus* and *C. carnea* can cause 50% or more *H. zea* mortality. In their field study, Bell and Whitcomb (1964) found that arthropod predators can cause up to 28% reduction of *H. zea* populations in cotton. Hence, management of beneficial arthropods in cotton agroecosystem can be a suitable tactic to incorporate in an Integrated Pest Management (IPM) system targeting *H. zea* in cotton.

1.5. Research Objectives

The main objective of this research is to evaluate the integration of *Bt* technology, conventional insecticides, biopesticides, trap cropping, beneficial arthropods, and action thresholds into a comprehensive and sustainable IPM system.

Objective 1- Devise economic thresholds toward bollworm management in *Bt* cotton and assess the benefits from treating *Bt* cotton with insecticide,

Objective 2- Evaluate and refine the current *H. zea* threshold recommendations and determine how each threshold affects *H. zea* control and profitability,

Objective 3- Investigate the potential for utilizing grain sorghum as a *Helicoverpa zea* trap crop and nursery crop for *H. zea* natural enemies and *Hear*NPV dissemination into cotton.

Objective 4- Establish a baseline-susceptibility of *H. zea* to *Hear*NPV and evaluate if *Bt* resistant *H. zea* are susceptible to *Hear*NPV.

1.6. Research Hypotheses

Hypothesis 1- Insecticide treatment triggered in *Bt* cotton based on relevant economic thresholds will favor efficient bollworm management and greater profitability,

Hypothesis 2- Refined action thresholds will generate more applicable treatment timings, offer efficient bollworm control, and enhance insecticide treatment benefit,

Hypothesis 3- Interplanting cotton with grain sorghum will divert cotton bollworm from cotton and increase the diversity and density of cotton bollworm natural enemies' population in cotton, and grain sorghum interplanted with cotton will serve as a source of *Hear*NPV and will favor persistent dissemination of the virus into the cotton canopy,

Hypothesis 4- *Helicoverpa zea* will be highly susceptible to *Hear*NPV and *H. zea* resistant to *Bt* proteins will not be cross-resistant to *Hear*NPV.

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2. DEVELOPMENT OF ECONOMIC THRESHOLDS TOWARD BOLLWORM, HELICOVERPA ZEA (BODDIE) (LEPIDOPTERA: NOCTUIDAE), MANAGEMENT IN BT COTTON AND ASSESSMENT OF THE BENEFITS FROM TREATING BT COTTON WITH INSECTICIDE[*]

2.1. Introduction

The introduction of transgenic cotton (*Gossypium hirsutum* L.) expressing insecticidal proteins from *Bacillus thuringi*ensis (Bt) in 1996 ushered in a new era in cotton insect pest management (Naranjo 2011). Global commercialization of Bt crops has reached 104.2 million hectares, and Bt cotton comprises approximately 90% of U.S. cotton production (James 2019). In the U.S., commercial Bt cotton varieties specifically target lepidopteran insect pests, which include tobacco budworm (*Chloridea virescens* (Fabricius)), pink bollworm (*Pectinophora gossypiella* (Saunders)), and bollworm (*Helicoverpa zea* (Boddie)) (Flint and Parks 1999, Luttrell and Jackson 2012, Braswell et al. 2019).

Currently, tobacco budworm has been functionally relegated to non-pest status, and pink bollworm has been eradicated in the U.S. in large part from wide-spread use of Bt cotton (Carrière et al. 2003, Blanco 2012, Perdue 2018). However, long-term suppression of bollworm has been less successful. Following the introduction of Bollgard® cotton expressing the single Bt protein Cry1Ac, outbreaks of bollworm often required mediation with foliar insecticides to prevent economic injury (Mahaffey et. al. 1995, Smith 1997). With the introduction of second

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[*] Calvin et al. 2021. Development of economic thresholds toward bollworm (Lepidoptera: Noctuidae), management in Bt cotton, and assessment of the benefits from treating Bt cotton with insecticide. J. Econ. Entomol. 114: 2493-2504.

generation dual-gene Bt cotton expressing Cry1Ac+Cry2Ab (Bollgard II®), Cry1Ac+Cry1F (WideStrike®), and Cry1Ab+Cry2Ae (TwinLink®), the need for remedial insecticide applications targeting bollworm was greatly reduced (Greenplate et al. 2000, Stewart et al. 2001, Moar et al. 2002, Fleming et al. 2018). However, unexpected injury incidences became increasingly common beginning in 2010 (Jackson et al. 2011, Greene et al. 2011, Siebert et al. 2011). Reduction in efficacy of dual-gene Bt cotton to bollworm has been attributed to field-evolved resistance to Bt Cry proteins (Dively et al. 2016, Yang et al. 2017, Reisig et al. 2018, Little et al. 2019, Kerns et al. 2019, Yu et al. 2021).

In 2015, third generation Bt cottons were introduced that express the vegetative insecticidal protein, Vip3Aa, in addition to the Cry proteins (Fleming et al. 2018). The currently available Bt cotton technologies expressing Vip3Aa include: Bollgard 3® (Cry1Ac+Cry2Ab+Vip3Aa19), WideStrike® 3 (Cry1Ac+Cry1F+Vip3Aa19) and TwinLink Plus™ (Cry1Ab+Cry2Ae+Vip3Aa19). The addition of Vip3Aa in pyramided Bt cotton has resulted in exceptional bollworm control (Kerns et al. 2019, Rabelo et al. 2020). However, unexpected injury events involving Vip3Aa expressing corn (Yang et al. 2018) and cotton (Kerns et al. 2015, Brown et al. 2019) have been reported. Additionally, a major resistance allele conferring high levels of Vip3Aa resistance in a field-derived strain of bollworm has been reported in Texas (Yang et al. 2020).

Because Bt cotton can experience unacceptable injury from bollworm regardless of the Bt technology deployed, there is continued need for bollworm monitoring and implementation of action thresholds (Reisig et al. 2019). Prior to the introduction of Bt cotton, it was reported that 10 or more bollworm/tobacco budworm larvae per 100 plants or 1.5 to 2.0 larvae per 3 meters of row (10 feet of row) were required to cause significant yield loss (Adkisson et al. 1964).

Following the release of Bollgard® cotton, Sullivan et al. (1998) defined treatment thresholds of 75 eggs or 30 small (less than 0.64 cm) or 3 large larvae (> 0.64 cm) or 5% boll damage per 100 plants. Additional studies on dual-gene cotton concluded that although insecticide applications to dual-gene cotton for bollworm management were not warranted, the action thresholds defined by Sullivan et al. (1998) for single-gene Cry1Ac cotton were applicable (Carter et al. 2012).

In the current study, we report the relationships between cotton yield and fruit loss due to bollworm feeding and bollworm larval density and compute economic injury levels and economic thresholds. Additionally, we report the yield response of second and third generation *Bt* cotton when subjected to insecticide applications targeting bollworm.

2.2. Materials and Methods

2.2.1. Field Plots and Yield

Tests were conducted across the Mid-South and in Texas at five to eight test sites per year from 2014-2020. The *Bt* cotton technologies evaluated included: TwinLink™ (TL; Cry1Ab+Cry2Ae), TwinLink Plus™ (TLP; Cry1Ab+Cry2Ae+Vip3Aa), Bollgard II® (BG2; Cry1Ac+Cry2Ab), Bollgard 3® (BG3; Cry1Ac+Cry2Ab+Vip3Aa), WideStrike® (WS; Cry1Ac+Cry1F), WideStrike 3® (WS3; Cry1Ac+Cry1F+Vip3Aa). A non-*Bt* variety (NBT) was included as a check. *Bt* technology entries and varieties utilized varied by year (Table 2-1).

All tests were designed as a factorial with Factor A being 5-7 cotton technology entries and factor B being entries either sprayed for bollworm with chlorantraniliprole at 75.1 g-AI/ha (Prevathon®, FMC Corporation, Philadelphia, PA) at first bloom and thereafter if re-infestation occurred, or non-sprayed. Plots were 4 rows wide (1 m between rows) with 12.2-15.2 m in length. Each factorial combination was replicated 4 times. Foliar applications were made in accordance with the occurrence of larvae in the NBT cotton plots at each individual location.

After the cotton had attained full maturity, the test sites were prepped for harvest using locally recommended harvest aids appropriate for the given environmental conditions. The middle two rows of each plot were harvested using two-row mechanical cotton pickers with integrated weighing scales. Yields were calculated in kgs-lint/ha.

2.2.2. Insect Sampling and Fruit Damage Assessment

Insect densities, and square and boll injury were determined prior to foliar treatment and weekly thereafter until bollworm infestations subsided. Plots were sampled utilizing a common technique used by many crop advisors. Within each plot, 20-25 plants were inspected depending on location. The number of plants inspected within a location and year remained the same. Each plant was partitioned into three sections based on approximate nodal position. The top section consisted of nodes 1-5, middle nodes 6-10 and lower nodes ≥11. The top section terminal was inspected for the presence of larvae, and 2 squares were inspected for the damage and larvae. Within the middle section, 2 medium-large sized squares, 2 white blooms, and 2 bloom-tagged (dried attached blossoms) or small bolls were inspected for damage and larvae, and within the lower section of the plant, 2 medium to large sized bolls were inspected for damage and larvae. The number of larvae, damaged squares, and damaged bolls were recorded in each plot. Larvae were categorized as small (first and second instar) or large (≥ third instar). Damaged squares or bolls were those where the larvae had completely penetrated the square calyx or boll carpel wall. Fruit damage and larval counts were averaged over the period from insecticide application to 18 to 24 days post insecticide application, depending on temporal persistence of bollworm infestation and crop damage at each year by test site.

2.2.3. Calculating Gain Threshold, Economic Injury Level, and Economic Threshold

The Gain Threshold (GT) was calculated based on Stone and Pedigo (1972), where management cost is divided by the crop's market value. The estimated management cost is the combined expense of the insecticide and insecticide application [US$/ha]. The application cost was calculated based on data from Texas Agricultural Custom Rates [TACR] (2020) and Custom Rates for Farm and Ranch Services in Mississippi [CRFRSM] (2020). The cost of Prevathon® was estimated by surveying farm supply retailers across the Mid-South and Texas. The market value of cotton is the value expressed as US$/kg-lint. Thus, GT is expressed as kg-lint/ha, but was converted to percentage GT by dividing the estimated control cost of an insecticide application by the crops value (yield potential [kg-lint/ha] × market value [US$/kg-lint]):

$$\%GT = (C^{-(V \times Y)}) \times 100$$

Where C is the estimated control cost of an insecticide application [US$/ha], V is the market value of the cotton [US$/kg-lint], and Y is the yield potential [kgs-lint/ha]. Thus, %GT is the percentage expressed as kg-lint/ha, and represents the percentage of yield loss necessary to pay for an insecticide application.

Linear regression models were used to relate percentage square, boll, or percentage large larvae to percentage of maximum yield. Replicates within a site were averaged to alleviate differences in yield response among test sites due to agronomic and environmental conditions; yield data were normalized by each year and site by setting the maximum yield to that of the highest yielding replicate at each year by site to 100%, and the treatments to its corresponding percentage.

To determine the relationship between fruit damage or larvae density and yield, a linear regression model was used where percentage change in yield was the dependent variable and percentage fruit damage or percentage large larvae was the independent variable using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA. The ROUT method (Q = 1) was used to detect outliers. We detected two outlying values each for percentage damaged squares and bolls, and three outlying values for large larvae per 100 plants. None of the outlying values were eliminated since their inclusion did not significantly alter the intercepts or slopes based on overlapping 95% confidence intervals. Each year by site was analyzed separately, and those that failed to generate a significant linear response ($P \geq 0.05$) were eliminated from further analysis. Eliminated data sets (n=20 out of 42 total data sets considered) invariably resulted from very low incidences of bollworm infestation or from weather related events that disrupted accurate yield assessment. The remaining year-by-site data sets were pooled for regression analysis.

The regression equations were used for estimating maximum increase in yield and provided a y-intercept and slope for calculating the economic injury level (EIL):

EIL (% fruit reduction or larvae) = $(\beta_0 – (\% \text{ yield potential - } \%GT))^{-\beta}{}_{1}$

Where β_0 is the y-intercept regression, β_1 is the slope of the regression and % change in yield potential is the percentage maximum yield – the percentage yield - %GT (Pedigo et al. 1986, Ragsdale et al. 2007). The economic threshold (ET) was set at 70% of the EIL and rounded to the nearest whole number.

2.2.4. Yield Response to Insecticide

To evaluate the benefit of spraying bollworms among *Bt* cotton technologies, yields were compared between insecticide sprayed and non-sprayed plots using the locations presented in Table 2-1. Because of inherent variability among location sites and years due to differing environmental conditions, bollworm incidence, and agronomic difference among cotton varieties, the spray vs non-sprayed yields within each *Bt* technology were analyzed by year, site and *Bt* technology. Yield data were analyzed using PROC GLIMMIX ($P < 0.05$) (SAS Institute 2011) with replicate block as the random effect and spray treatment as the fixed effect. The SLICEDIFF option of the LSMEANS statement was used to separate sprayed vs non-sprayed for each *Bt* technology. The results generated from these analyses were then used to prepare a binomial data set (with all test sites or with test sites where NBT yield significant benefit) allowing for comparison among *Bt* technologies using the Kruskal-Wallis test (SAS institute 2013). When significant differences were detected, Wilcoxon post hoc test ($P < 0.05$) were used for pairwise comparisons between means.

2.2.5. Economic Threshold Validation

Based on preliminary data, trials were conducted at seven locations across the Mid-South (Starkville, MS; Stoneville, MS; Pine Bluff, AR; Rohwer, AR; Winnsboro, LA; St. Joseph, LA; and Jackson, TN) from 2014 to 2020 to field validate the ET. Plots were 4 rows wide (1 m between rows) × 12.2-15.2 m in length arranged as a 3 × 3 factorial with 4 replications. Factor A consisted of cotton technologies: NBT, WS or BG2. Factor B consisted of either a non-treated, sprayed at an ET of 6% injured fruiting form (equal number of squares and bolls), or a preventative threshold sprayed at first occurrence of bollworm egg lay and/or small larvae. The insecticide application rate at all locations was chlorantraniliprole (Prevathon®, FMC Corporation, Princeton, NJ) at 75.1 g-AI/ha.

Square and boll injury were determined prior to foliar treatment and weekly thereafter using the previously described sampling procedure. The middle two rows of each plot were machine harvested and yields were determined. Profitability (P) was determined for each location based on the equation:

$$P\ (US\$^{-ha}) = V - ((C_I \times A) + C_T))$$

Where V is the crop value [US$/ha] based on yield [kg-lint/ha] and market value [US$/kg-lint], C_I is the cost of the insecticide and insecticide application [US$/ha] and A is the number of applications. C_T is the cost of the *Bt* technology [US$/ha].

Because of inherent differences in varietal yield potential and because insecticide applications were triggered independently at each site, data were analyzed by site and *Bt* technology. Data were analyzed using PROC GLIMMIX ($P < 0.05$) with the spray treatment and spray treatment x *Bt* technology as the fixed effects and replicate block as the random effect. Data were analyzed using PROC GLIMMIX ($P < 0.05$) with the spray treatment as the fixed effect and replicate block as the random effect. Where significant ($P < 0.05$) LSMEANS were separated using Tukey's HSD ($P < 0.05$).

2.3. Results2.3.1. Gain Threshold, Economic Injury Level, and Economic Threshold

For computing C for GT values, application cost was estimated using the average cost of aerial and ground applications for Texas and Mississippi in 2020, which calculated to average US$16.31/ha [US$6.60/ac] (Texas Agricultural Custom Rates [TACR] 2020, Custom Rates for Farm and Ranch Services in Mississippi [CRFRSM] 2020) (Table 2.2). Prevathon® is the most commonly used insecticide for managing bollworm in the Mid-South and Texas and is commonly applied at 1.02-1.46 l/ha [14-20 fl-oz/ac], and averages 1.29 l/ha [17.6 fl-oz/ac]. Determining true cost of the insecticide was difficult because many retailers offer rebates and other incentives. We estimated the cost of Prevathon® at US$31.28/l [US$0.93/fl-oz]. V is the crop value set at US$1.10-$2.20/kg-lint [US$0.50-$1.00/lbs-lint] in US$0.11/kg-lint [US$0.05/lbs-lint] increments representing the variability in cotton crop value across years (USDA-NASS 2021). Y is the crop yield potential and was varied to span a broad range of yield environments and were set at 713.74, 1,070.62, 1,249.05, 1,427.49, and 1,784.36 kg-lint/ha [500, 800, 1,200, 1,500 and 1,800 lbs-lint/ac].

The GT is based on the estimated market price of the expected, reasonable yield potential that may be lost to a pest before management is justified; thus GT = Management Cost (US$/ha) / Market Value (US$/ha) (Stone and Pedigo 1972). Across all variables, the GT mean ± SEM was 35.99 ± 1.09 kg-lint//ha, and ranged from 25.75 to 51.49 kg-lint/ha. The %GT across variables necessary to equal an insecticide application was 3.12 ± 0.17%, and ranged from 1.44 to 7.21% (Table 2.2).

Yields across all years, locations and plots averaged 1,211.55 kg-lint/ha. Across all data, the percentage of damaged squares and damaged bolls averaged 11.45 ± 1.22 and 3.74 ± 0.54 for squares and bolls respectively. Large bollworm larvae averaged 4.17 ± 0.74 per 100 plants.

When analyzed across years and locations, there was a significant negative linear relationship between the percentage of maximum yield and percentage square or boll damage (square damage: $F = 87.60$; df = 1, 208; $P < 0.0001$; boll damage: $F = 57.23$; df = 1, 218; $P < 0.0001$). Based on the slopes of the regression models, the percentage yield loss was 0.3704 ± 0.0396 kg-lint/ha for each percentage increase in square damage (Fig 2.1a), and 0.6890 ± 0.0911 kg-lint/ha for each percentage increase in boll damage (Fig 2.1b). Thus, the value of a single damaged boll was equal to 1.86 damaged squares. Because bolls represent a physiologically more mature fruiting structure than squares, a higher value in potential yield contribution is expected. However, value in potential yield contribution would also be influenced by square and boll size, which we did not categorize. Thus, the 1.86 value would vary depending on the fruit developmental size sampled. Because the slopes of the linear regression models significantly differed between squares and bolls, when determining the relationship between percentage damaged fruiting form (squares and bolls combined), the bolls were weighted. There was a significant negative linear relationship between the percentage of maximum yield and percentage fruiting form damage, $F = 75.46$; df = 1, 208; $P < 0.0001$. The slope of the model suggests that for every percentage of damaged fruiting form, with equal sampling of squares and bolls, there is a 0.3983 ± 0.046 kg-lint/ha yield reduction (Fig 2.1d).

Using a static control cost of US$56.76/ha, and variable crop yield potentials and market values, the EIL for square damage ranged from 3.90 to 19.48% (Table 2.3). Based on these calculated EILs, the corresponding ETs, set at 70% of the EIL, ranged from approximately 3 to 14% damaged squares. The EIL for bolls ranged from 2.09 to 10.47% damaged, with corresponding ETs ranging from approximately 1 to 7% damaged bolls. The fruiting form EILs ranged from 3.62 to 18.11% damage, and the corresponding ETs ranged from approximately 3 to 13% damage.

Percentage maximum yield had a negative linear relationship to the number of large bollworm larvae per 100 plants ($F = 46.27$; df = 1, 217; $P < 0.0001$). The percentage yield loss was 0.7681 ± 0.1129 kg-lint/ha for each large larva (Fig 2.1d). Small larvae were not included since they may not have had sufficient time to succumb to *Bt* or insecticide toxicity. The EIL for large larvae per 100 plants ranged from 1.88 to 9.39 and their corresponding ETs ranged from 1-7 (Table 2.3).

2.3.2. Yield Response to Insecticide

Across all years and test sites, NBT, WS, BG2, TL, TLP, BG3 and WS3 significantly benefited ($P < 0.05$) in increased yield from treating with Prevathon®, relative to not being treated, for 63.9, 41.7, 16.7, 12.5, 9.5, 11.1 and 11.1% of the sites, respectively (Table 2-4). The NBT benefited from Prevathon® applications at only 63.9% of the sites, and the remaining

39.1% of sites invariably had low bollworm infestations or adverse environmental conditions that interfered with accurate assessment. If the low bollworm infestation sites are not considered, then the percentage of sites where significant ($P < 0.05$) yield increases were detected were 100, 60.0, 26.1, 20.0, 14.3, 9.1 and 17.4% of the sites for NBT, WS, BG2, TL, TLP, BG3, and WS3, respectively (Table 2-4). Hence, when only high bollworm infestation sites are considered, there was approximately 1.5-fold increase in percentage of sites where *Bt* technologies benefited in increased yields, with the exception of BG3 which exhibited a 1.2-fold reduction. Based on the binomial response in yield from treating with insecticide relative to the non-spray (spraying the *Bt* technology either provided a significant ($P < 0.05$) benefit or did not), there was a significant difference among *Bt* technologies in the incidence of yield benefit from spraying either when all test sites were included (Chi-Square = 43.17; df = 6; $P < 0.0001$) or when only high bollworm infestation sites were considered (Chi-Square = 54.60; df = 6; $P < 0.0001$). When considering all test sites, the yield increase from insecticide applications in BG2, BG3, TL, TLP, WS3 was significantly lower than NBT, and none of these *Bt* technologies differed from one another (Table 2.4). However, the yield increase from treating NBT and WideStrike with Prevathon® was similar ($Z = 1.68$; $P = 0.63$). Using only test sites where NBT yielded significant benefit from treating with insecticide, none of the *Bt* technologies differed from one another, and they were all significantly different than the NBT (Table 2.4).

2.3.3. Economic Threshold Validation

The *Bt* technology cost (C_T) was difficult to estimate because cotton seed companies have adopted a seamless technology fee assessment, which means they do not separate the cost associated with herbicide and *Bt* technologies. We estimated *Bt* technology cost at US$66.72/ha [US$27.00/ac] based on the *Bt* technology fees reported for the Mid-South and Texas (Cook and

Threet 2020). The insecticide and application cost were estimated as previously described, where the insecticide cost was estimated per application at US$43.66/ha [US$17.67/ac] and application cost as US$16.31/ha [US$6.60/ac].

There was a significant test site effect ($F = 147.51$; df = 6; $P < 0.0001$), thus each site was analyzed independently. Across test sites, the ET was exceeded in the NBT entry at every site (Table 2.5). At Rohwer, AR and Jackson, TN the NBT required two insecticide applications whereas the remaining sites were treated once. The WS entry reached ET once at every site except Starkville, MS, which never reached ET. The BG2 entry reached ET only once at the Stoneville, MS, Starkville, MS, and Rohwer, AR sites. All technology entries received preventive sprays, each *Bt* technology received two preventive sprays at the Rohwer, AR site, and the NBT at Jackson, TN required two preventive sprays. When utilizing the ET across all sites, the WS and BG2 entries resulted in a 25 and 75% reduction in insecticide applications, respectively.

There was a treatment × *Bt* technology interaction at the majority of the locations except at Stoneville, MS and Rohwer, AR (Table 2.5). At Stoneville, MS, there were no differences in profitability among spray treatment timings within any of the *Bt* technologies. At Starkville, MS, the ET was reached for the NBT and BG2, which each required a single insecticide application while WS never reached ET. Profitability did not differ for any treatments within the NBT and WS. In BG2, however, ET exhibited significantly greater profit than the preventive spray. ET was not significantly different than the untreated. At the Pine Bluff, AR and Winnsboro, LA sites, ET was reached for the NBT and WS, but not for BG2. At these sites, profitability did not differ for any treatments within either *Bt* entries, but profit within the NBT for the ET and preventive treatments exceeded that of the untreated treatments. At Rower, AR, the ET was reached for all of the *Bt* technology entries. Profitability did not differ for any treatments within the WS and BG2, whereas, within the NBT, preventive treatments exhibited significantly greater profitability than both the untreated and the ET treatments. At Jackson, TN and St. Joseph, LA sites, ET was reached for the NBT and WS, but not for BG2. ET was reached twice at Jackson, TN and once at St. Joseph, LA. For Jackson, TN, profit did not differ for any treatments within the BG2. For WS, the ET and preventive treatments did not differ in profitability but both did differ from the untreated treatment. For the NBT, preventive treatments did not differ in profitability to both the untreated and ET treatments; however, ET exhibited greater profit than the untreated treatments. For St. Joseph, LA, there was no significant difference in profitability among treatments within BG2. Within WS, preventive treatments increased profits above the untreated treatment while preventive treatment and ET did not differ. ET and preventive treatments did not differ in profitability, however, they both exhibited significantly greater profit than the untreated treatment (Table 2.5).

2.4. Discussions

Regardless of the *Bt* technology utilized, *Bt* cotton may exhibit unacceptable injury from bollworm infestation, making the implementation of action thresholds vital for long-term management of the pest (Reisig et al. 2019). Similar to some previous studies (Adkisson et al. 1964, Sullivan et al. 1998, Carter et al. 2015, Del Pozo-Valdivia et al. 2021), our data suggests that square and/or boll injury, as well as larval density, can be used as indicators to determine when insecticide application targeting bollworm in cotton should be initiated. However, neither the ET based on square damage nor boll damage alone should be considered viable ETs since none of the data utilized in the model represented pre-bloom or post-physiological crop cutout bollworm infestations. These ETs should only be utilized from early bloom through late bloom to reflect the physiological period when our data was collected. During this period, it is standard practice for both squares and bolls to be sampled for bollworm damage in a fashion similar to the sampling procedure we utilized. Because the slopes of the linear regression models significantly differed between squares and bolls (1.86 damaged squares equaled 1 damaged boll), boll damage estimates should be weighted accordingly. Therefore, the fruit EIL is based on sampling an equal number of squares and bolls, but the boll damage values are weighted (Table 2.3). For ease of field utility, when sampling an unequal number of squares and bolls, damaged bolls could be counted as 2 damaged squares and the square threshold utilized, but some slight accuracy in ET computation will be sacrificed.

The calculated EILs and corresponding ETs in the current study provides a matrix of values that vary depending on the crop value as well as the crop yield potential. This flexibility in EIL and ET allows producers and crop protection advisors the ability to customize the ET based on yield potential, market value and control cost. ETs calculated for low market value and low yield potential cotton were consistently greater for each individual variable-damaged square, damaged boll, and damaged fruiting form. For general threshold and validation purposes, we chose a 6% fruit injury ET because it represents the rounded mean ET (5.56%) across all yield potentials and market values utilized within our tabulations (Table 2.3).

Sullivan et al. (1998) suggested treatment thresholds of 75 eggs or 30 small larvae (less than 0.64 cm), 3 large larvae (> 0.64 cm), or 5% boll damage per 100 plants. In that study, they did not consider damaged squares into the computation of the ET. However, our data suggest that damaged squares are a viable factor for estimating yield reduction and may be utilized for determination of ET. The determination of a threshold based on damaged squares is also supported by Del Pozo-Valdivia et al. (2021) where a > 5% damaged squares threshold was suggested, similar to the 6% (rounded mean damaged square ET) damaged squares threshold calculated in this current study. The economic threshold (rounded mean larvae ET) of 2.88 large larvae per 100 plants computed in the current study was similar to that suggested by Sullivan et al. (1998) of 3 larvae per 100 plants. Sullivan et al. (1998) determined that a threshold of 75 eggs per 100 plants was a more effective threshold, and thresholds based on eggs have often been recommended for use in NBT cotton. However, factors associated with the survival of bollworm larvae, including eggs predation and parasitism, natural mortality of neonates, dispersal of neonates, and bollworm/tobacco budworm (*Chloridea virescens*) eggs ratio complicate the implementation of a threshold based on eggs (Del Pozo-Valdivia et al. 2021). In a caged study conducted before the introduction of *Bt* cotton, a threshold of 10 larvae or greater per 100 plants was recommended (Adkisson et al. 1964). Because they did not report larval size, it can be assumed this larval threshold included small and large larvae, making comparison between this recommended larval threshold and our computed larval threshold difficult. Additionally, utilizing small larvae numbers in a threshold for *Bt* cotton does not allow for the *Bt* technology to fully exhibit efficacy in that the bollworm neonates should ingest the *Bt* expressing cotton tissue for the *Bt* toxin to be effective; when sufficient toxin is ingested by susceptible bollworm neonates, second instar bollworm larvae is not anticipated (Reisig et al. 2019, Del Pozo-Valdivia et al. 2021).

Economic thresholds currently recommended for bollworm management in cotton vary throughout much of the southern Cotton Belt although many states have a common egg threshold recommendation. For instance, in Mississippi and Louisiana thresholds of 20% eggs, 4 larvae per 100 plants, or 6% fruit injury are recommended for dual-gene *Bt* cotton, and 4 larvae (≥ 0.32 cm) per 100 plants, or 6% fruit injury for triple-gene *Bt* cotton (LSU AgCenter 2020, MSU-Extension

2021). In Tennessee, thresholds of 4 larvae ≥ 0.64 cm per 100 plants, or 6% fruiting form injury are recommended (UT-Extension 2018). In South Carolina thresholds of 50 eggs per 100 plants, 3 ≥ large larvae (0.64 cm) per 100 plants, or 5%square/boll damaged are recommended for both dual and triple-gene cotton (CU-Cooperative Extension Service, 2021). In North Carolina, thresholds of 25% eggs on 100 leaves or fruiting structure for dual-gene cotton, and 4% damaged bolls or 3 ≥ 2nd instar larvae (0.32 cm) per 100 fruits for triple-gene *Bt* cotton are recommended (Reisig and Huseth 2023). In Arkansas, thresholds of 25% eggs, or 5% damaged fruiting form, or 2-3 large larvae (0.64 cm) per 4.3 row meters (14 row feet) are recommended for dual-gene cotton, and 5% damaged fruiting form, or 2-3 large larvae (0.64 cm) per 4.3 row meters (14 row feet) are recommended for triple-gene cotton (Insecticide Recommendation for Arkansas, 2019). The recommended threshold in Texas is 6% damaged squares and/or bolls with larvae present for both dual and triple-gene cotton (Vyavhare et al. 2018).

The fruit damage and larval thresholds currently recommended by state Extension services, as well as those suggested by Del Pozo-Valdivia et al. (2021), are similar to the values we report in the current study (Table 2-3), although our data suggest that thresholds using only bolls should usually not exceed 4% (averaging approximately 3% across market prices and yield potentials). Del Pozo-Valdivia et al. (2021) findings support this conclusion. Many state Extension services have adopted egg thresholds for non-*Bt* and dual-gene cotton, although some believe egg thresholds are undesirable since they do not permit the *Bt* technologies to demonstrate efficacy nor do they account for evolving levels of *Bt* resistance (Reisig et al. 2019), and they would likely need to be adjusted based on the efficacy of each *Bt* technology. Utilizing damage and/or larval density thresholds often results in larval “escapes”, where the bollworm larvae ultimately end up residing within the plant canopy or within fruiting structures where they are protected from insecticide application (Sullivan et al. 1998). However, utilizing egg thresholds is not justified, and not currently recommended by any state Extension service for cotton expressing Vip3Aa, or for cotton grown in areas were widespread *Bt* resistance has not been documented. Additionally, we do not recommend utilizing a threshold based on large larvae; although we calculated such a threshold in the current study, we believe making an application decision based on large larvae would likely result in poor control as most ≥ 2nd instar bollworms in cotton will be found protected within fruiting structures or the plant canopy (Reisig et al. 2019).

Among *Bt* cotton technologies, we were unable to detect differences in frequencies of increased yields from treating for bollworms with insecticides. However, the frequency of yield increase from spraying NBT was significantly greater than all of the *Bt* technologies, with the exception of WS. Other studies report WS to be less effective against bollworm infestations compared to other dual-gene cotton varieties (Carter et al. 2015, Kerns et al. 2018, Fleming et al. 2018, Rabelo et al. 2020). Bioassays have demonstrated that bollworm larvae surviving on BG2 cotton exhibit lower body weight relative to larvae from WS cotton (Rabelo et al. 2020), which probably results in less feeding and crop injury. Thus, increases in yield following insecticide applications targeting bollworms in WS are expected to be more frequently observed than cotton technologies expressing Cry2A and Vip3Aa *Bt* proteins. However, bollworm resistance to Cry2Ab2 is common, as are control failures associated with *Bt* cottons expressing Cry2A proteins (Dively et al. 2016, Reisig and Kurtz 2018). Thus, although BG2 can be an effective technology, it should be scouted for bollworm and bollworm injury for insecticide application decision making. The inability of WS to continually provide suitable control is likely due to the ineffectiveness of Cry1F and the widespread field-evolved resistance of bollworm to Cry1Ac and

Cry2Ab (Ali et al. 2006, Tabashnik et al. 2012, Crespo et al. 2016, Kaur et al. 2019, Yang et al. 2019). Despite the widespread resistance, the Cry2A *Bt* proteins appear to offer utility towards managing bollworms (Kerns et al. 2018), a trend we also observed in the current study. Additionally, high variability in yield response from treating with insecticides was observed especially for NBT, WS and TL. This observed variability is most likely explained by the differences in bollworm pressure across sites observed throughout the study and the difference in frequency of *Bt*-resistance within bollworm populations across sites. Since incidence of selection pressure varies among locations, ratios of susceptible and *Bt*-resistant bollworms may consequently vary (Fleming et al. 2018).

All *Bt* technology tested in this current study exhibited an increase in yield from spraying (Table 2.4). Although triple-gene *Bt* cotton varieties experienced significant yield increase from treating with insecticides less frequently (9.5 and 11.1%), their frequencies were close to most dual-gene *Bt* cotton varieties (12.5 and 16.7%) with exception of WS (41.7 %). Results from other studies demonstrated that unexpected bollworm injury may occur in all currently available *Bt* technologies (Fleming et al. 2018, Brown et al. 2019). Besides field-evolved resistance of bollworm to Cry *Bt* proteins (Ali et al. 2006, Tabashnik et al. 2012, Crespo et al. 2016, Kaur et al. 2019), alleles conferring resistance to bollworm against Vip3Aa have been reported (Yang et al. 2020). Moreover, occurrence of unexpected injury involving Vip3Aa expressing corn (Yang et al. 2018, Dively et al. 2021) and cotton (Kerns et al. 2015; Brown et al. 2019) have been reported. Additionally, results from Dively et al. (2021) study involving Vip3Aa expressing sweet corn suggested that bollworm susceptibility to Vip3Aa is declining. It is likely that susceptibility of bollworm to this currently most effective *Bt* protein is also being threatened by the lack of implementation of appropriate insect resistance management (IRM) strategy (Reisig and Kurtz 2018).

Based on profitability of utilizing the 6% fruiting form injury ET relative to not treating or treating preventatively, as expected, the NBT benefitted the most from insecticide applications. At five of the seven locations, treatments triggered at an ET and/or preventively exhibited significantly greater profitability relative to the untreated. For WS, however, ET and/or preventive treatments were more profitable than not treating at 2 out of 7 locations. In contrast, for BG2, neither ET nor preventive treatments differed in profitability relative to the untreated at any of the seven locations. Profitability is significantly influenced by the amount of yield increase generated with regard to insecticide application and cost of the *Bt* technology. The degree of bollworm protection afforded by an insecticide application in NBT cotton, relative to a *Bt* cotton, is expected to be more pronounced even where *Bt* resistance occurs since a proportion of the bollworms will most likely still be susceptible to the *Bt* proteins.

The results of the current studies suggest that using a percent bollworm fruiting form injury threshold is viable, and utilizing current crop market price, yield potential, and control cost can further refine that threshold. Field validation provided strong evidence that the use of a 6% fruiting form injury threshold favored a decrease in insecticide applications and resulted in profitability equivalent to preventive sprays. Thus, incorporating a vetted ET into an IPM program targeting bollworm can be highly beneficial. Additionally, implementation of this threshold can result in decrease insecticide use which consequently may lead to reduced environmental impacts. Since chlorantraniliprole has long residual activity (Adams et al. 2016), and field-evolved resistance has already been detected for several target pests (Silva et al. 2018, Wang et al. 2018, Yeole et al. 2018), potential development of bollworm resistance to chlorantraniliprole is a concern. Therefore, the adoption of an effective economic threshold will hopefully reduce the unnecessary applications of chlorantraniliprole and reduce resistance selection pressure. Future research addressing the benefit of insecticide treatments targeting bollworm in *Bt* cotton, as well as further evaluation of the ETs would be beneficial in order to produce stronger conclusions favoring the most efficient management of bollworm.

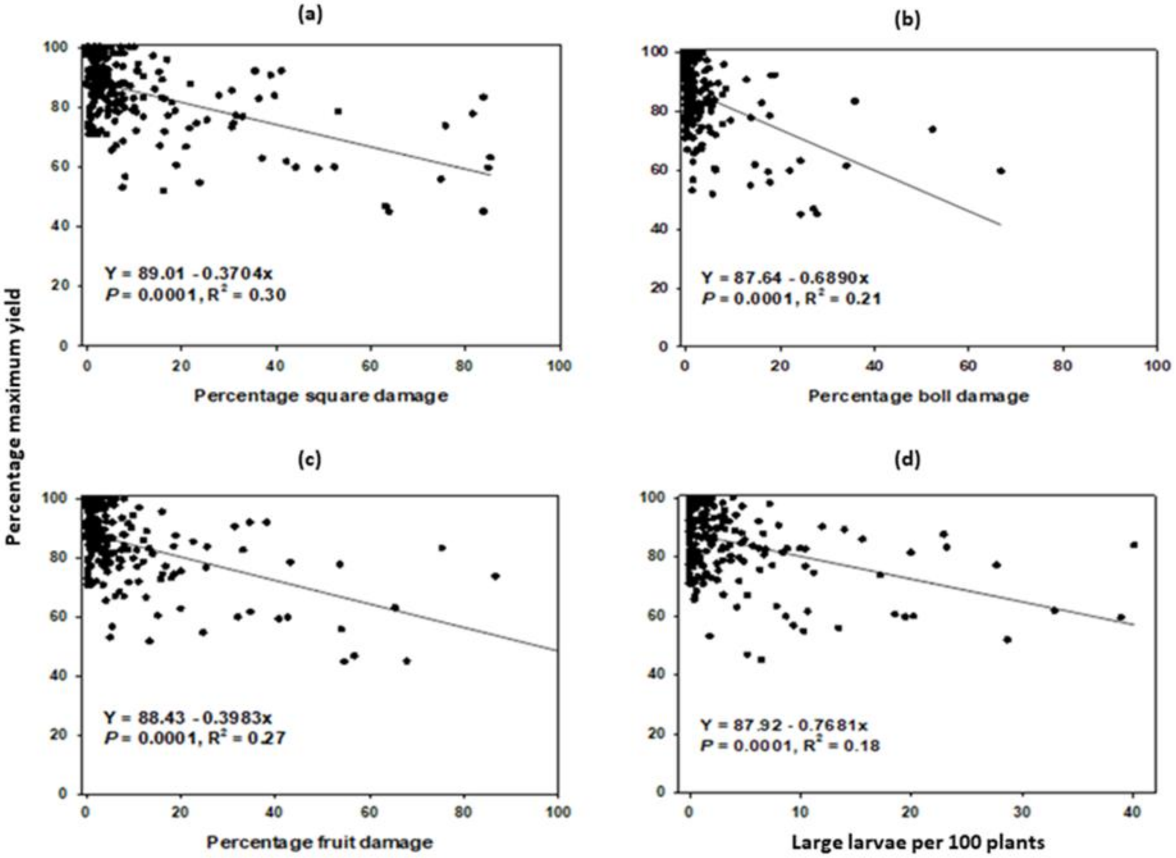

Figure 2.1 Regression analysis of the correlation of percentage maximum yield as influenced by percentage square damage (a); percentage boll damage (b); percentage fruiting form damage (c); large larvae per 100 plants (d).

Table 2.1 Cotton varieties, *Bt* technology and number of trial sites by year.

Bt technology[1]	2014	2015	2017	2018	2019	2020
NBT	PHY 315RF	PHY 315RF	DP 1441RF	DP 1822XF	DP 1822XF	DP 1822XF
TL	ST 5289GLT	ST 5289GLT	ST 494GLT	ST 5122GLT	--	--
TLP	--	--	ST 5575GLTP	ST 5471GTLP	ST 5471GTLP	ST 5471GTLP
BG2	DP 1133B2RF	ST 5288B2RF	ST 4946GLB2	ST 1518B2XF	ST 1518B2XF	ST 1518B2XF
BG3	--	--	--	DP 1835B3XF	DP 1835B3XF	DP 1835B3XF
WS	PHY 499WRF	PHY 499WRF	PHY 333WRF	PHY 333WRF	--	--
WS3	PHY 495W3RF	PHY 495W3RF	PHY 330WRF3	PHY 330WRF3	PHY 330W3FE	PHY 330W3FE
Sites number	5	7	7	8	8	7

[1] NBT = Non-*Bt*, TL = TwinLink™ (Cry1Ab+Cry2Ae), TLP = TwinLink™ Plus (Cry1Ab+Cry2Ae+Vip3Aa), BG2 = Bollgard® 2 (Cry1Ac+Cry2Ab), BG3 = Bollgard® 3 (Cry1Ac+Cry2Ab+Vip3Aa), WS = WideStrike® (Cry1Ac+Cry1F), WS3 = WideStrike® 3 (Cry1Ac+Cry1F+Vip3Aa)

Table 2.2 Variable for calculating gain threshold (GT).

Control cost (C) US$/ha [US$/ac][a]	Yield potential (Y) kg-lint/ha [lbs-lint/ac]	Market price (V) US$/kg-lint [US$/lbs-lint]	Gain threshold (GT) kg-lint/ha [lbs-lint/ac]	Percentage Gain threshold (%GT)
56.76 [22.97]	713.74 [800]	1.10 [0.50]	40.99 [45.94]	7.21
		1.21 [0.55]	37.26 [41.76]	6.56
		1.32 [0.60]	34.16 [38.28]	6.01
		1.43 [0.65]	31.53 [35.34]	5.55
		1.54 [0.70]	29.28 [32.81]	5.15
		1.65 [0.75]	27.32 [30.63]	4.81
		1.76 [0.80]	25.62 28.71]	4.51
		1.87 [0.85]	24.11 [27.02]	4.24
		1.98 [0.90]	22.77 [25.52]	4.01
		2.09 [0.95]	21.57 [24.18]	3.80
		2.20 [1.00]	20.49 [22.97]	3.61
	1,070.62 [1,200]	1.10 [0.50]	40.99 [45.94]	4.81
		1.21 [0.55]	37.26 [41.76]	4.37
		1.32 [0.60]	34.16 [38.28]	4.01
		1.43 [0.65]	31.53 [35.34]	3.70
		1.54 [0.70]	29.28 [32.81]	3.44
		1.65 [0.75]	27.32 [30.63]	3.21
		1.76 [0.80]	25.62 28.71]	3.01
		1.87 [0.85]	24.11 [27.02]	2.83
		1.98 [0.90]	22.77 [25.52]	2.67
		2.09 [0.95]	21.57 [24.18]	2.53
		2.20 [1.00]	20.49 [22.97]	2.40
	1,249.05 [1,400]	1.10 [0.50]	40.99 [45.94]	4.12
		1.21 [0.55]	37.26 [41.76]	3.75
		1.32 [0.60]	34.16 [38.28]	3.44
		1.43 [0.65]	31.53 [35.34]	3.17
		1.54 [0.70]	29.28 [32.81]	2.94
		1.65 [0.75]	27.32 [30.63]	2.75
		1.76 [0.80]	25.62 28.71]	2.58
		1.87 [0.85]	24.11 [27.02]	2.42
		1.98 [0.90]	22.77 [25.52]	2.29
		2.09 [0.95]	21.57 [24.18]	2.17
		2.20 [1.00]	20.49 [22.97]	2.06

Table 2.2 Continued.

Control cost (C) US$/ha [US$/ac][a]	Yield potential (Y) kg-lint/ha [lbs-lint/ac]	Market price (V) US$/kg-lint [US$/lbs-lint]	Gain threshold (GT) kg-lint/ha [lbs-lint/ac]	Percentage Gain threshold (%GT)
	1,427.49	1.10 [0.50]	40.99 [45.94]	3.61
	[1,600]	1.21 [0.55]	37.26 [41.76]	3.28
		1.32 [0.60]	34.16 [38.28]	3.01
		1.43 [0.65]	31.53 [35.34]	2.77
		1.54 [0.70]	29.28 [32.81]	2.58
		1.65 [0.75]	27.32 [30.63]	2.40
		1.76 [0.80]	25.62 28.71]	2.25
		1.87 [0.85]	24.11 [27.02]	2.12
		1.98 [0.90]	22.77 [25.52]	2.00
		2.09 [0.95]	21.57 [24.18]	1.90
		2.20 [1.00]	20.49 [22.97]	1.80
	1,784.36	1.10 [0.50]	40.99 [45.94]	2.89
	[2,000]	1.21 [0.55]	37.26 [41.76]	2.62
		1.32 [0.60]	34.16 [38.28]	2.40
		1.43 [0.65]	31.53 [35.34]	2.22
		1.54 [0.70]	29.28 [32.81]	2.06
		1.65 [0.75]	27.32 [30.63]	1.92
		1.76 [0.80]	25.62 28.71]	1.80
		1.87 [0.85]	24.11 [27.02]	1.70
		1.98 [0.90]	22.77 [25.52]	1.60
		2.09 [0.95]	21.57 [24.18]	1.52
		2.20 [1.00]	20.49 [22.97]	1.44

[a] Control cost estimated with insecticide cost set at US$31.28/l and application cost of US$16.31/ha, equivalent to US$0.93/fl-oz and US$6.60/ac for insecticide and application, respectively.

Table 2.3 EIL and ETs for bollworm injured fruiting form and larvae based on control cost, and yield potential[a]

Yield potential kg-lint/ha [lbs-lint/ac]	Market Price US$/kg-lint [US$/lb-lint]	Percentage damaged						Larvae/100 plants	
		Squares		Bolls		Fruit[b]			
		EIL	ET	EIL	ET	EIL	ET	EIL	ET
713.74 [800]	1.10 [0.50]	19.48	13.64	10.47	7.33	18.11	12.68	9.39	6.57
	1.21 [0.55]	17.71	12.40	9.52	6.66	16.47	11.53	8.54	5.98
	1.32 [0.60]	16.23	11.36	8.73	6.11	15.09	10.56	7.83	5.48
	1.43 [0.65]	14.98	10.49	8.05	5.64	13.93	9.75	7.22	5.05
	1.54 [0.70]	13.91	9.74	7.48	5.24	12.94	9.06	6.71	4.70
	1.65 [0.75]	12.98	9.09	6.98	4.89	12.07	8.45	6.26	4.38
	1.76 [0.80]	12.17	8.52	6.54	4.58	11.32	7.92	5.87	4.11
	1.87 [0.85]	11.46	8.02	6.16	4.31	10.65	7.46	5.52	3.86
	1.98 [0.90]	10.82	7.57	5.82	4.07	10.06	7.04	5.22	3.65
	2.09 [0.95]	10.25	7.18	5.51	3.86	9.53	6.67	4.94	3.46
	2.20 [1.00]	9.74	6.82	5.24	3.67	9.06	6.34	4.70	3.29
1070.62 [1200]	1.10 [0.50]	12.98	9.09	6.98	4.89	12.07	8.45	6.26	4.38
	1.21 [0.55]	11.80	8.26	6.35	4.45	10.98	7.69	5.69	3.98
	1.32 [0.60]	10.82	7.57	5.82	4.07	10.06	7.04	5.22	3.65
	1.43 [0.65]	9.99	6.99	5.37	3.76	9.29	6.50	4.82	3.37
	1.54 [0.70]	9.27	6.49	4.99	3.49	8.62	6.03	4.47	3.13
	1.65 [0.75]	8.66	6.06	4.65	3.26	8.05	5.64	4.17	2.92
	1.76 [0.80]	8.12	5.68	4.36	3.05	7.55	5.29	3.91	2.74
	1.87 [0.85]	7.64	5.35	4.11	2.88	7.10	4.97	3.68	2.58
	1.98 [0.90]	7.21	5.05	3.88	2.72	6.71	4.70	3.48	2.44
	2.09 [0.95]	6.83	4.78	3.67	2.57	6.36	4.45	3.30	2.31
	2.20 [1.00]	6.49	4.54	3.49	2.44	6.04	4.23	3.13	2.19
1249.05 [1400]	1.10 [0.50]	11.13	7.79	5.98	4.19	10.35	7.25	5.37	3.76
	1.21 [0.55]	10.12	7.08	5.44	3.81	9.41	6.59	4.88	3.42
	1.32 [0.60]	9.27	6.49	4.99	3.49	8.62	6.03	4.47	3.13
	1.43 [0.65]	8.56	5.99	4.60	3.22	7.96	5.57	4.13	2.89
	1.54 [0.70]	7.95	5.57	4.27	2.99	7.39	5.17	3.83	2.68
	1.65 [0.75]	7.42	5.19	3.99	2.79	6.90	4.83	3.58	2.51
	1.76 [0.80]	6.96	4.87	3.74	2.62	6.47	4.53	3.35	2.35
	1.87 [0.85]	6.55	4.59	3.52	2.46	6.09	4.26	3.16	2.21
	1.98 [0.90]	6.18	4.33	3.32	2.32	5.75	4.03	2.98	2.09
	2.09 [0.95]	5.86	4.10	3.15	2.21	5.45	3.82	2.82	1.97
	2.20 [1.00]	5.56	3.89	2.99	2.09	5.17	3.62	2.68	1.88

Table 2.3 Continued.

Yield potential kg-lint/ha [lbs-lint/ac]	Market Price US$/kg-lint [US$/lb-lint]	Percentage damaged						Larvae/100 plants	
		Squares		Bolls		Fruit[b]			
		EIL	ET	EIL	ET	EIL	ET	EIL	ET
1427.49 [1600]	1.10 [0.50]	9.74	6.82	5.24	3.67	9.06	6.34	4.70	3.29
	1.21 [0.55]	8.85	6.20	4.76	3.33	8.23	5.76	4.27	2.99
	1.32 [0.60]	8.12	5.68	4.36	3.05	7.55	5.29	3.91	2.74
	1.43 [0.65]	7.49	5.24	4.03	2.82	6.97	4.88	3.61	2.53
	1.54 [0.70]	6.96	4.87	3.74	2.62	6.47	4.53	3.35	2.35
	1.65 [0.75]	6.49	4.54	3.49	2.44	6.04	4.23	3.13	2.19
	1.76 [0.80]	6.09	4.26	3.27	2.29	5.66	3.96	2.94	2.06
	1.87 [0.85]	5.73	4.01	3.08	2.16	5.33	3.73	2.76	1.93
	1.98 [0.90]	5.41	3.79	2.91	2.04	5.03	3.52	2.61	1.83
	2.09 [0.95]	5.13	3.59	2.76	1.93	4.77	3.34	2.47	1.73
	2.20 [1.00]	4.87	3.41	2.62	1.83	4.53	3.17	2.35	1.65
1784.36 [2000]	1.10 [0.50]	7.79	5.45	4.19	2.93	7.24	5.07	3.76	2.63
	1.21 [0.55]	7.08	4.96	3.81	2.67	6.59	4.61	3.42	2.39
	1.32 [0.60]	6.49	4.54	3.49	2.44	6.04	4.23	3.13	2.19
	1.43 [0.65]	5.99	4.19	3.22	2.25	5.57	3.90	2.89	2.02
	1.54 [0.70]	5.56	3.89	2.99	2.09	5.17	3.62	2.68	1.88
	1.65 [0.75]	5.19	3.63	2.79	1.95	4.83	3.38	2.50	1.75
	1.76 [0.80]	4.87	3.41	2.62	1.83	4.53	3.17	2.35	1.65
	1.87 [0.85]	4.58	3.21	2.46	1.72	4.26	2.98	2.21	1.55
	1.98 [0.90]	4.33	3.03	2.33	1.63	4.02	2.81	2.09	1.46
	2.09 [0.95]	4.10	2.87	2.20	1.54	3.81	2.67	1.98	1.39
	2.20 [1.00]	3.90	2.73	2.09	1.46	3.62	2.53	1.88	1.32

[a] Control cost estimated with insecticide cost set at US$31.28/l and application cost of US$16.31/ha, equivalent to US$0.93/fl-oz and US$6.60/ac for insecticide and application, respectively.

[b] Equal number of squares and bolls sampled.

Table 2.4 Percent yield increase as affected by Prevathon® (0.067 g-AI/ha).

Bt technology	Number of test sites	Sum of scores ± SD	Mean score*	Percentage of sites with significant yield increase
All test sites				
NBT	36	4870.50 ± 231.25	135.29 a	63.90
WS	24	2727.00 ± 195.81	113.63 ab	41.70
BG2	36	3213.00 ± 231.25	89.25 b	16.70
TL	24	2044.50 ± 195.81	85.19 b	12.50
TLP	21	1728.00 ± 184.77	82.29 b	9.50
BG3	18	1509.00 ± 172.53	83.83 b	11.10
WS3	36	3018.00 ± 231.25	83.83 b	11.10
Test sites where NBT significantly benefitted from treating with insecticide				
NBT	23	2311.50 ± 131.24	100.50 a	100.00
WS	15	1135.50 ± 110.10	75.70 b	60.00
BG2	23	1257.50 ± 131.24	54.67 b	26.10
TL	15	763.50 ± 110.10	50.90 b	20.00
TLP	14	663.00 ± 106.86	47.36 b	14.30
BG3	11	485.50 ± 96.00	44.14 b	9.10
WS3	23	1133.50 ± 131.24	49.28 b	17.40

*Mean scores = mean ranks assigned to the observations generated by the Kruskal-Wallis test. Mean score with the same letter are not significantly different (Wilcoxon $\alpha = 0.05$).

Table 2.5 Profitability US$/ha among *Bt* technology across seven sites as affected by Prevathon® applied at ET or preventively at 0.067 g-AI/ha and number of Prevathon® applications.

		Bt technology and number of insecticide applications (App.)					
Site	Treatment	Non-*Bt*	App.	WideStrike	App.	Bollgard II	App.
Starkville, MS	Non-treated	1580.15 ± 37.76 abc	0	1766.35 ± 115.56 a	0	1517.42 ± 80.22 bc	0
	ET	1731.46 ± 58.01 ab	1	1799.09 ± 72.14 a	0	1737.63 ± 50.03 ab	1
	Preventive	1749.31 ± 129.76 ab	1	1468.32 ± 80.22 a	1	1460.42 ± 67.22 c	1
	Bt × Trt (*P* = 0.04)						
Stoneville, MS	Non-treated	1832.85 ± 112.81 a	0	2424.43 ± 237.18 a	0	2461.57 ± 185.98 a	0
	ET	2012.93 ± 116.72 a	1	2265.61 ± 167.58 a	1	2240.62 ± 131.82 a	1
	Preventive	1998.81 ± 103.06 a	1	2259.97 ± 145.21 a	1	2257.57 ± 122.76 a	1
	Bt × Trt (*P* = 0.61)						
Pine Bluff, AR	Non-treated	2013.59 ± 50.56 d	0	2220.47 ± 104.66 cd	0	2459.44 ± 146.64 abc	0
	ET	2643.64 ± 103.98 a	1	2353.83 ± 82.29 bc	1	2349.40 ± 132.34 bc	0
	Preventive	2557.28 ± 103.29 ab	1	2437.14 ± 69.88 abc	1	2432.20 ± 211.57 abc	1
	Bt × Trt (*P* = 0.01)						
Winnsboro, LA	Non-treated	887.45 ± 60.07 c	0	1131.51 ± 108.83 ab	0	955.79 ± 119.91 bc	0
	ET	1245.23 ± 218.30 a	1	1191.22 ± 128.29 ab	1	1168.06 ± 226.85 ab	0
	Preventive	1233.94 ± 217.76 a	1	1195.74 ± 168.27 ab	1	977.12 ± 144.82 bc	1
	Bt × Trt (*P* = 0.03)						
Rohwer, AR	Non-treated	1681.04 ± 43.64 b	0	1763.02 ± 144.26 a	0	1845.54 ± 144.43 a	0
	ET	1724.08 ± 78.62 b	2	1705.42 ± 94.49 a	1	1953.80 ± 177.75 a	1
	Preventive	2101.81 ± 103.19 a	2	1844.13 ± 46.39 a	2	1914.16 ± 170.55 a	2
	Bt × Trt (*P* = 0.32)						
Jackson, TN	Non-treated	2352.41 ± 51.94 cd	0	2281.42 ± 124.94 d	0	2572.89 ± 108.88 bc	0
	ET	2816.75 ± 98.42 a	2	2579.23 ± 63.97 abc	1	2661.32 ± 84.89 ab	0
	Preventive	2574.26 ± 124.65 abc	2	2635.34 ± 52.79 ab	1	2552.03 ± 59.10 bc	1
	Bt × Trt (*P* = 0.02)						

Table 2.5 Continued.

		Bt technology and number of insecticide applications (App.)					
Site	Treatment	Non-*Bt*	App.	WideStrike	App.	Bollgard II	App.
St. Joseph, LA	Non-treated	906.52 ± 120.57 b	0	1032.15 ± 304.33 b	0	1122.90 ± 220.40 a	0
	ET	1330.05 ± 85.10 a	1	990.25 ± 246.95 ab	1	1107.09 ± 223.99 a	0
	Preventive	1405.56 ± 111.08 a	1	1423.81 ± 139.10 a	1	1230.03 ± 243.97 a	1
	Bt × Trt (*P* = 0.03)						

Where there is no significant ($P \geq 0.05$) Treatment × *Bt* technology interaction, means in a column within a site and *Bt* technology followed by the same letter are not significantly different ($P \geq 0.05$); where there was a significant ($P < 0.05$) Treatment × *Bt* technology interaction, means within a site followed by the same letter are not significantly different ($P \geq 0.05$).

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3. PROFITABILITY OF COTTON, *GOSSYPIUM HIRSITUM* (L.), WHEN CONTROLLING BOLLWORM, *HELICOVERPA ZEA* (BODDIE), WITH VARIABLE TREATMENT THRESHOLDS

3.1. Introduction

Varieties of cotton, *Gossypium hirsutum* (L.) (Malvales: Malvaceae), containing genes from *Bacillus thuringiensis* (*Bt)* (Bacillales: Bacillaceae) predominate the Cotton Belt across the United States as the primary means of controlling lepidopteran pests, including *Helicoverpa zea* (Boddie) and *Chloridea virescens* (F.) (Lepidoptera: Noctuidae), in the crop (Fleming et al. 2018). Currently, commercialized varieties of *Bt* cotton express either only crystalline (Cry) proteins, or Cry proteins pyramided with vegetative insecticidal proteins (Vip) (Vyavhare and Kerns 2022). Crops expressing these *Bt* proteins have offered valuable benefits including reduced chemical insecticide use and crop yield protection (Carpenter 2010, Hutchison et al. 2010, Yu et al. 2011, Edgerton et al. 2012, Kathage and Qaim 2012, Lu et al. 2012). However, the success of *Bt* crops in insect pest management has led to extensive use of the technology. This use has placed a strong selection pressure on target pest populations resulting in the evolution of resistance in *H. zea* and consequently threatening these benefits (Gould 1998, Huang et al. 2011, Tabashnik et al. 2013).

Resistance in agricultural pests has been described as field-evolved resistance and practical resistance. Field-evolved resistance is a genetically mediated decline in the susceptibility of a population to a pesticide after being extensively exposed to the pesticide in the field. Practical resistance is an outcome of field-evolved resistance and occurs when field-evolved resistance causes a decrease in the efficacy of a pesticide leading to practical consequences for pest control (Tabashnik et al. 2014). Numerous studies have demonstrated the occurrence of widespread practical resistance in *H. zea* to pyramided *Bt* cotton containing Cry1Ac, Cry2Ab, and Cry1F *Bt* proteins (Reisig et al. 2018, Yang et al. 2018, Yang et al. 2022). The addition of Vip3Aa in pyramided cotton has resulted in exceptional control of *H. zea* (Kerns et al. 2019, Rabelo et al. 2020). However, unexpected injuries in corn (Yang et al. 2018, Dively et al. 2021) and cotton (Kerns et al. 2015, Brown et al. 2019) expressing Vip3Aa have been reported. A strain of *H. zea* collected from corn expressing Vip3Aa20 in Texas demonstrated the ability to survive elevated concentrations of Vip3Aa51 in diet overlay assays and on *Bt* cotton tissue expressing Cry1Ac+Cry1F+Vip3Aa19 (Yang et al. 2019). Additionally, major resistance alleles conferring high levels of Vip3Aa resistance in field-derived strains of *H. zea* have been documented in Texas, Louisiana, and Mississippi (Yang et al. 2020, Santiago-Gonzalez et al. 2023). Moreover, a 3-year monitoring study across 18 to 30 sites in the United States and eastern Canada demonstrated that the phenotypic frequency of resistance in *H. zea* to Vip3Aa is gradually increasing (Dively et al. 2021).

Because of the issues surrounding *Bt* resistance, reliance on insecticides to manage *H. zea* in *Bt* cotton has become common and widespread (Kerns et al. 2018, Cook 2018, Reisig et al. 2019). Currently, chlorantraniliprole (Diamides: IRAC MoA group 28) is the most widely used insecticide to manage *H. zea* in cotton. Chlorantraniliprole is highly effective and quick-acting, has little negative impact on insect pests' natural enemies, and provides good residual control, making them a suitable choice for producers (Richardson et al. 2020). With the exclusive reliance on chlorantraniliprole for control of *H. zea* in cotton and other crops, along with its long residual activity and high selection pressure, there is a great concern that resistance to chlorantraniliprole may rapidly develop (Adams 2016, Richardson et al. 2020). For instance, field-evolved resistance to chlorantraniliprole has already been reported for several lepidopteran pests, including *S. frugiperda*; diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae); tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae); and beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Wang et al. 2012, Silva et al. 2018, Yeole et al. 2018, Boaventura et al. 2019). In Brazil, populations of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), a close relative of *H. zea*, subjected to a diagnostic dose of chlorantraniliprole showed an increase in survivorship from 0% in 2014 to approximately 60% in 2018, indicating a rapid evolution of resistance in that species towards chlorantraniliprole (Pereira et al. 2020). There is a possibility that a similar pattern of evolution of resistance could occur in *H. zea* if appropriate insecticide resistance management action is not taken in a timely fashion.

Control of *H. zea* with foliar insecticide sprays has been inconsistent due to problems associated with insecticide resistance and action threshold timing (Kerns et al. 2017, Reisig et al. 2019). An action threshold is a pest density or degree of damage at which control action should be taken to prevent an increase in the pest population density before yield loss occurs at the economic injury level (Stern et al. 1959). Prior to the widespread incidence of unexpected *H. zea* injury in *Bt* cotton, most action thresholds were based on the number of large larvae present. This approach provided time for natural mortality and efficacy of *Bt* cotton to reduce populations of *H. zea* before prompting supplemental applications of insecticide (Reisig et al. 2019). A threshold based on fruiting forms injury with larvae present was devised and suggested for management of *H. zea* in *Bt* cotton (Calvin et al. 2021). Thresholds based on densities of large larvae are also recommended by several university Extension services (UT-Extension 2018, UA-Research & Extension 2019, MSU-Extension 2021, CU-Cooperative Extension Service 2021, Reisig and Huseth 2023, LSU AgCenter 2023). However, the utilization of thresholds based on damage to fruiting forms may result in inadequate control because they allow time for larvae to disperse into secluded areas of the plant canopy and within fruiting structures to avoid contact with the insecticide (Kerns et al. 2017, Vyavhare and Kerns 2022). Consequently, action thresholds for *H. zea* in *Bt* cotton have changed throughout much of the Cotton Belt. Many university Extension services have adopted action thresholds based on egg counts for *H. zea* management in non-Bt and dual-gene *Bt* cotton (UA-Research & Extension 2019, CU-Cooperative Extension Service 2021, MSU-Extension 2021, Reisig and Huseth 2023, LSU AgCenter 2023). However, because of the uncertainty of *Bt* efficacy, insecticide resistance, spray application timing, impact on beneficial arthropods, and economic benefit, there continues to be uncertainty and a lack of scientifically vetted data to support reliance on various adopted thresholds and treatment recommendations. Thus, there is a fundamental need to reassess action thresholds in *Bt* cotton and validate current recommendations.

In this study, we assessed the current recommended thresholds for controlling *H. zea* in cotton for Mississippi, South Carolina, and Texas. Additionally, we evaluated how each threshold affected profitability.

3.2. Materials and Methods

3.2.1. Test Locations

These experiments were conducted at three distinct geographical and environmental locations that are representative of the Cotton Belt across the southern United States. The test locations include College Station, TX; Stoneville, MS; and Blackville, SC. These experiments were repeated at each location over a period of two years (2020 and 2021).

3.2.2. Experimental Design and Treatments

For these experiments, three distinct *Bt* cotton technologies and five distinct action thresholds were utilized. The *Bt* cotton technologies included: 1) Non-Bt (NBT; DP 1822 XF), 2) Bollgard® 2 (BG2; Cry1Ac+Cry2Ab; DP 1646 B2XF), and 3) Bollgard® 3 (BG3; Cry1Ac+Cry2Ab+Vip3Aa; DP 1851 B3XF) planted in replicated (4) plots. Each plot was 4 or 8 rows wide and 12.2 m (40 ft) long with a row spacing of 0.97-1.02 m (38-40 inches). Planting dates in 2020 and 2021 were 21 and 22 April in College Station, TX; 18 May and 27 April in Stoneville, MS; and 27 and 22 May in Blackville, SC, respectively. The action thresholds evaluated included: 1) non-treated, 2) foliar spray triggered at 20% oviposition (20% of plants with at least one egg), 3) foliar spray triggered at 6% square and/or boll injury with any size larvae present, 4) foliar spray triggered at ≥3% large larvae (3rd instar larvae or bigger found on 3% of plants sampled), and 5) preventive treatment beginning at first bloom and repeated every two weeks until the *H. zea* infestation subsided. Treatments 2, 3, and 4 were sprayed as necessary based on the average of all four replications. The action thresholds were evaluated separately for each *Bt* technology. These thresholds represent current action threshold recommendations for *H. zea* from Cooperative Extension Service in the southern United States. Where an action threshold was triggered, chlorantraniliprole (Prevathon®, FMC Corporation, Philadelphia, PA) was applied at a rate of 75.1 g-AI/ha with high-clearance sprayers delivering water at 93.5 L/ha (10 gal/ac) and 3.51-4.22 kg/cm or 344.74 - 413.69 kPa (50 - 60 Psi) through TXVS-6 hollow-cone tips or using a CO_2-pressurized backpack sprayer with a 2-row boom equipped with 4 nozzles spaced 50.8 cm (20 inches) apart and calibrated to deliver 93.5 L/ha (10 gal/ac) at 193.05 kPa (28 Psi). Cotton was grown using standard production practices for each location, but no insecticides were applied that would impact *H. zea*.

3.2.3. Field Sampling

For each plot, 25 random plants per plot were sampled weekly beginning at first bloom until *H. zea* infestations subsided. For each plant, the terminal was inspected for evidence of *H. zea* feeding and presence. Four squares (2 small from the top 5 nodes and 2 large from below the top 5 nodes) and four bolls (2 small bolls approximately 1 cm in diameter with bloom tags – dried/attached blossoms – and 2 large bolls approximately 2.0-2.5 cm in diameter with no bloom tags) were sampled on each plant for evidence of damage from and the presence of *H. zea* larvae. Injury to squares and bolls was only recorded as positive when the budding tissue was penetrated, when the fruit-feeding injury would result in square abortion, or when the carpel wall of the boll was penetrated. The size of each *H. zea* larva for all sampling was recorded as small (1st and 2nd instar) or large (3rd, 4th, or 5th instar). Additionally, when inspecting the various plant structures, the number of *Heliothine* eggs was recorded for each plant. Yields were measured as kgs-lint/ha after harvesting the middle 2 rows of each plot using a mechanized harvester.

3.2.4. Ratio of *H. zea* and *C. virescens*

We collected data to determine the ratio of *H. zea* to *C. virescens* at 2 of the 3 locations (College Station, TX; and Blackville, SC). In College Station, TX, approximately 100 *Heliothine* larvae were collected from a strip of NBT cotton planted adjacent to the experimental plots. Upon collection, the larvae were immediately placed individually into 29 ml plastic condiment cups containing a laboratory-based meridic diet (WARD'S Stonefly Heliothis diet, Rochester, NY). The larvae were brought to the laboratory and allowed to develop into adults for speciation to *H. zea* or *C. virescens*. In Blackville, SC, the ratio of *H. zea* to *C. virescens* was determined using data from pheromone traps. These data served to determine if treatments based on 20% oviposition were primarily directed toward the intended target – *H. zea*.

3.2.5. Profitability Calculation

Profitability (P) for each threshold regime within a *Bt* technology was determined for each location within each year. Profitability was determined based on the equation: P (US$/ha) = V − ($C_I$ × A); where V is the crop value (US$/ha) based on yield (kg-lint/ha) and market value (US$/kg-lint), C_I is the cost of insecticide and insecticide application, and A is the number of insecticide applications. This profitability equation is a modified version of that described in Calvin et al. (2021). The insecticide and application costs used in the computation of the profitability were US$45.96/ha (US$18.6/ac) and US$16.31/ha (US$6.60/ac), respectively as described in Calvin et al. (2021). The crop value was estimated at US$2.22/kg-lint (US$1.00/lb-lint) (USDA-NASS 2022).

3.2.6. Statistical Analyses

To account for the non-homogeneity of variance, data for fruiting forms injury and large larvae were *square root* transformed, and the yield and profitability data were transformed using logarithmic (*log*) transformation. All variables including the fruiting forms injury, large larvae, yield, and profitability data were analyzed by *Bt* technology with years and locations combined using an analysis of variance (ANOVA). Action threshold was a fixed effect and year, location, and replicate (year*location) were random effects. The percentage reduction in insecticide applications was also analyzed by *Bt* technology using an analysis of variance (ANOVA). Action threshold was a fixed effect and replicate was a random effect. For this data set, each location by year was considered as a replicate (PROC GLIMMIX. Version 9.4, SAS Institute Inc., Cary, NC). The Kenward-Roger method (Kenward and Roger 1997) was used to compute denominator degrees of freedom for the test of fixed effects for all variables. The Tukey-Kramer adjustment ($\alpha = 0.05$) was utilized to allow the interpretation of pairwise differences between means.

3.3. Results

3.3.1. Ratio of *H. zea* to *C. virescens*

In College Station, TX, the ratio of *H. zea* to *C. virescens* was 30:0 and 26:1 in 2020 and 2021, respectively. In Blackville, SC, the ratio of *H. zea* to *C. virescens* was approximately 4:1 and 3:1 in 2020 and 2021, respectively.

3.3.2. Evaluation of Damaged Fruiting Forms, Large Larvae, Yield, and Profitability

3.3.2.1. NBT Cotton

For the NBT cotton, significant differences in the percentage of damaged fruiting forms ($F = 27.92$; df = 4, 467.9; $P < 0.0001$), percentage of large larvae ($F = 9.48$; df = 4, 466.9; $P < 0.0001$), yield ($F = 15.61$; df= 4, 92; $P < 0.0001$), and profitability ($F = 11.5$; df =4, 92; $P < 0.0001$) were detected among thresholds. We observed a similar trend among the thresholds for the percentage of damaged fruiting forms and the percentage of large larvae. The NBT cotton treated using the ≥ 3% large larvae, 6% fruiting forms injury, 20% oviposition thresholds or treated preventively had a percentage of damaged fruiting forms and a percentage of large larvae significantly lower than that of the non-treated NBT cotton (Fig 3.1A, B). The NBT cotton treated using the ≥ 3% large larvae, 6% fruiting forms injury, and 20% oviposition thresholds were comparable in both percentage of damaged fruiting forms and incidence of large larvae. The NBT cotton treated preventively had the lowest levels of damaged fruiting forms and large larvae. However, the ≥ 3% large larvae threshold exhibited equivalent *H. zea* control to that of the preventive spray (Fig 3.1A, B). The NBT cotton treated using the ≥ 3% large larvae, 6% fruiting forms injury, 20% oviposition thresholds, or treated preventively exhibited yields that were statistically greater than that of the non-treated NBT cotton. The NBT cotton treated preventively exhibited the highest yield but equivalent to that of the NBT cotton treated based on a 6% fruiting forms injury threshold (Fig 3.1C). The NBT cotton treated using the ≥ 3% large larvae, 6% fruiting forms injury, 20% oviposition thresholds or treated preventively showed equivalent profitability that was significantly more profitable than that of the non-treated NBT cotton (Fig 3.1D).

3.3.2.2. BG2 Cotton

For the BG2 cotton, there were significant differences among thresholds in the percentage of damaged fruiting forms ($F = 13.90$; df = 4, 468.5; $P < 0.0001$) and the percentage of large larvae ($F = 5.77$; df = 4, 469.1; $P = 0.0002$). The BG2 cotton treated based on a 20% egg lay threshold or treated preventively exhibited the lowest incidence of percentage damaged fruiting forms and percentage of large larvae (Fig 3.2A, B). The thresholds did not differ for yield ($F = 2.45$; df = 4, 92; $P = 0.0516$) or profitability ($F = 1.46$; df = 4, 92; $P = 0.2216$).

3.3.2.3. BG3 Cotton

For the BG3 cotton, the thresholds did not differ in either the percentage of damaged fruiting forms ($F = 0.40$; df = 4, 467; $P = 0.8076$), percentage of large larvae ($F = 1.82$; df = 4, 489; $P = 0.1242$), yield ($F = 1.10$; df = 4, 92; $P = 0.3615$), or profitability ($F = 1.24$; df= 4, 92; $P = 0.2999$) (Fig 3.3).

3.3.3. Comparison of the Thresholds in Insecticide Application Reduction

The thresholds of 20% oviposition, 6% fruiting forms injury, and ≥ 3% large larvae provided substantial reductions in insecticide applications relative to the preventive spray. Across all years and test locations, the 20% oviposition threshold resulted in insecticide application reduction ranging from 50 to 100% across all *Bt* cotton technologies for an average of 70.9, 76.4, and 70.9% for NBT, BG2, and BG3, respectively (Table 3-1). Across all years and test locations, the ≥ 3% large larvae threshold provided reductions in insecticide applications ranging from 0 to 66.7% within the NBT cotton and 25 to 100% within the BG2 cotton for an average of 41.7 and 73.6% for NBT and BG2, respectively. The ≥ 3% large larvae threshold provided a 100% reduction in insecticide applications within the BG3 cotton in all years and at all locations (Table 3-1). Across all years and test locations, the 6% fruiting forms injury threshold resulted in a reduction in insecticide applications ranging from 0 to 66.7% within the NBT cotton for an average of 47.2%, and 66.7 to 100% within the BG2 and BG3 cotton for an average of 79.2 and 94.5% for BG2 and BG3, respectively (Table 3-1). When the percentage reduction in insecticide applications among thresholds was compared using an ANOVA, a significant difference among thresholds was observed within NBT ($F = 41.33$; df = 4, 20; $P < 0.0001$), BG2 ($F = 25.30$; df = 4, 20; $P < 0.0001$), and BG3 ($F = 102.22$; df = 4, 20; $P < 0.0001$) cotton. For NBT cotton, the 20% oviposition threshold resulted in a greater percentage reduction in insecticide applications than the ≥ 3% large larvae threshold. The 6% fruiting forms injury threshold had a percentage reduction in insecticide applications comparable to both the 20% oviposition and ≥ 3% large larvae thresholds. For BG2 cotton, the reduction in insecticide applications was similar for the thresholds of 6% fruiting forms injury, 20% oviposition, and ≥ 3% large larvae. For BG3 cotton, the ≥ 3% large larvae and 6% fruiting forms injury thresholds provided a comparable reduction in insecticide applications, but greater than that of the 20% oviposition threshold (Table 3-1).

3.4. Discussions

Helicoverpa zea was highly represented in the populations at an estimated 96 to 100% in College Station, TX, and 75 to 80% in Blackville, SC, indicating that the insecticide applications primarily targeted *H. zea*. Although predominant, *Helicoverpa zea* pressure was relatively low in the field during the study, which may have some impacts on our observations as described in previous studies (Francis 2021, Del Pozo-Valdivia et al. 2021).

To the best of our knowledge, this is one of the few studies to evaluate comprehensively the various adopted economic thresholds utilized in decision-making for *H. zea* management in cotton. Similar to the observations made by Carter et al. (2015), our data show that the benefit of treating cotton with an insecticide targeting *H. zea* was pronounced for NBT cotton and negligible for BG2 cotton. The results of this current study indicate that the efficacy of a threshold and/or the benefit of spraying cotton with an insecticide varies depending on the *Bt* technology used. These findings are consistent with the results of several other studies (Calvin et al. 2021, Del Pozo-Valdivia et al. 2021, Francis 2021).

Although the control of *H. zea* in NBT cotton was equivalent among all tested thresholds (20% oviposition, 6% fruiting forms injury, and ≥ 3% large larvae) the threshold based on larval counts appeared to be the most effective in terms of *H. zea* control compared with the preventive spray. The larval threshold received an equivalent number of insecticide applications to the 6% fruiting forms injury threshold but a 41.2% greater number of insecticide applications than the 20% oviposition threshold (Table 3-1). Thus, the ≥ 3% large larvae threshold was not practically more effective than the 20% oviposition threshold. The 6% fruiting forms injury threshold appeared to be the most effective in terms of yield relative to the preventive spray. Regardless, NBT cotton treated based on any of the tested thresholds resulted in comparable yields. These results are consistent with results from a previous study where insecticide applications triggered on either an egg threshold or fruiting forms injury threshold resulted in comparable *H. zea* control and cotton yields for the NBT cotton (Durant 1991). In contrast, Francis (2021) found that controlling *H. zea* in NBT cotton using a threshold based on fruiting forms injury resulted in a greater yield than using an egg threshold. As expected, treating NBT cotton with insecticide using any of the assessed thresholds resulted in superior profitability than non-treated NBT cotton. The preventive spray provided comparable profitability to the thresholds based on 20% oviposition, 6% injury to fruiting forms, or ≥ 3% large larvae but required insecticide application more frequently. This trend was also observed in a previous study when evaluating the efficacy of a threshold of 6% injury to fruiting forms relative to a preventive spray (Calvin et al. 2021). Although we did not detect an increase in profitability by using the 20% oviposition, 6% fruiting forms injury, or ≥ 3% large larvae threshold relative to the preventive spray, the adoption of either of these thresholds will play a significant role in reducing the resistance selection pressure of chlorantraniliprole on *H. zea*.

Thresholds based on oviposition, injury to fruiting forms, and larvae incidence applied to BG2 cotton tended to provide different levels of control, with the 20% oviposition threshold appearing to be the most effective and equivalent to the preventive spray. In addition to providing better *H. zea* control, the 20% oviposition threshold also received an equivalent number of insecticide applications as the 6% fruiting forms injury and ≥ 3% large larvae thresholds (Table 3-1). Although the thresholds provided varying levels of *H. zea* control, spraying BG2 cotton based on any threshold did not result in statistically greater yield or profitability relative to the non-treated BG2 cotton. It was unexpected to not detect a positive effect of insecticide applications on yield and profitability for the BG2 cotton in the current study because *H. zea*, being highly resistant to Cry1 and Cry2 *Bt* proteins, has the potential to cause substantial damage to and measurable yield loss in BG2 cotton (Gore et al. 2000, Reisig et al. 2018, Yang et al. 2018, Dorman et al. 2021, Santiago-Gonzalez et al. 2022, Yang et al. 2022). However, it is possible that infestations of and damage from *H. zea* in non-treated BG2 cotton in the current study were not severe enough to significantly affect yield. Additionally, because cotton has an indeterminate growing pattern, it can often compensate for the early season loss of fruiting forms caused by *H. zea* by reallocating assimilates to other retained fruiting sites (Kerby and Buxton 1981, Jones et al. 1996), especially if good growing conditions extend past summer months (Gore et al. 2000). Furthermore, results from a comprehensive study involving dose-response bioassays using multiple populations of *H. zea* collected across the southeastern United States showed that the vulnerability of crops expressing Cry *Bt* proteins could be variable (Reisig et al. 2023).

As observed in other studies (Francis 2021, Del Pozo-Valdivia et al. 2021), yields and profitability were not improved by controlling *H. zea* in BG3 cotton with any of the action thresholds used in our research. Because the Vip3Aa Bt protein is still highly effective against *H. zea* (Rabelo et al. 2020, Niu et al. 2021, Yang et al. 2022), this was not unexpected. Although BG3 cotton can occasionally incur unexpected injury by *H. zea* (Kerns et al. 2015, Brown et al. 2019) and there is widespread resistance to Cry *Bt* proteins (Yang et al. 2022), the benefit of spraying *Bt* cotton with insecticides targeting *H. zea* can be inconsistent. This is likely because the Vip3Aa *Bt* protein contained in triple-gene *Bt* cotton is still very effective in controlling *H. zea*, and the Cry2Ab *Bt* protein contained in the dual-gene cotton can still provide some measurable control of *H. zea* (Kerns et al. 2018). In a comprehensive study involving the pairing of field and laboratory data, Yang et al. (2022) found that reduced susceptibility of *H. zea* to the Vip3Aa protein in bioassays did not result in decreased efficacy of the protein in the field.

Considering the number of insecticide applications each threshold required to control *H. zea* for a particular *Bt* cotton technology across years and locations of the study, any of the thresholds (20% oviposition, 6% fruiting forms injury, and the ≥ 3% large larvae) resulted in a measurable reduction in insecticide applications relative to treating preventively. However, our data suggest that it was more efficient to manage *H. zea* in the NBT cotton based on 20% oviposition or a 6% fruiting forms injury threshold; in the dual-gene *Bt* cotton, the ≥ 3% large larvae, 20% oviposition, and 6% fruiting forms injury thresholds was equivalently effective; while it was more beneficial to manage *H. zea* in triple-gene *Bt* cotton using ≥ 3% large larvae or 6% fruiting forms injury threshold. Therefore, the 6% fruiting forms injury threshold is a suitable common threshold for control of *H. zea* across all currently grown cotton technologies. The 20% oviposition or ≥ 3% large larvae threshold may serve as alternative thresholds to manage *H. zea* in dual-gene cotton; however, only the ≥ 3% large larvae threshold should be used as an alternative threshold to manage *H. zea* in triple-gene *Bt* cottons. Use of the egg threshold is only advisable for NBT and dual-gene *Bt* cottons in regions where the *H. zea* pressure is elevated, the ratio of *H. zea* to *C. virescens* is high, and control failures in dual-gene *Bt* cottons are common (Reisig et al. 2019, Vyavhare and Kerns 2022). The use of an egg threshold could be premature under some circumstances because it does not provide time for natural mortality of *H. zea* to occur and for the *Bt* traits to provide some level of *H. zea* control (Reisig et al. 2019). It is reported that natural mortality in *H. zea* populations is high in cotton, with 71 to 95% natural mortality of *H. zea* eggs or first instar larvae (Sansone and Smith 2001a,b). However, the other thresholds are not exempt from disadvantages. The implementation of thresholds based on injury or larval density could result in surviving *H. zea* larvae hiding within the cotton canopy and/or fruiting structures and protected from insecticide applications (Sullivan et al. 1998, Reisig et al. 2019).

The results of this study suggest that the action thresholds of 20% oviposition, 6% fruiting forms injury, and the ≥ 3% large larvae thresholds are viable and can be implemented according to the *Bt* technology used or, in an alternative fashion, as conditions allow for *H. zea* management in cotton. The availability of several alternative action thresholds to manage *H. zea* in cotton provides producers and/or pest managers with flexibility in choice of sampling technique to determine need for insecticide application targeting the pest.

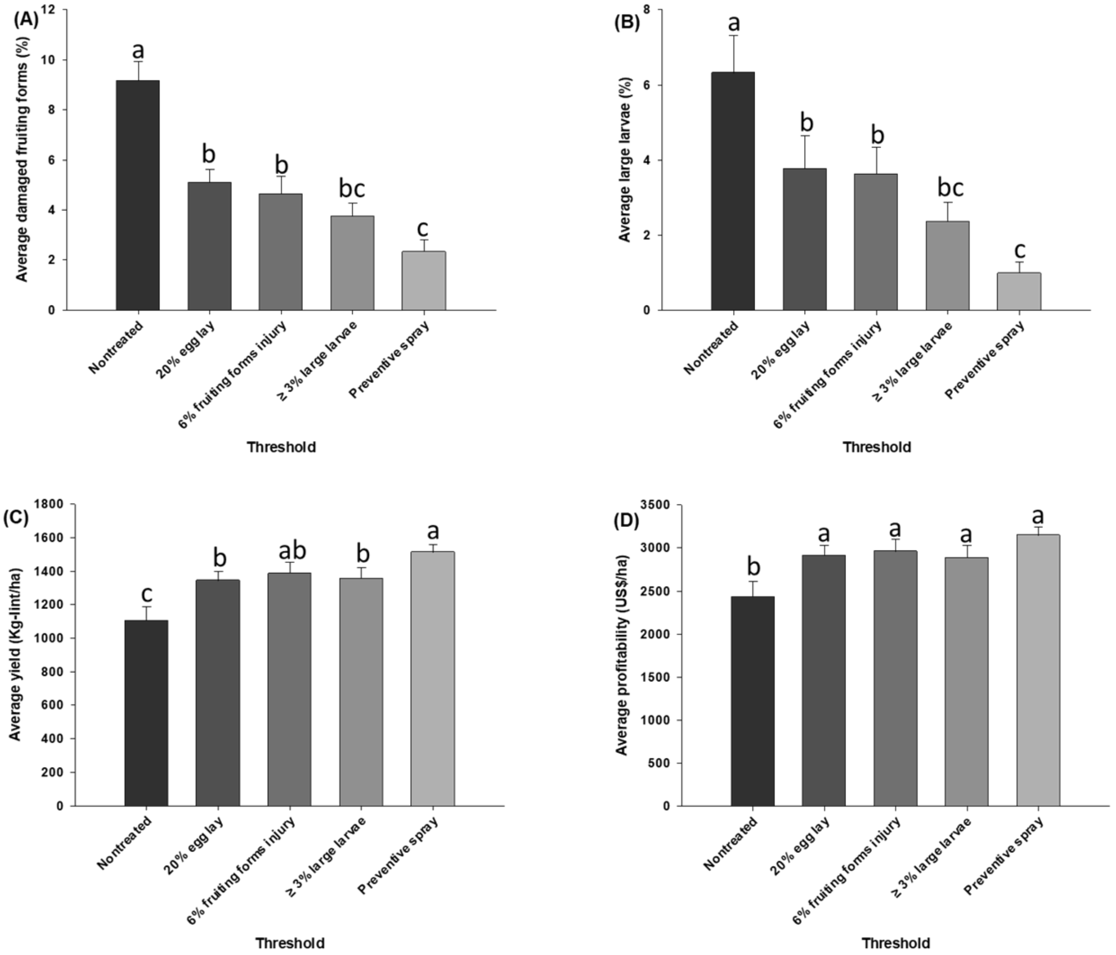

Figure 3.1 Mean percentage of damaged fruiting forms ± SE (A), percentage of large larvae (*Helicoverpa zea*) per 100 plants ± SE (B), yield (kg-lint/ha) ± SE (C), and profitability (US$/ha) ± SE among thresholds within NBT cotton, across two years (2020 and 2021) and three sites (TX, MS, and SC) as affected by the average number of chlorantraniliprole applications at a rate of 75.1 g-AI/ha. Bars with the same letter within a graph are not significantly different (*P* > 0.05).

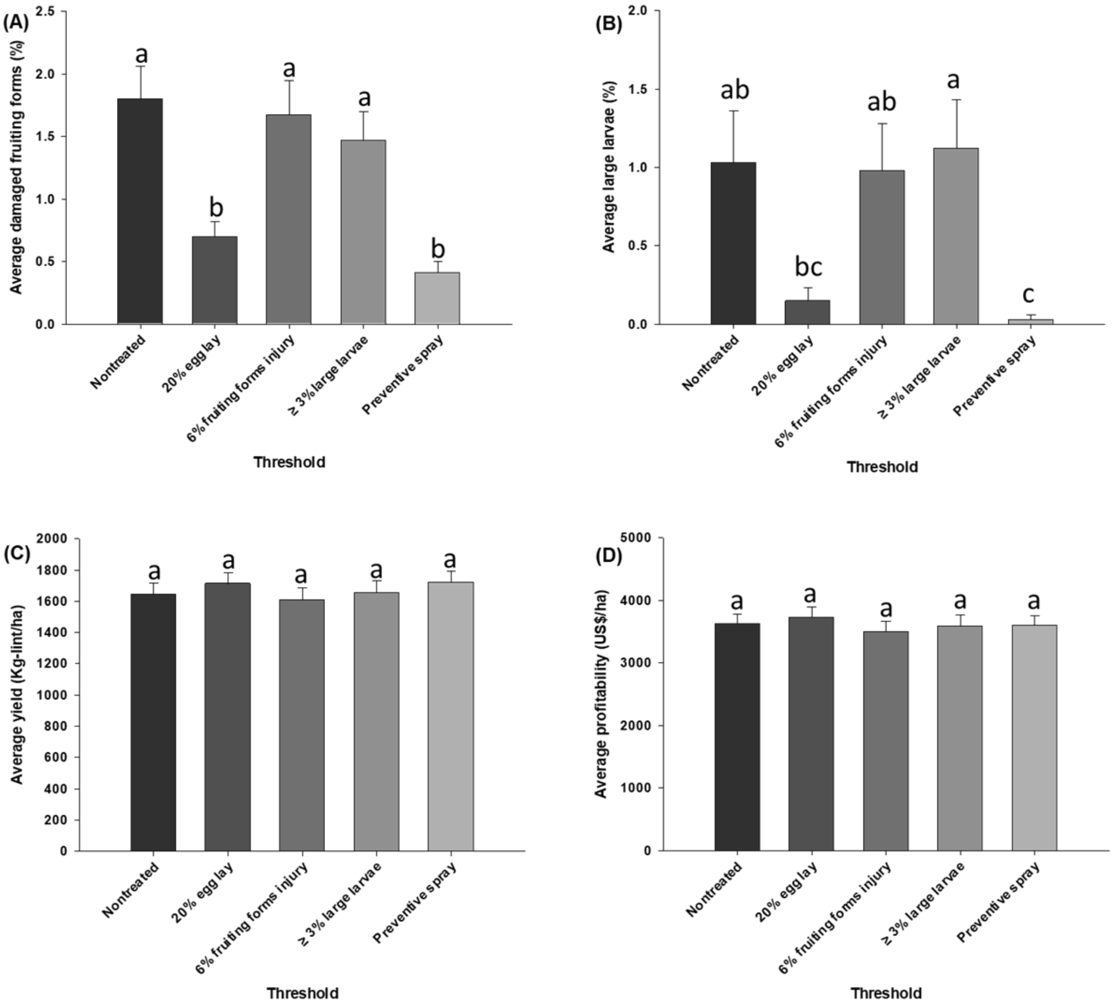

Figure 3.2 Mean percentage of damaged fruiting forms ± SE (A), percentage of large larvae (*Helicoverpa zea*) per 100 plants ± SE (B), yield (kg-lint/ha) ± SE (C), and profitability (US$/ha) ± SE among thresholds within BG2 cotton, across two years (2020 and 2021) and three sites (TX, MS, and SC) as affected by the average number of chlorantraniliprole applications at a rate of 75.1 g-AI/ha. Bars with the same letter within a graph are not significantly different (*P* > 0.05).

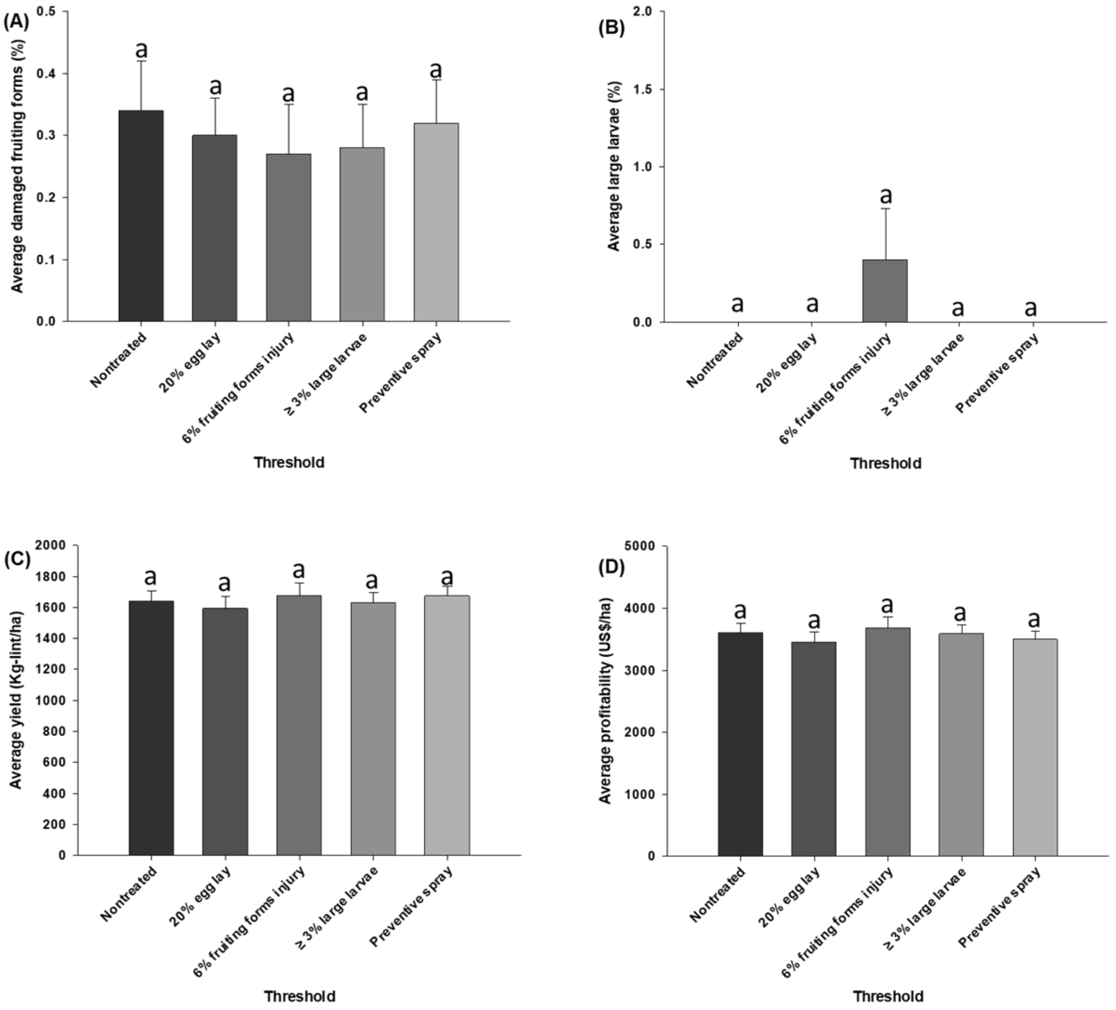

Figure 3.3 Mean percentage of damaged fruiting forms ± SE (A), percentage of large larvae (*Helicoverpa zea*) per 100 plants ± SE (B), yield (kg-lint/ha) ± SE (C), and profitability (US$/ha) ± SE among thresholds within BG3 cotton, across two years (2020 and 2021) and three sites (TX, MS, and SC) as affected by the average number of chlorantraniliprole applications at a rate of 75.1 g-AI/ha. Bars with the same letter within a graph are not significantly different (*P* > 0.05).

Table 3.1 Number of insecticide applications (app.) triggered in non-Bt (NBT), dual-gene Bt (BG2), and triple-gene Bt cotton (BG3) and percentage reduction in insecticide applications (reduction in app. [%]) relative to the preventive spray for *Helicoverpa zea* for each year, location, and threshold.

Bt technology	Threshold	College Station, TX				Stoneville, MS				Blackville, SC				Average across years and locations	
		2020		2021		2020		2021		2020		2021			
		App.	Reduction in app. [%]	App.	Reduction in app. [%]	App.	Reduction in app. [%]	App.	Reduction in app. [%]	App.	Reduction in app. [%]	App.	Reduction in app. [%]	App.	Reduction in app. [%][SE]
NBT	Non-treated	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0.0	100.0 ± 0.0 a
	20% oviposition	1	50.0	0	100.0	1	75.0	1	66.7	1	66.7	1	66.7	0.8	70.9 ± 6.7 b
	6% fruiting forms injury	2	0.0	1	66.7	2	50.0	1	66.7	2	33.3	1	66.7	1.5	47.2 ± 10.9 bc
	≥3% large larvae	2	0.0	2	33.3	2	50.0	1	66.7	2	33.3	1	66.7	1.7	41.7 ± 10.3 c
	Preventive spray	2	0.0	3	0.0	4	0.0	3	0.0	3	0.0	3	0.0	3.0	0.0 ± 0.0 d
BG2	Non-treated	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0.0	100.0 ± 0.0 a
	20% oviposition	1	50.0	0	100.0	1	75.0	1	66.7	1	66.7	0	100.0	0.7	76.4 ± 8.2 a
	6% fruiting forms injury	0	100.0	0	100.0	1	75.0	1	66.7	1	66.7	1	66.7	0.7	79.2 ± 6.7 a
	≥3% large larvae	1	50.0	0	100.0	3	25.0	0	100.0	0	100.0	1	66.7	0.8	73.6 ± 13.0 a
	Preventive spray	2	0.0	3	0.0	4	0.0	3	0.0	3	0.0	3	0.0	3.0	0.0 ± 0.0 b
BG3	Non-treated	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0.0	100.0 ± 0.0 a
	20% oviposition	1	50.0	1	66.7	1	75.0	1	66.7	1	66.7	0	100.0	0.8	70.9 ± 6.7 b
	6% fruiting forms injury	0	100.0	0	100.0	0	100.0	0	100.0	1	66.7	0	100.0	0.2	94.5 ± 5.5 a
	≥3% large larvae	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0.0	100.0 ± 0.0 a
	Preventive spray	2	0.0	3	0.0	4	0.0	3	0.0	3	0.0	3	0.0	3.0	0.0 ± 0.0 c

Means with the same letter within a *Bt* technology are not significantly different ($P > 0.05$).

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4. THE POTENTIAL FOR UTILIZING GRAIN SORGHUM AS A *HELICOVERPA ZEA* TRAP CROP AND NURSERY CROP FOR *H. ZEA* NATURAL ENEMIES AND *HEAR*NPV DISSEMINATION INTO COTTON

4.1. Introduction

The introduction and widespread adoption of genetically modified corn, *Zea maize* L. (Poales: Poaceae) and cotton, *Gossypium hirsutum* L. (Malvales: Malvaceae), producing *Bacillus thuriengiensis* (*Bt*) (Bacillales: Bacillaceae) proteins has resulted in effective *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) control while causing marginal to no harm to non-target organisms (Carpenter 2010, Yu et al. 2011). However, with the occurrence of resistance in *H. zea* to one or more *Bt* proteins, remedial insecticide sprays are often required to prevent unacceptable injury in *Bt* cotton (Tabashnik and Carriere 2015, Dively et al. 2016, Kerns et al. 2018, Reisig et al. 2018, Reisig et al. 2019, Little et al. 2019, Yang et al. 2019, Kaur et al. 2019). In the U.S., because of widespread issues with pyrethroid resistance, insecticides containing chlorantraniliprole are the primary means for managing *H. zea* in cotton (Musser et al. 2017, Reisig et al. 2019, Vyavhare and Kerns 2022). Currently, there are numerous reports of field-evolved resistance of lepidopteran pests to chlorantraniliprole (Roditakis et al. 2015). However, to date, no chlorantraniliprole resistance has been reported for *H. zea*, but because of the heavy reliance on this insecticide for *H. zea* management in cotton, grain sorghum, soybean, and other crops, there is concern that resistance may develop (Calvin et al. 2021, Musser et al. 2022, Allen et al. 2023). Thus, it is beneficial to develop additional management tactics targeting *H. zea* in cotton.

Implementation of intercropping (also known as polyculture) systems have demonstrated utility for insect pest management. Intercropping involves the simultaneous cultivation of two or more companion crop species in one field (Stomph et al. 2020). The companion crops may serve as a repellent, a trap crop, and/or natural enemy recruiter (Smith and McSorley 2000, VanTine and Verlinden 2003, Jones and Gillett 2005, Rodriguez-Saona 2012). This ecosystem service provided by the intercropping system may promote insect pest suppression in the main crop, thus reducing/delaying the need for insecticide applications (King and Coleman 1989, Tillman and Mullinix 2004, Knutson and Ruberson 2005, Safarzoda et al. 2014).

An intercropping system aimed at trap cropping involves cultivating a crop of interest simultaneously with another crop that is more preferred by the pests of concern; this favors the diversion of the pest from the main crop. The adoption of this system has resulted in the successful management of multiple key pests in several economic crops including *H. zea* in cotton (Tillman and Mullinix 2004, Ratnadass et al. 2009, Pinero and Manandhar 2015, Sarkar et al. 2018).

Reports from several studies conducted in various regions in the world showed that grain sorghum, *Sorghum bicolor* L. Moench (Poales: Poaceae), may serve as an effective diversionary trap crop for *H. zea* and *Helicoverpa armigera* (*Hübner*) (Lepidoptera: Noctuidae) from cotton, and as a source of *H. zea* natural enemies (Tillman and Mullinix 2004, Duraimurugan and Repgupathy 2005). Thus, the implementation of an intercropping system of cotton with grain sorghum may divert *H. zea* from cotton to the grain sorghum, while providing a valuable source of beneficial arthropods that may disperse from the grain sorghum into the cotton (Jones and Gillett 2005).

Grain sorghum may also serve as an effective source for *Hear*NPV dissemination to cotton (Roome 1975, Roome and Daoust 1975, Tillman and Mullinix 2004, Duraimurugan and Repgupathy 2005). *Helicoverpa armigera* nucleopolyhedrovirus is a viral pesticide that is specific to Heliothines, including *H. zea* (Gettig and McCarthy 1982). In the U.S., *Hear*NPV has demonstrated high efficacy for *H. zea* management in soybean, *Glycine max* (L.) Merr. (Black et al. 2022). In soybean, *Hear*NPV has been found to be very persistent in the canopy (Black et al. 2019), but in cotton, *Hear*NPV persistence has not been sustained. This lack of persistence is thought to be primarily due to the pH of dew on cotton leaves resulting in virus deactivation as the dew dries (Yearian and Young 1974, Young et al. 1977, McLeod et al. 1997). Although initial *Hear*NPV infection of *H. zea* larvae in cotton is possible, it is unlikely an epizootic event will persist. Thus, the challenge of effectively integrating *Hear*NPV into cotton IPM is to devise a system where an epizootic nursery source of *Hear*NPV can be initiated for persistent horizontal biotic and/or abiotic transmission into cotton.

This current study has two objectives. The first objective is to investigate the potential for utilizing grain sorghum as a trap crop for *H. zea* and a nursery crop for *H. zea* natural enemies. The second objective is to investigate the potential for utilizing grain sorghum as a nursery crop for *Hear*NPV dissemination into the cotton canopy to manage *H. zea*.

4.2. Materials and Methods

4.2.1. Locations, Experimental Design, and Treatments.

These experiments were conducted at three distinct geographical and environmental locations that are representative of the southern U.S. Cotton Belt. The sites include College Station, TX; Stoneville, MS; and Blackville, SC. Experiments were conducted over two years with the first year serving as a proof-of-concept experiment and the second year serving as a validation experiment. The cotton used in these experiments was a non-Bt variety, DP 1822 XF (Bayer CropScience LP, St. Louis, MO). The grain sorghum used consisted of equal blends of seed from six varied maturing hybrids (Table 1). The seed was blended to extend the bloom period to approximately 21 days to promote extended *H. zea* ovipositing attractiveness of the grain sorghum. All the grain sorghum hybrids utilized were supplied by S&W Seed Company, Longmont, CO.

4.2.2. Proof-of-Concept Experiment

This experiment was conducted in 2020 and consisted of three treatments at each location. Each field was separated from one another by at least 0.5 kilometers. Two fields were interplanted with grain sorghum and cotton in replicated strips of 8 rows wide (with a row spacing of 0.97-1.02 m) and 60.96 m long. A third field consisted of a solid cotton block of 64 rows wide (with a row spacing of 0.97-1.02 m) and 61 m long. Each interplanted field had four replicated strips of cotton and sorghum planted following an alternate pattern. Each test location served as a field replicate. Grain sorghum was planted 7-10 days after planting cotton to closely time the expected period of bloom of the earliest maturing grain sorghum with the expected initial week of bloom of the cotton.

All three fields and crops were grown using standard production practices but were not treated with insecticides that may eliminate *H. zea*. In one of the interplanted fields, the blooming grain sorghum was treated with *Hear*NPV (Heligen®, AgBiTech, Fort Worth, TX) at 0.1 l/ha targeting 1st and 2nd instar *H. zea* larvae. The treatment was applied by ground using a high-clearance sprayer calibrated to deliver a spray volume of 93.54 l/ha. The interplanted nontreated field served as a non-*Hear*NPV comparison. The cotton-only field served as a non-sorghum comparative treatment allowing evaluation of the effectiveness of grain sorghum as a

H. zea trap crop and natural enemy nursery. Pre-treatment data and samples were collected from all fields before the *Hear*NPV application and at 7, 14, and 21 days post-application.

Beneficial arthropods and *H. zea* larvae were sampled from grain sorghum using the beat-bucket method (Merchant and Teetes 1992). Four locations within each replicate were sampled. At each location, 25 heads were sampled (100 heads total per replicate) by bending the sorghum panicle into a 2.5-gallon bucket and vigorously shaking it against the bucket walls to dislodge *H. zea* larvae and beneficial arthropods. Samples were collected into 1-gallon Zip-Loc bags and returned to the laboratory for counting. The number of *H. zea* larvae were recorded and sized as small (1st and 2nd instar) or large (3rd, 4th, and 5th instar). The beneficial arthropods were identified into families and counted. The *H. zea* samples and the beneficial arthropods samples (pooled by family) were stored at -80 ^{o}C until they were tested for *Hear*NPV infection utilizing polymerase chain reaction (PCR). An additional beat bucket sample consisting of 100 sorghum heads was collected from each sorghum replicate. When available, ≥3rd instar *H. zea* larvae from this sample were collected into 29 mL Solo condiment cups containing laboratory-based meridic diet (WARD'S Stonefly Heliothis diet, Rochester, NY). Collected larvae were brought to the laboratory and held for parasitoid emergence and identification.

Cotton within the cotton-sorghum interplanting was sampled using three methods: visual sampling, beat-bucket sampling, and drop-cloth sampling. The visual sampling method is primarily aimed at detecting incidences of fruiting forms injury and eggs, and the drop cloth method was used to aid in collecting the *H. zea* larvae used to determine *Hear*NPV infection as well as determining *H. zea* parasitism. For the visual sampling method, each replicated strip was sampled by inspecting 25 individual plants using the method described by Calvin et al. (2021). For each plant, the terminal was inspected for evidence of *H. zea* feeding and the presence of *H.*

zea larvae. Four squares were sampled from each plant, 2 small upper canopy (first 5 nodes), and 2 lower canopy squares for evidence of injury and the presence of *H. zea* larvae. Four bolls were sampled on each plant, 2 small bolls (approximately 1-cm in diameter) with bloom tags (dried-attached blossoms) and 2 larger bolls (approximately 2-2.5 cm in diameter) with no bloom tags. Injury to squares and bolls was only recorded as positive when the budding tissue was penetrated, when the fruit-feeding injury would result in square abortion, or when the carpel wall of the boll was penetrated. The size of each *H. zea* larvae for all sampling was recorded as small (1st and 2nd instar) or large (3rd, 4th, and 5th instar). Additionally, when inspecting the various plant structures, the presence or absence and the number of Heliothine eggs were recorded for each plant.

Predators within the cotton plots were sampled using methods described by Knutson et al. (2008). A 5-gallon bucket was held at a 45° angle to the ground and the sample plants were grasped near the base and quickly bent into the bucket. Ten beat-bucket samples per replicated strip of cotton were taken, with 3 plants sampled per beat bucket. The plants were rapidly beaten against the side of the bucket 12-16 times for 3-4 seconds then were removed from the bucket. The leaves and fruiting forms that remained in the bucket and the dislodged predators were collected in 1-gallon Zip-Loc bags and brought to the laboratory for identification and counting. Leaves and fruiting forms dislodged were examined for predators. Additionally, four drop-cloth samples were collected per replicated strip of cotton. Black drop-clothes of 0.97 m long by 0.76 m wide were utilized. Approximately 1.5 m of cotton was vigorously shaken causing *H. zea* to dislodge and drop on the drop-cloth. Dislodged fruits and leaves were examined for the presence of *H. zea* larvae. The ≥3rd instar *H. zea* larvae from one-half of the larvae collected from each replicated strip were collected into 29 mL Solo condiment cups (WARD'S Stonefly Heliothis diet, Rochester, NY) containing laboratory-based meridic diet; these larvae were brought to the laboratory and allowed to develop to estimate parasitism. The other half of each sample and the collected predators were pooled and stored at -80 ^{o}C. These samples were then analyzed to estimate *Hear*NPV infection using polymerase chain reaction (PCR). When the number of *H. zea* larvae collected in a sample was low, all larvae collected were considered for PCR analysis. Throughout the sampling period, precautions were taken to minimize anthropogenic dispersal of *Hear*NPV.

4.2.3. Validation Experiment

The validation experiment was conducted similarly to the proof-of-concept experiment but instead of the grain sorghum being interplanted with cotton, it was planted on the edge of the field to simulate a practical means of implementation for growers. At each location, three approximately 2.0 ha blocks of cotton were utilized, with each block being separated from one another by at least 0.5 Km. Two of the fields were bordered on the predominantly upwind side with 8-12 rows of blended grain sorghum. Sorghum was planted 7-10 days after planting cotton to time the bloom on the earliest maturing sorghum with the first week of bloom on the cotton. Planting the sorghum upwind from the cotton minimized the potential for herbicide drift from the cotton into the sorghum and maximized the potential for arthropods and *Hear*NPV dispersal from the sorghum into the cotton. Each geographic location served as a field replicate. Both crops were grown using standard production practices but were not treated with insecticides that may eliminate *H. zea*. The blooming sorghum in one of the cotton-sorghum fields was treated with *Hear*NPV (Heligen®, AgBiTech, Fort Worth, TX) at a rate of 0.1 l/ha targeting 1st and 2nd instar larvae. The treatment was applied using a high-clearance sprayer calibrated to deliver a spray volume of 93.54 l/ha. The untreated field bordered with sorghum served as a non-

*Hear*NPV comparison. The cotton-only field served as a non-sorghum treatment to evaluate the effectiveness of grain sorghum as a *H. zea* trap crop and natural enemy nursery. Pre-treatment data and samples were collected from all fields before the *Hear*NPV application and at 7, 14, and 21 days post-application.

Sorghum was sampled as described in the proof-of-concept experiment. Four locations, with 25 sorghum heads per location, were sampled within the strip of sorghum. As previously described, *H. zea* larvae and beneficial arthropod density were determined for each sample date. In both the cotton-only and cotton bordered by sorghum fields, the cotton was sampled based on replicated transects originating from the sorghum planting or the edge of the predominant upwind edge for the cotton-only planting. Each field was divided into equally spaced grids and the transects were divided into 4 equally spaced transects along those grids (Fig. 1). Data were collected along each transect at 7.6 m, 15.2 m, 30.5 m, 61.0 m, and 91.4 m. At each transect location, 10 plants were visually sampled, and 5 beat-bucket and 2 drop-cloth samples were taken as previously described. As in the proof-of-concept experiment, fruiting forms injury numbers, eggs, *H. zea* larvae, predators, parasitized larvae density, and *Hear*NPV infection were determined for each sample transect distance by replicate by sample date. The data were collected, and the samples were processed as previously described in the proof-of-concept experiment. Precautions were taken to minimize anthropogenic dispersal of *Hear*NPV. Samples were taken in the untreated field first then in the *Hear*NPV treated field starting from the furthest to the closest transect to the sorghum block at each date.

4.2.4. *Hear*NPV Infection Analysis

*Hear*NPV infection of *H. zea* larvae was determined using methods described by Black et al. (2019). For each sample, *Hear*NPV occlusion bodies were purified and extracted, and the

DNA was subsequently separated and extracted utilizing a DNA extraction kit (DNeasy Blood and Tissue Kit: Qiagen, Germantown, MD). Extracted DNA was amplified with *Hear*NPV polyhedrin-specific primers HzSpolh-2F (5′-CCCTACTTTGGGCAAAACC-3′) and HzSpolh-2R (5′-TCGGTTTGGTTGGTCGCATA-3′) (IDT, Coralville, IA) utilizing a Veriti™ 96-Well Thermal Cycler (Applied Biosystem, Foster City, CA). A volume of 50 µl of PCR mixture was used and consisted of 1 µl extracted DNA sample, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM each primer, 1× GoTaq Flexi Buffer, and 1.25 U of GoTaq DNA polymerase (Promega, Madison, WI). To confirm the effective amplification of the target gene, a positive control and a negative control consisting of *Hear*NPV and deionized water, respectively, were included in each individual thermocycler run. Once amplified, samples were visualized using a 4200 TapeStation with D1000 ScreenTape Assay (Agilent Technologies, Inc, Waldbronn, Germany) for *Hear*NPV confirmation. *Hear*NPV presence was confirmed when a band was present at 400 base pairs (bp). For the *HearNPV*-positive samples, PCR products were sequenced (Eurofins, Louisville, KY) to confirm the *Hear*NPV polyhedron sequence.

4.2.5. Statistical Analyses

For the proof-of-concept experiment, the percentage of fruiting forms injury, beneficial arthropods, parasitized larvae, eggs, and *H. zea* larvae were compared between treatments using a multiple Student’s t-test (PROC TTEST. Version 13.1, SAS Institute Inc., Cary, NC). For the validation experiment, the percentage of fruiting forms injury, beneficial arthropods, and *H. zea* larvae were compared between treatments and between distances within treatment using a multiple Student’s t-test (PROC TTEST. Version 13.1, SAS Institute Inc., Cary, NC). To compare the virus detection frequency between treatments, the Kruskal–Wallis test (Version 13.1, SAS Institute Inc., Cary, NC) was performed.

4.3. Results

4.3.1. Proof-of-Concept Experiment

When the cotton-only was compared with the non-treated cotton-sorghum, no significant differences were detected for the percentage of damaged fruiting forms (*t* = 1.42, df = 76.806, *P* = 0.1591), percentage of eggs (*t* = 1.48, df = 64.723, *P* = 0.1435), percentage of small larvae (*t* = 0.89, df = 86, *P* = 0.3781), percentage of large larvae (*t* = 0.86, df = 75.8, *P* = 0.3942), and the number of beneficial arthropods (*t* = -1.07, df = 86, *P* = 0.288). However, significant differences were detected in the percentage of parasitized larvae with cotton-only exhibiting a greater incidence of parasitized larvae (Fig 4.2f; *t* = 2.03, df = 43, *P* = 0.0484).

When the cotton-only was compared with the *Hear*NPV-treated cotton-sorghum, no significant differences were detected in the percentage of damaged fruiting forms (*t* = 1.88, df = 69.031, *P* = 0.0642), percentage of eggs (*t* = 1.45, df = 78.588, *P* = 0.1521), percentage of large larvae (*t* = 1.39, df = 73.512, *P* = 0.1676), number of beneficial arthropods (*t* = 0.23, df = 86, *P* = 0.8165), or percentage of parasitized larvae (*t* = 0.82, df = 37, *P* = 0.4179). Significant differences were detected for the percentage of small larvae (*t* = 2.18, df = 63.927, *P* = 0.0328) with cotton-only exhibiting greater incidence of small larvae (Fig 4.2c).

The non-treated cotton-sorghum did not differ from *Hear*NPV-treated cotton-sorghum in either the percentage of damaged fruiting forms (*t* = 0.49, df = 86, *P* = 0.6278), percentage of eggs (*t* = 0.13 , df = 77.868, *P* = 0.8944), percentage of small larvae (*t* = 1.27, df = 69.662, *P* = 0.2072), percentage of large larvae (*t* = 0.66, df = 86, *P* = 0.509), number of beneficial arthropods (*t* = 1.28, df = 78.158, *P* = 0.2057), or percentage of parasitized larvae (*t* = -0.77, df = 40, *P* = 0.4449).

4.3.2. Validation Experiment: Comparison of Treatment

For this experiment, the percentage of eggs and parasitized larvae were not evaluated due to the incompleteness of the data for these variables. Significant differences between cotton-only and non-treated cotton-sorghum were not observed for either the percentage of damaged fruiting forms (*t* = -0.56, df = 390.42, *P* = 0.5792), percentage of small larvae (*t* = -0.92, df =398, *P* =0.3585), percentage of large larvae (*t* = 1.53, df = 398, *P* = 0.1261), or the number of beneficial arthropods (*t* = 1.08, df = 332.67, *P* = 0.2826).

Surprisingly, the cotton-only plots had significantly fewer damaged fruiting forms (Fig.3a; *t* = -2.76, df = 398, *P* = 0.006) and small larvae (Fig 4.3b: *t* = -3.01, df = 361.55, *P* = 0.0028) than cotton from the *Hear*NPV-treated cotton-sorghum plots. However, *Hear*NPV-treated cotton-sorghum plots resulted in a greater number of beneficial arthropods in the cotton (Fig 4.3d; *t* = -2.04, df = 396.6, *P* = 0.0416). There was no significant difference between the two treatments for the percentage of large larvae (*t* = -0.25, df = 398, *P* = 0.8024).

*Hear*NPV-treated cotton-sorghum resulted in a significantly greater number of fruiting forms injury (Fig 4.3a; *t* = -2.39, df = 398, *P* = 0.0174), small larvae (Fig 4.3b; *t* = -2.23, df = 365.84, *P* = 0.0265), as well as a greater number of beneficial arthropods (Fig 4.3d; *t* = -3.27, df = 357.57, *P* = 0.0012) than the non-treated cotton-sorghum, but the two treatments did not differ in large larvae incidence (Fig 4.3c; *t* = -1.78, df = 398, *P* = 0.0766).

4.3.3. Validation Experiment: Comparison of Distance

Within the cotton-only field, there was no difference between any of the distances for damaged fruiting forms, small larvae, or large larvae (*P* > 0.05; Fig 4.3a, b, c). However, beneficial arthropod incidence was statistically greater at 7.6 m distance than at 15.2 m and 30.5

m distances and significantly greater at 91.4 m distance than at 15.2 m distance (*P* < 0.05; Fig 4.3d).

Within the non-treated cotton-sorghum, the 7.6 m and 15.2 m distances had a lower incidence of fruiting forms injury than the 30.5 m distance; the 7.6 m and 15.2 m distances exhibited fewer incidences of fruiting forms injury than the 61.0 m distance (*P* < 0.05; Fig 4.3a). The 61.0 m distance had fewer small larvae than the 15.2 m and 30.5 m distances and the 30.5 m distance exhibited a greater incidence of small larvae (*P* < 0.05; Fig 4.3b). The 7.6 m distance had fewer large larvae than the 30.5 m and 61.0 m distances and the 15.2 m distance exhibited large larvae incidence significantly fewer than the 30.5 m, 61.0 m, and 91.4 m distances (*P* < 0.05; Fig 4.3c). The 30.5 m distance had a significantly greater number of beneficial arthropods than the 61.0 m distance (*P* < 0.05; fig 4.3d).

Within the *Hear*NPV-treated cotton-sorghum, none of the distances differed in the number of damaged fruiting forms, large larvae, or beneficial arthropods (*P* > 0.05; Fig 4.3a, b, c). However, for small larvae, the 15.2 m distance had significantly fewer small larvae than at 91.m distance (*P* < 0.05; Fig 4.3b).

4.3.4. Beneficial Arthropods Observed

A variety of predators and parasitoids of *H. zea* were observed in cotton in both years of the study (Table 4.2). Minute pirate bugs (Hemiptera: Anthocoridae), fire ants (Hymenoptera: Formicidae), lady beetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae, Hemerobiidae), cotton fleahopper (Hemiptera: Miridae), big-eyed bug (Hemiptera: Geocoridae), and spiders (Araneae: Thomisidae, Salticidae, Araneidae, and Oxyopidae) were the most occurring predators. Tachinid flies (Diptera: Tachinidae) and braconid wasps (Hymenoptera: Braconidae) were the most occurring parasitoids.

4.3.5. PCR Analysis

4.3.5.1. *Helicoverpa zea* Samples

In 2020, *Hear*NPV was not detected in *H. zea* samples collected from pre-treated cotton of any treatment. However, the virus was detected in *H. zea* samples collected throughout the subsequent sampling dates for all treatments (Fig 4.4a). Based on the Kruskal-Wallis test results, there was a difference in the *Hear*NPV prevalence between the cotton-only and non-treated cotton-sorghum (χ^2 = 3.8571, df = 1, *P* = 0.0495) with cotton-only having greater prevalence of *Hear*NPV (Fig 4.4b). Additionally, there was a significant difference between non-treated cotton-sorghum and *Hear*NPV-treated cotton-sorghum (χ^2 = 3.8571, df = 1, *P* = 0.0495) with *Hear*NPV-treated cotton-sorghum exhibiting greater incidence of *Hear*NPV (Fig 4.4b). There was no statistical difference between the cotton-only and *Hear*NPV-treated cotton-sorghum (χ^2 = 0, df = 1, *P* = 1).

In 2021, *Hear*NPV was detected in *H. zea* samples collected from cotton at all sampling dates for both treated cotton-sorghum and non-treated cotton-sorghum. Additionally, throughout the subsequent sampling dates, the virus was detected in *H. zea* samples collected from both fields and across most distance locations except at 91.4 m distance in the *HearNPV*-treated field. However, *Hear*NPV was not detected in any *H. zea* samples collected from the cotton-only field (Fig 4.5a). We observed a statistical difference in *Hear*NPV frequency between the cotton-only and non-treated cotton-sorghum (χ^2 = 7.8125, df = 1, *P* = 0.0052) with non-treated cotton-sorghum exhibiting greater *Hear*NPV incidence (Fig 4.5b). Additionally, there was a significant difference between non-treated cotton-sorghum and *Hear*NPV-treated cotton-sorghum (χ^2 = 6.9018, df = 1, *P* = 0.0086) with non-treated cotton-sorghum exhibiting greater *Hear*NPV incidence (Fig 4.5b). No statistical difference between the cotton-only and *Hear*NPV-treated cotton-sorghum were observed (χ^2 = 3.7156, df = 1, *P* = 0.0539).

4.3.5.2. Beneficial Arthropod Samples

In 2020, none of the beneficial arthropod samples collected from the cotton-only and non-treated cotton-sorghum fields were positive for *Hear*NPV while the virus was detected in 7 samples collected from the treated cotton-sorghum field. Arthropods in the family Chrysopidae Coccinellidae, Pentatomidae, and Reduviidae were the only arthropod groups that appeared to be carriers for *Hear*NPV (Table 4.3). In 2021, the virus was detected in beneficial arthropod samples collected from both treated and non-treated cotton-sorghum fields. The arthropod groups that carried the virus were spiders (Thomisidae, Salticidae, Araneidae, and Oxyopidae), Formicidae, Anthocoridae, Reduviidae, Coccinellidae, and Pentatomidae. Coccinellids, pentatomids, and reduviids were the only arthropod groups in which the virus was detected consistently in both years of the study (Table 4.3).

4.4. Discussions

Several studies have reported the utility of intercropping for insect pest management. Growing crops in an intercropping setting may favor pest diversion and increase natural enemy populations (Roome 1975, Roome and Daoust 1975, Tillman and Mullinix 2004, Duraimurugan and Repgupathy 2005, Jones and Gillett 2005). Based on the results of this current study, growing cotton in an intercropping system did not result in consistent increase in *H. zea* control and beneficial arthropods relative to the cotton-only treatment. Surprisingly, the cotton-sorghum treatment exhibited a significantly lower percentage of parasitized larvae relative to the cotton-only. It appears that the sorghum not only trapped some *H. zea* but also its parasitoids. Hence, the results of this study did not show evidence that sorghum could serve as a *H. zea* trap crop and a source of *H. zea* natural enemies. However, a previous study has found sorghum to be a desirable diversionary *H. zea* trap crop and favored measurable *H. zea* control, but, similarly to our study, sorghum did not serve as a source for *H. zea* natural enemies (Tillman and Mullinix 2004).

Additionally, the results of our current study did not provide sufficient evidence to support our hypothesis that grain sorghum interplanted with cotton will serve as a source of *Hear*NPV and will favor persistent dissemination of the virus into the cotton canopy. Surprisingly, *Hear*NPV was detected in samples collected from all treatments indicating that the virus is naturally occurring in the locations where this current study was conducted. In the first year of the study, *Hear*NPV was more prevalent in the treated cotton-sorghum field compared with the non-treated cotton-sorghum field, but the virus became more prevalent in the non-treated cotton-sorghum field in the second year of the study. However, we observed an interesting pattern. When *Hear*NPV was more prevalent in the treated field, it exhibited fewer incidences of fruiting forms injury and larvae and when *Hear*NPV was more prevalent in the non-treated field, there was a reduction in fruiting forms injury and larvae. This indicates that the presence of *Hear*NPV that comes from either natural sources or nearby *Hear*NPV-treated grain sorghum may favor some level of *H. zea* suppression in cotton. However, previous studies have demonstrated that *Hear*NPV applied to nearby grain sorghum favored a greater level of *H. armigera* control in cotton comparing to when applied directly to cotton and facilitated the persistence of the virus in cotton canopy (Roome 1975, Roome and Daoust 1975, Duraimurugan and Repgupathy 2005).

Several factors could have impacted the results of this study. For instance, to maintain isolation, the fields (treatments) were planted distantly from each other. Thus, the field for each individual treatment could have been exposed to significantly different levels of *H. zea* pressure and had considerably varied densities of beneficial arthropods. The natural occurrence of the virus could have also been inherently varied among field locations. In College Station, we observed a higher *H. zea* pressure in the *Hear*NPV-treated cotton-sorghum field than the non-treated cotton-sorghum and the cotton-only fields in 2021. This condition might have caused the data to be biased. Additionally, *H. zea* population in these locations could have had varied levels of susceptibility to *Hear*NPV. Resistance to Cry Bt proteins in *H. zea* is widespread (Tabashnik and Carriere 2015, Dively et al. 2016), and laboratory bioassays showed that *H. zea* strains resistant to cry *Bt* proteins are significantly less susceptible to the *Hear*NPV relative to the *Bt* susceptible strain (Calvin et al. 2023). This situation has caused this study to be extremely challenging.

Our data suggests that the effectiveness of using sorghum as a trap crop, and natural enemy and *Hear*NPV nursery will not consistently result in beneficial outcomes.

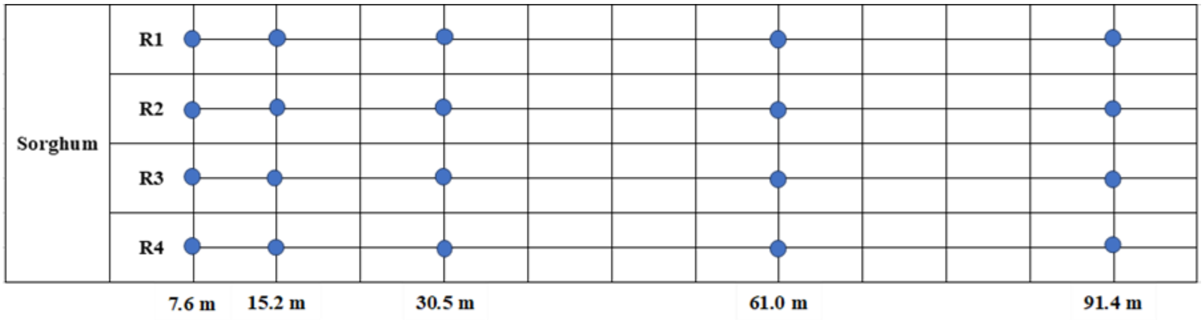

Figure 4.1 Distribution of transect locations within the cotton fields. R1 = transect replicate 1, R2 = transect replicate 2, R3 = transect replicate 3, and R4 = transect replicate 4.

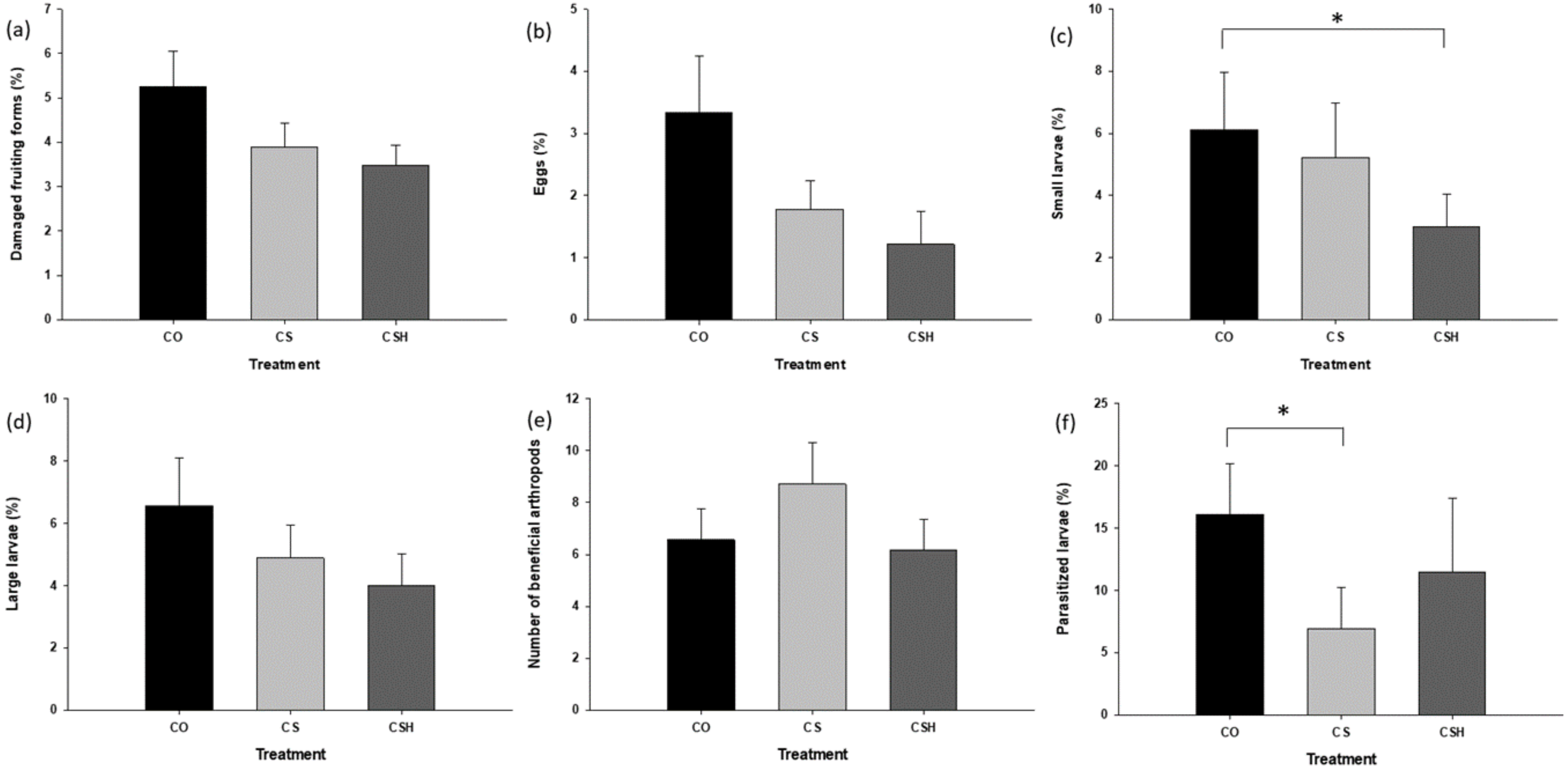

Figure 4.2 Means (±SE) percentage damaged fruiting forms per 100 fruits (a), means (±SE) percentage eggs per 100 plants (b), means (±SE) percentage of small larvae per 100 plants (c), means (±SE) percentage of large larvae per 100 plants (d), means (±SE) number of beneficial arthropods (e), and means (±SE) percentage parasitized larvae (f) as affected by grain sorghum and *Hear*NPV in 2020. CO = cotton only, CS = cotton intercropped with grain sorghum, and CSH = cotton intercropped with grain sorghum treated with *Hear*NPV. The asterisks indicate the comparisons were significantly different ($P \leq 0.05$).

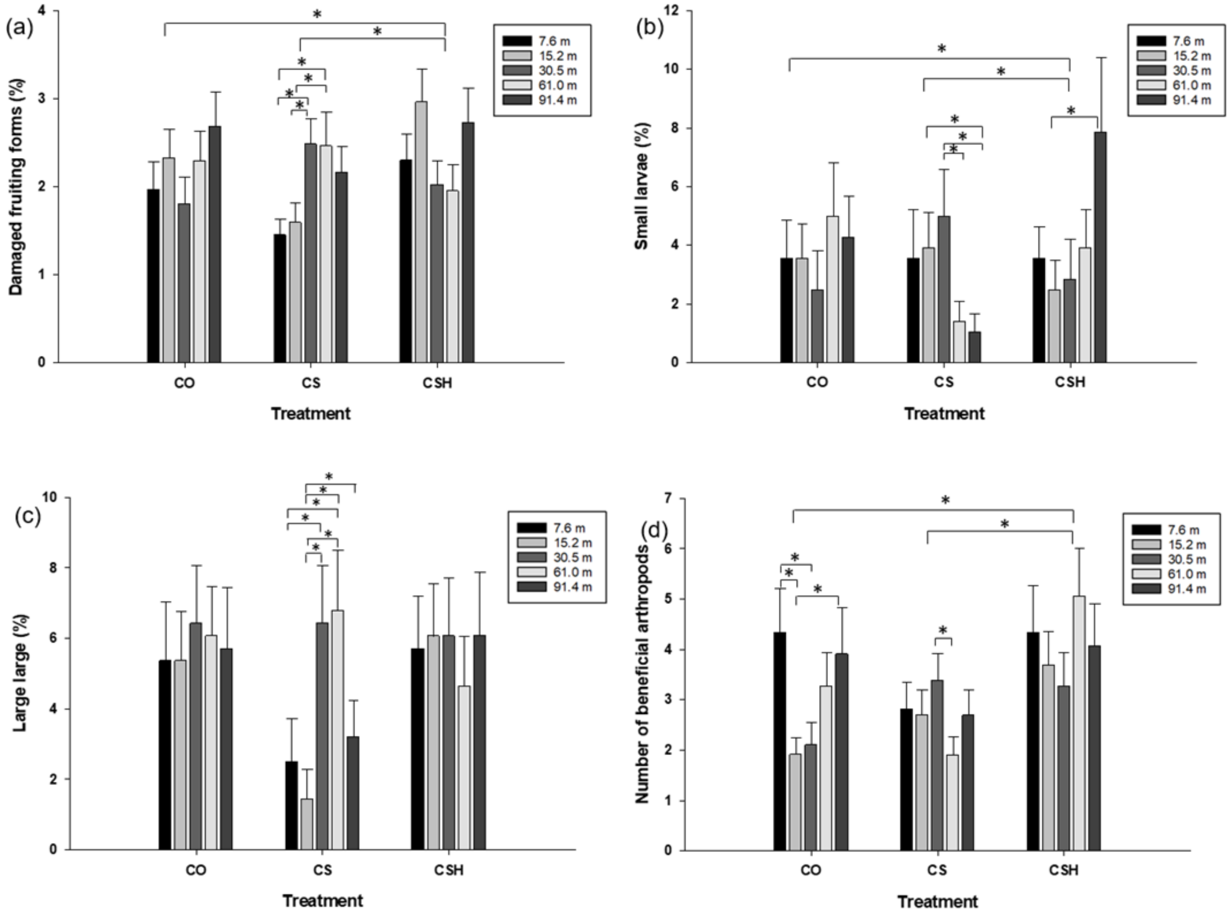

Figure 4.3 Means (±SE) percentage of damaged fruiting forms (a), means (±SE) percentage of small larvae (b), means (±SE) percentage of large larvae (c), and means (±SE) number of beneficial arthropods (d) between paired treatments and between paired distance within treatment affected by grain sorghum and *Hear*NPV in 2021. CO = cotton only, CS = cotton intercropped with grain sorghum, and CSH = cotton intercropped with grain sorghum treated with *Hear*NPV. The asterisks indicate the comparisons were significantly different (*P* ≤ 0.05).

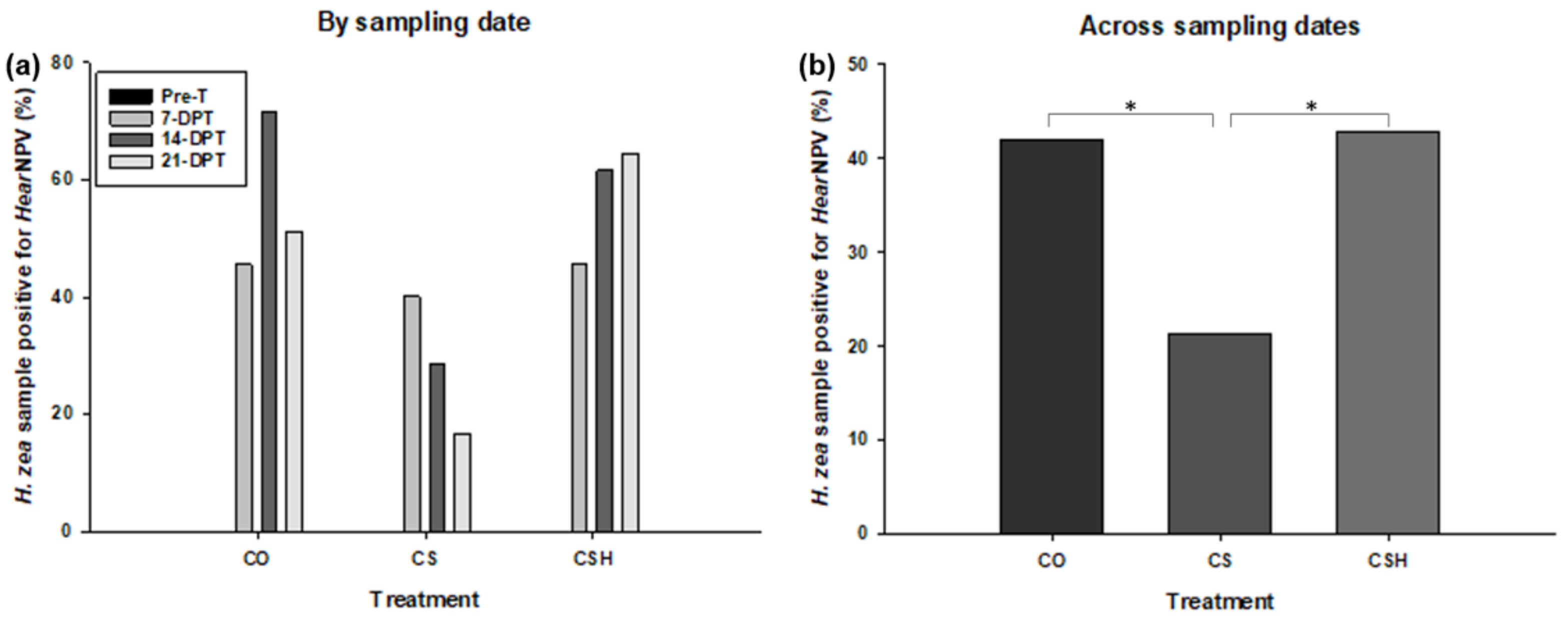

Figure 4.4 Percentage of *H. zea* sample that were tested positive for *Hear*NPV in 2020. Graph (a) displays data by sampling date; graph (b) displays data across sampling dates. CO = cotton only, CS = cotton intercropped with grain sorghum, and CSH = cotton intercropped with grain sorghum treated with *Hear*NPV. Only data across sampling dates were considered for statistical analysis. There was no significant difference between any treatment comparisons (*P* > 0.05).

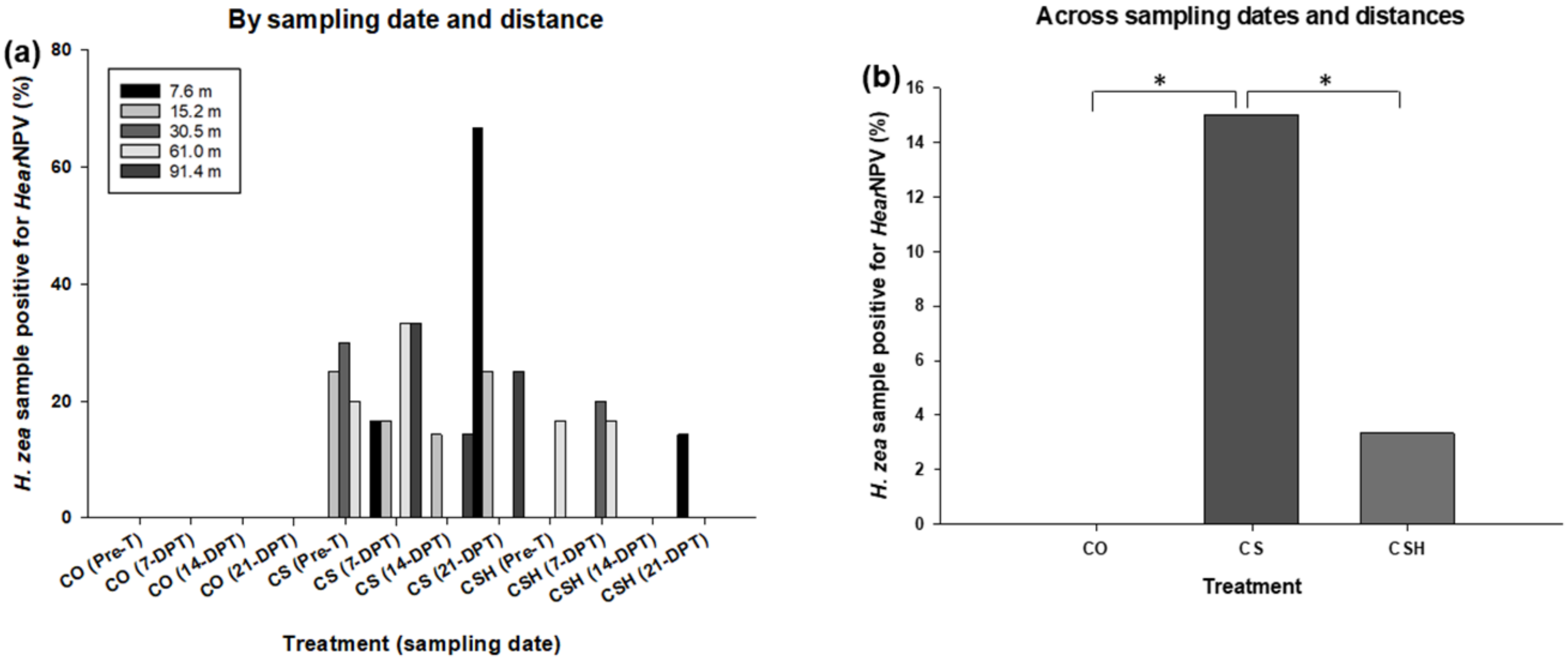

Figure 4.5 Percentage of *H. zea* sample that were tested positive for *Hear*NPV in 2021. Graph (a) displays data by sampling date and distance; graph (b) displays data across sampling dates and distances. CO = cotton only, CS = cotton intercropped with grain sorghum, and CSH = cotton intercropped with grain sorghum treated with *Hear*NPV, Pre-T = Pre-treatment, DPT = days post-treatment. Only data across sampling dates and distances were considered for statistical analysis. The asterisks indicate the comparisons were significantly different (P ≤ 0.05).

Table 4.1 Grain sorghum hybrids utilized.

Sorghum hybrid	Minimum days to 50% bloom	Maximum days to 50% bloom
SP 78M30	72	76
SP 74M21	69	74
SP 68M57	66	71
SP 31A15	54	58
SP 43M80	58	62
251	50	54

Table 4.2 Beneficial arthropods that occurred in cotton and grain sorghum in 2020 and 2021.

Order	Family	Common name	Benefit
Araneae	Thomisidae	Crab spider	Predator
	Salticidae	Jumping spider	Predator
	Araneidae	Orb-weaver spiders	Predator
	Oxyopidae	Lynx spider	Predator
Coleoptera	Coccinellidae	Lady beetle	Predator
Diptera	Syrphidae	Hoverfly	Predator
	Tachinidae	Tachinid fly	Parasitoid
Hemiptera	Pentatomidae	Spined soldier bug	Predator
	Reduviidae	Assassin bug	Predator
	Geocoridae	Big-eyed bug	Predator
	Anthocoridae	Minute pirate bug	Predator
	Miridae	Cotton fleahopper	Predator
	Nabidae	Damsel bug species	Predator
Hymenoptera	Formicidae	Fire ant	Predator
	Braconidae	Braconid wasp	Parasitoid
Neuroptera	Chrysopidae	Green lacewings	Predator
	Hemerobiidae	Brown lacewings	Predator

Table 4.3 Beneficial arthropods that tested positive for *Hear*NPV in 2020 and 2021.

Year	Arthropod groups	n[a]	No. positive sample	% positive sample	n[a]	No. positive sample	% positive sample	n[a]	No. positive sample	% positive sample
		Cotton-only			Non-treated cotton-sorghum			Treated cotton-sorghum		
2020	Chrysopidae	5	0	0	15	0	0	31	4	12.9
	Coccinellidae	10	0	0	28	0	0	52	1	1.9
	Pentatomidae	0	0	0	1	0	0	4	1	25
	Reduviidae	0	0	0	1	0	0	2	1	50
	Combined	15	0	0	45	0	0	89	7	7.9
2021	Coccinellidae	24	0	0	50	3	6	43	2	4.7
	Pentatomidae	0	0	0	3	2	66.7	2	0	0
	Reduviidae	1	0	0	4	1	25	1	1	100
	Formicidae	19	0	0	57	2	3.5	72	3	4.2
	Anthocoridae	4	0	0	31	0	0	40	1	2.5
	Spiders*	11	0	0	58	6	10.3	60	1	1.7
	Combined	59	0	0	203	14	6.9	218	8	3.7

[a] Denotes sample size.

*Spiders include Thomisidae, Salticidae, Araneidae, and Oxyopidae.

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5. BASELINE SUSCEPTIBILITY OF FIELD AND LABORATORY STRAINS OF *HELICOVERPA ZEA* (BODDIE) TO *HEAR*NPV AND CROSS-RESISTANCE TO BT PROTEINS

5.1. Introduction

Helicoverpa zea (Boddie) is a polyphagous insect that is a pest of many crops including corn (*Zea mays* (L.)), sorghum (*Sorghum bicolor* (L.) Moench), cotton (*Gossypium spp*.), soybean (*Glycine max* (L.) Merr.), tomato (*Solanum lycopersicum* (L.)), and hemp (*Cannabis sativa* (L.)) (Delahaut et al. 2004, Capinera 2020, Foster 2017, Reay-Jones 2019, Ajayi and Samuel-Foo 2021). *Helicoverpa zea* is primarily managed with conventional insecticides and *Bt* plant-incorporated protectants (Vemula et al. 2012, Towles et al. 2021). This pest is notorious for developing resistance to insecticides and has developed resistance to insecticides from several insecticide classes including organochlorines (IRAC MoA group 2A), organophosphates (IRAC MoA group 1B), and pyrethroids (IRAC MoA group 3A). Additionally, *H. zea* has recently developed resistance to Cry1 and Cry2 *Bt* proteins (IRAC MoA group 11A) (Vemula et al. 2012, Adams et al. 2016, Olmstead et al. 2016, Reisig et al. 2018, Yang et al. 2018, Reisig et al. 2019). The ability of *H. zea* to develop resistance to insecticides renders the management of the pest challenging.

Implementation of integrated pest management (IPM) approaches can play a major role in the effective management of *H. zea* by mitigating the already established resistance as well as delaying the onset of resistance to new classes of insecticides (Hoy 1998, Martinez 2015). *Helicoverpa armigera nucleopolyhedrovirus* (*Hear*NPV), is an entomopathogenic virus in the family Bacculoviridae (Blissard and Theilmann 2018). *Hear*NPV is specific to lepidopterans in the subfamily Heliothinae, which includes *H. zea* (Gettig and McCarthy 1982*). Hear*NPV-based insecticides are target-host specific, selective, and have a novel mode of action (IRAC MoA group 31) (Muraro et al. 2022, Blissard and Theilmann 2018). Considering the characteristics of *Hear*NPV, the insecticide can potentially serve as an effective tool to incorporate into an IPM program targeting *H. zea* and has already seen limited adoption to manage *H. zea* in soybean, grain sorghum, and hemp in the U.S., and its potential to manage *H. zea* in cotton has also been investigated (OSU Extension service, Musser et al. 2016, Stewart et al. 2022).

*Hear*NPV is a foliar-applied spray like conventional insecticides and is activated upon larval ingestion. The virus infects the host in two phases namely primary and secondary infection. Initially, the larvae ingest the virus occlusion bodies (OBs) (protein structures that protect the virus from environmental conditions) which travel through the digestive tract to reach the midgut. Once in the midgut, the alkaline content of the midgut activates the virus by degrading the occlusion bodies allowing the release of the occlusion-derived virions which then cross the peritrophic membrane, infect the midgut cells, and replicate. In the second phase of the infection, the replicated virus then egresses as budded virus and ultimately spread throughout the host's muscle, fat body, hemocytes, and tracheal cells causing it to liquefy (O'Reilly et al. 1992, Hunter-Fujita et al. 1998, Blissard and Theilmann 2018). Once the host larva dies and liquefies, millions of viral particles are released into the environment where horizontal and abiotic transmissions can occur (Boucias and Pendland 1998).

Resistance to baculovirus in insects has been reported. *Helicoverpa zea* and *Spodoptera littoralis* (Boisduval) have shown resistance to *Autographa californica* multiple nucleopolyhedrovirus (*Ac*MNPV) infections. These insects become resistant to the virus by melanizing and encapsulating the tracheal epidermis, a mechanism that prevents secondary infection from occurring (Rohrmann 2019). Additionally, there are several reports in Germany of field resistance of *Cydia pomonella* (L.) to *Cydia pomonella* granulovirus (*Cp*GV). In resistant *C. pomonella*, the virus is able to cross the peritrophic membrane, however, the larva provokes a systemic blockade preventing the occurrence of viral DNA replication within the midgut cells (Asser-Kaiser et al. 2007, Asser-Kaiser et al. 2011). Additionally, resistance in *Heliothis subflexa* (Guenée) to *Baculovirus heliothis* has also been reported, however, the mechanism of resistance is not known (Ignoffo et al. 1985).

The development of new insecticides is difficult since this process is lengthy, expensive, and subject to stringent regulatory requirements (Sparks 2013). Considering the ability of lepidopteran hosts to develop resistance to baculoviruses, it is crucial to devise proactive insecticide resistance management (IRM) programs for *Hear*NPV. Implementation of proactive IRM is essential to maintain the long-term efficacy of pesticides and baseline susceptibility of pests to pesticides, a strategy allowing for resistance monitoring, is crucial for effective IRMs (Miller et al. 2010, Pereira et al. 2020). Therefore, it is important to characterize the baseline susceptibility of *H. zea* to *Hear*NPV prior to its widespread adoption.

The objective of this study is to characterize the baseline susceptibility of several *H. zea* strains to *Hear*NPV and evaluate cross-resistance between *Hear*NPV and *Bt* proteins.

5.2. Materials and Methods

A series of bioassays were conducted to determine the current level of susceptibility of *Bt*-susceptible and *Bt*-resistant *H. zea* strains, and field-collected populations to *Hear*NPV.

5.2.1. Laboratory Insect Strains

The susceptible strain (SS) was obtained from Benzon (Benzon Research, Carlisle, PA) and is susceptible to Cry1Ac, Cry2Ab2, Cry1F, and Vip3Aa *Bt* proteins (Yang et al. 2020a, Yang et al. 2021b). The CRY-RR strain is resistant to Cry1Ac, Cry1F, and Cry2Ab2 *Bt* proteins, but is susceptible to Vip3Aa *Bt* protein (Yang et al. 2021a). The VIP-RR-70 and VIP-RR-15 strains are resistant to Vip3Aa *Bt* proteins but are susceptible to Cry1Ac, Cry1F, and Cry2Ab2 *Bt* proteins (Yang et al. 2020a). The TRE-RR strain is resistant to Cry1Ac, Cry2Ab2, and Vip3Aa proteins (Yang et al. 2023) (Table 1). The CRY-RR, VIP-RR-70, VIP-RR-15, and TRE-RR strains reside at Texas A&M University, College Station, TX. The resistant (RR) *H. zea* strains had been backcrossed with SS and reselected for resistance to produce resistant strains that are genetically similar to the SS strain. The CRY-RS strain is a reciprocal cross between the susceptible strain and the CRY-RR strain.

5.2.2. Field-Collected Insect Strains

In 2021, the field-collected populations were collected from Intrasect (Cry1Ab + Cry1F), DoublePro (Cry1A.105 + Cry2Ab2), or Trecepta (Cry1A.105 + Cry2Ab2+Vip3Aa) corn (Tables 2 and 3). Laboratory bioassays found that the Thrall, TX, and Winnsboro, LA populations were resistant to Cry1Ac, and Cry2Ab2, but were susceptible to Vip3Aa (Table 1). The Malone, TX population was resistant to Cry1Ac, but was susceptible to Cry2Ab2 and Vip3Aa. The Alexandria, LA population was not tested for resistant to Cry1Ac, but was susceptible to Cry2Ab2, and Vip3Aa. In 2022, the field-collected populations were collected from non-Bt corn or crimson clover (Tables 2 and 3). In 2022, the Epps, LA, Leland, LA, and Taylor, TX populations were resistant to Cry1Ac and Cry2Ab2, but susceptible to Vip3Aa, and the Mariana, AR population was resistant to Cry1Ac, but susceptible to Cry2Ab2 and Vip3Aa (Table 1).

5.2.3. Preliminary Bioassay for *Hear*NPV Concentrations Determination

The *Hear*NPV inoculum consisted of the commercial insecticide Heligen® (AgBiTech, Fort Worth, TX). The active constituent of Heligen® is occlusion bodies (OBs) of the nucleopolyhedrovirus of *Helicoverpa* spp. at a concentration of 7.5×10^9 OBs/mL.

To determine the suitable concentrations of *Hear*NPV to cause *H. zea* mortality ranging from 0 to 100%, a preliminary bioassay utilizing *Hear*NPV concentrations of 1x10, 2x10, 3x10, $1x10^2$, $2x10^2$, $3x10^2$, $1x10^3$, $2x10^3$, $3x10^3$, $1x10^4$, $2x10^4$, $3x10^4$, $1x10^5$, $2x10^5$, and $3x10^5$ OBs/mL as well as a non-treated control. Based on the results, concentrations of $0.7x10^3$, $2x10^3$, $0.7x10^4$, $2x10^4$, $0.4x10^5$, and $1x10^5$ OBs/mL were selected to perform the reported dose-response bioassays.

5.2.4. Dose-Response Bioassays

For this experiment, a diet-overlay bioassay was performed. Seven treatments including 6 distinct *Hear*NPV concentrations ($0.7x10^3$, $2x10^3$, $0.7x10^4$, $2x10^4$, $0.4x10^5$, and $1x10^5$ OBs/mL) and a non-treated control consisting of deionized water were utilized. A red food dye (McCormick Culinary, Hunt Valley, MD) at a concentration of 1 µL/mL was added to each treatment solution allowing the visualization of the solution on the diet. For the bioassays conducted in 2021, 29 mL solo condiment cups (Dart Container Corporation, Mason, MI) were utilized. Six mL of liquid diet (Southland Product, Inc., Lake Village, AR, USA) were dispensed into each cup. Each treatment replicate consisted of 14 to 30 larvae depending on the larvae availability. In 2022 we transitioned to using 128-well bioassay trays (C-D International, Pitman, NJ) and 1 mL of liquid diet was dispensed into each well of the tray. Each treatment replicate consisted of 12 to 16 larvae depending on the larvae availability. When the diet was cool, each cup or well was infested with a single second instar *H. zea* larva using a fine paint brush. After infestation, 100 µL of solution of each respective treatment was overlaid on the diet into each cup (2021), or 50 µL of solution of each respective treatment were overlaid on the diet into each well (2022) and immediately covered with the lid (2021) or air vented covers (C-D International, Pitman, NJ) (2022). The trays were kept in a climate control room at 25 °C and a photoperiod of 16:8 (L:D). Larval mortality data were collected daily until 10 days post-inoculation. Any larvae that were found dead at 1- or 2-day post inoculation were considered dead from handling and/or drowning and were excluded from the analysis. Each bioassay was replicated 3 to 5 times depending on larvae availability.

5.2.5. Time-Response Bioassays

*Hear*NPV was evaluated individually for each *H. zea* population at a concentration of $1x10^5$ OBs/mL which is the concentration that provided 100% mortality in the SS strain. The bioassays were conducted as previously described for the dose-response bioassays. Each treatment replicate comprised of 14 to 30 larvae, and 12 to 16 larvae in 2021 and 2022, respectively. Larval mortality data were collected daily until 10 days post-inoculation. The bioassays were replicated 3 to 5 times.

5.2.6. Resistance and Time Ratio Determination

The resistance and time ratios were determined for each of the tested *H. zea* strains and field-collected populations. The resistance ratio was calculated by dividing the LC_{50} value of the test strain by the LC_{50} value of the susceptible strain. The time ratio was calculated by dividing the LT_{50} value of the test strain by the LT_{50} value of the susceptible strain.

5.2.7. Data Analyses

Probit analysis (PROC PROBIT. Version 13.1, SAS Institute Inc., Cary, NC) was utilized to determine LC_{50} and LT_{50} values as well as the 95% confidence interval for each *H. zea* strain.

Larvae mortality was corrected based on the SS mortality utilizing Abbott's formula (Abbott 1925). A correlation analysis was performed utilizing Pearson's correlation coefficient (GraphPad Prism 9.5.0, GraphPad Software, San Diego, CA) to determine the correlation between *Bt* and *Hear*NPV resistance ratios.

5.3. Results

5.3.1. Dose-Response Bioassays

In 2021 dose-response bioassays the CRY-RR strain and the field-collected strains including, Thrall, TX, Malone, TX, Winnsboro, LA, and Alexandria, LA, exhibited *Hear*NPV LC_{50} values and resistance ratios significantly greater than that of the SS strain based on non-overlapping LC_{50} Confidence Intervals (CIs). The *Hear*NPV resistance ratios ranged from 9.5 to 22.7-fold (Table 5-2). These *H. zea* strains, except for Alexandria, LA, are resistant to both Cry1Ac and Cry2Ab2 *Bt* proteins (Table 5-1). The Alexandria, LA strain was evaluated for resistance to only Cry2Ab2 and Vip3Aa and was susceptible to both *Bt* proteins. The VIP-RR-70 strain, which is susceptible to both Cry1Ac and Cry2Ab2 (Table 5-1), exhibited a *Hear*NPV LC_{50} and a resistance ratio comparable to that of the SS strain based on overlapping LC_{50} CIs (Table 5-2).

In 2022 dose-response bioassays, we observed an increase in susceptibility for the SS and CRY-RR strains relative to the 2021 dose-response bioassays. Additionally, the CRY-RR, CRY-RS, VIP-RR-15, and TRE-RR strains exhibited *Hear*NPV LC_{50} and resistance ratios similar to that of the SS strain based on overlapping LC_{50} CIs. However, the field-collected strains including Epps, LA, Leland, MS, Marianna, AR, and Taylor, TX exhibited *Hear*NPV LC_{50} and resistance ratios significantly greater than that of the SS strain based on non-overlapping LC_{50}

CIs. Their resistance ratios ranged from 1.8 to 175-fold (Table 5-2). The field collected *H. zea* strains were reported to be resistant to Cry1Ac and/or Cry2Ab2. (Table 5-1).

5.3.2. Time-Response Bioassays

In 2021 time-response bioassays, all *H. zea* strains exhibited *Hear*NPV LT_{50} values and time ratios that were statistically greater than that of the SS strain based on non-overlapping LT_{50} CIs. The numerical differences between the test strains LT_{50} and that of the SS strain were slight, ranging from 1.39 to 5.04 days. In 2022 time-response bioassays, all the laboratory *H. zea* strains and most of the field-collected *H. zea* strains including Epps, LA, Leland, MS, and Taylor, TX had LT_{50} and time ratios that were statistically longer than that of the SS strain based on non-overlapping LT_{50} CIs. The numerical differences between the LT_{50} values of these test strains and that of the SS strain were slight, ranging from 0.48 to 5.69 days. However, based on non-overlapping LT_{50} CIs, the Marianna, AR, strain had a statistically shorter LT_{50} value relative to the SS strain. The numerical difference between the LT_{50} values of Marianna, AR, and SS strains was only 0.6 day (Table 5-3).

5.3.3. Correlation Between *Hear*NPV and Bt Proteins Resistance Ratios

The *Hear*NPV resistance ratios and the resistance ratios for Cry1Ac, Cry2Ab2, or Vip3Aa were pooled and paired to create data sets for the correlation analysis. Because there was a statistical outlier (Epps, LA, resistance ratio) for the Cry1Ac and *Hear*NPV pair, the correlation analysis was performed for this group utilizing both the complete data set and the data set with the outlier removed.

There was a significant positive correlation between Cry2Ab2 and *Hear*NPV resistance ($r = 0.67$, $P = 0.0089$). When the complete data set was considered in the analysis, there was no significant correlation between Cry1Ac and *Hear*NPV resistance ratios ($r = 0.02$, $P = 0.9438$).

However, there was a highly significant positive correlation between Cry1Ac and *Hear*NPV resistance ratios when the outlier was removed (*r* = 0.81, *P* = 0.0015). The correlation between the Vip3Aa and *Hear*NPV resistance ratios was not significant (*r* = -0.17, *P* = 0.5566).

5.4. Discussions

*Hear*NPV has been seen limited adoption for managing *H. zea* in several crops in the U.S. (OSU Extension service, Musser et al. 2016, Popham et al. 2016, Stewart et al. 2022, Black et al. 2022). Further adoption of *Hear*NPV may play a significant role in alleviating insecticide resistance in this pest. Making efforts to establish insect resistance management programs for *Hear*NPV can help mitigate the risk of the evolution of *Hear*NPV resistance (Hoy 1998, Martinez 2015).

Based on the results of the current study, the *H. zea* strains that exhibited resistance to Cry1Ac and Cry2Ab2 *Bt* proteins were slightly less susceptible to *Hear*NPV relative to the genetically similar SS strain. Additionally, correlation analysis indicated positive correlations between *Hear*NPV and Cry1Ac or Cry2Ab2 resistance ratios. Moreover, we observed a significant positive correlation between the resistance ratios for Cry1Ac and Cry2Ab2, and the resistance ratios for *Hear*NPV. This suggests that there is some cross-resistance between Cry1Ac and Cry2Ab2 resistance and *Hear*NPV resistance in *H. zea*. *Helicoverpa zea* strains with resistance to Vip3Aa (VIP-RR-70, VIP-RR-15, and TRE-RR) were as susceptible to *Hear*NPV as the SS strain. Thus, the reduced susceptibility to *Hear*NPV is associated with the resistance to Cry1Ac and Cry2Ab2 *Bt* proteins in *H. zea* but not associated with resistance to Vip3Aa *Bt* protein or triple-resistant (Cry1, Cry2, and Vip3Aa proteins) in *H. zea*. However, the TRE-RR strain is resistant to Cry1, Cry2 and Vip3Aa *Bt* proteins. The reason the TRE-RR strain, remained highly susceptible to *Hear*NPV is not clear, but appears to be associated with resistance to Vip3Aa. In a similar study involving *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren), the Cry2Ab+Vip3A-resistant strains exhibited comparable *Hear*NPV LC_{50} values to their respective reference susceptible strain. Although there were no differences in LC_{50} values for Cry1Ac or Cry2Ab-resistant strains and the susceptible strain of *H. armigera* and *H. punctigera,* the LC_{50} values for the Cry1Ac or Cry2Ab-resistant strains were numerically elevated (Windus et al. 2021). Such results support our findings.

All the field-collected strains tested in this current study had reduced susceptibility to *Hear*NPV relative to the SS strain and most were resistant to Cry1Ac and Cry2Ab2 (Kerns et al. 2022, Kerns et al. 2023). Cry1 and Cry2 resistance in feral *H. zea* is common and widespread in the southern U.S. (Yang et al. 2020b, Yang et al. 2021a). Additionally, *H. zea* has been found to be highly resistant to the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (*Ac*MNPV) (Rohrmann 2019). Hence, it is possible that with widespread and extensive *Hear*NPV use and the absence of appropriate IRM programs, *H. zea* may evolve resistance to *Hear*NPV. In contrast, since the *H. zea* genotypes that were susceptible to Cry *Bt* proteins but resistant to Vip3Aa, or resistant to both Cry and Vip3Aa proteins were susceptible to *Hear*NPV, the virus may serve a significant role in mitigating the evolution of Vip3Aa resistance. We also observed that *Hear*NPV required a slightly greater amount of time to cause 50% mortality in all tested *H. zea* strains relative to the SS strain. Since the difference in time is slight, it will most likely not be a significant factor in reduced *Hear*NPV performance in *H. zea*.

Several tactics can be implemented to alleviate the onset of *Hear*NPV resistance in *H. zea.* These tactics should include insect resistance monitoring, and the adoption of varied control measures such as insecticide rotation, biological control, mechanical control, sanitation, and tank-mixes of *Hear*NPV with one or more insecticides having distinct mode of action (PES:

Pesticide Environmental Stewardship, Wilen et al. 2021). Although reduced susceptibility in some *H. zea* strains was observed, integrating *Hear*NPV in a well-designed IPM program can be beneficial. Because of its unique mode of action, *Hear*NPV can significantly aid in insect resistance management of insecticides commonly used for *H. zea* management (Muraro et al. 2022). Beneficial arthropods are a key component of IPM (Godfrey et al. 2013) and *Hear*NPV as a selective insecticide has no impact on natural enemies (Gettig and McCarthy 1982). Hence, the adoption of *Hear*NPV will preserve beneficial organisms, maximize biological control, and avoid insecticide-induced secondary pest outbreaks (Godfrey et al. 2013, Blissard and Theilmann 2018, Muraro et al. 2022). Additionally, *Hear*NPV-based insecticides are cost effective relatively to most synthetic insecticides used for *H. zea* control which is attractive to agricultural producers. Moreover, Vip3Aa-resistant *H. zea* have shown high susceptibility to *Hear*NPV and the adoption of *Hear*NPV may aid in delaying the evolution of Vip3Aa resistance which currently remains the sole *Bt* protein that consistently provides effective *H. zea* control (Yang et al. 2022).

Overall, the results of this study suggest that the incorporation of *Hear*NPV into IPM programs for *H. zea* management may prove to be valuable addition for insecticide and *Bt* resistance management and pest management.

Table 5.1 *Bt* resistance ratio of field-collected and laboratory *H. zea* strains.

Insect strain	Cry1Ac	Cry2Ab2	Vip3Aa39	Reference
CRY-RR	779.3*	387.8*	0.4	Yang et al. 2021a
CRY-RS	128.1*	135.4*	1.5	Yang et al. 2021a
VIP-RR-70	6.3	1.9	> 892.8*	Yang et al. 2023
VIP-RR-15	2.0	1.2	> 892.8*	Yang et al. 2023
TRE-RR	264.8*	495.3*	> 95.7*	Yang et al. 2023
Thrall, TX	2787333*	64.8*	< 0.09	Kerns et al. 2023
Malone, TX	539.6*	67.3*	< 0.09	Kerns et al. 2022
Taylor, TX	55028.6*	12.0*	0.2	Kerns et al. 2022
Winnsboro, LA	154.6*	79.4*	0.21	Kerns et al. 2022
Alexandria, LA	/	5.31	< 0.09	Kerns et al. 2022
Epps, LA	351.1*	740.7*	1.9	Kerns et al. 2023
Leland, MS	72150.0*	13.2*	0.04	Kerns et al. 2023
Marianna, AR	1498.6*	8.0	0.3	Kerns et al. 2023

*Indicates significant resistance ratios (≥10-fold).

Table 5.2 Dose-response of laboratory and field-collected *H. zea* strains to *Hear*NPV.

Insect strain	Host	n[a]	LC$_{50}$ (95% FL) (OBs/mL)	Slope ± SE	x^2	df	Resistance ratio (RR)[b]
			2021				
SS	Lab diet	840	6.5x10^{3} (4.9x10^{3}, 8.4x10^{3})	1.7 ± 0.2	119	22	1
CRY-RR	Lab diet	840	71.0x10^{3} (54.6x10^{3}, 99.8x10^{3})	1.4 ± 0.1	104	28	10.9*
VIP-RR-70	Lab diet	1050	10.5x10^{3} (8.0x10^{3}, 14.0x10^{3})	1.3 ± 0.1	128	28	1.6
Thrall, TX	Intrasect corn	560	147.3x10^{3} (64.4x10^{3}, 3625.0x10^{3})	1.3 ± 0.4	9.5	22	22.7*
Malone, TX	DoublePro corn	616	62.6x10^{3} (43.1x10^{3}, 100.0x10^{3})	1.2 ± 0.2	60.4	22	9.5*
Winnsboro, LA	DoublePro corn	630	66.9x10^{3} (33.3x10^{3}, 241.3x10^{3})	0.8 ± 0.2	25.2	22	10.3*
Alexandria, LA	Trecepta corn	336	134.4x10^{3} (54.2x10^{3}, 1623.9x10^{3})	0.9 ± 0.2	14.7	16	20.7*
			2022				
SS	Lab diet	448	2.8x10^{3} (2.1x10^{3}, 3.7x10^{3})	1.9 ± 0.2	86.6	22	1
CRY-RR	Lab diet	448	3.9x10^{3} (2.4x10^{3}, 5.7x10^{3})	1.6 ± 0.2	55	21	1.4
CRY-RS	Lab diet	896	3.1x10^{3} (2.2x10^{3}, 4.2x10^{3})	1.2 ± 0.1	108	22	1.1
VIP-RR-15	Lab diet	448	1.8x10^{3} (0.8x10^{3}, 3.1x10^{3})	1.0 ± 0.2	39.2	22	0.7
TRE-RR	Lab diet	238	1.6x10^{3} (0.9x10^{3}, 2.4x10^{3})	1.2 ± 0.2	47.7	16	0.6
Epps, LA	Crimson clover	336	490.0x10^{3} (100.0x10^{3}, 4.9x10^{8})	0.6 ± 0.2	10.2	16	175.0*
Leland, MS	NBT corn	448	5.5x10^{3} (4.0x10^{3}, 7.5x10^{3})	1.7 ± 0.2	89.6	22	2.0*
Marianna, AR	NBT corn	448	5.0x10^{3} (4.0x10^{3}, 6.3x10^{3})	1.8 ± 0.1	147	22	1.8*
Taylor, TX	NBT corn	448	10.2x10^{3} (6.2x10^{3}, 17.0x10^{3})	0.6 ± 0.1	45.4	22	3.6*

[a] Total number of larvae assayed.

[b] Resistance ratios were calculated by dividing the LC$_{50}$ value of the test population by the LC$_{50}$ of the SS strain.

* Indicates significant resistance ratios based on non-overlapping 95% CIs.

Table 5.3 Time-response of laboratory and field-collected *H. zea* strains to *Hear*NPV at a concentration of 1x10^{5} OBs/mL.

Insect strain	Host	n[a]	LT_{50} (95% FL) (dpi)[b]	Slope ± SE	x^2	df	Time ratio (TR)[c]
			2021				
SS	Lab diet	240	5.4 (5.2, 5.5)	17.5 ± 1.7	100.3	38	1
CRY-RR	Lab diet	240	8.7 (8.0, 9. 6)	5.3 ± 0.7	60.8	38	1.6*
VIP-RR-70	Lab diet	300	6.7 (6.6, 6.9)	7.4 ± 0.4	377	48	1.3*
Thrall, TX	Intrasect corn	160	10.4 (9.7, 11.6)	5.7 ± 0.7	70.7	38	1.9*
Malone, TX	Double Pro corn	176	8.1 (7.7, 8.7)	7.8 ± 1.0	67.2	38	1.5*
Winnsboro, LA	Double Pro corn	180	7.9 (7.3, 8.6)	4.7 ± 0.5	79.8	38	1.5*
Alexandria, LA	Trecepta corn	96	7.5 (5.8, 11.3)	2.5 ± 0.6	19.64	21	1.4*
			2022				
SS	Lab diet	128	4.6 (4.5, 4.7)	34.1 ± 4.6	56.2	38	1
CRY-RR	Lab diet	128	5.5 (5.2, 5.8)	12.0 ± 1.5	64.8	38	1.2*
CRY-RS	Lab diet	256	6.3 (6.1, 6.5)	9.8 ± 0.7	175.33	38	1.4*
VIP-RR-15	Lab diet	128	5.6 (5.3, 5.8)	8.6 ± 0.7	140.64	38	1.2*
TRE-RR	Lab diet	64	6.0 (5.5, 6.5)	8.5 ± 1.2	51.2	28	1.3*
Epps, LA	Crimson clover	96	10.3 (9.6, 11.7)	7.3 ± 1.2	39.2	28	2.2*
Leland, MS	NBT corn	128	5.1 (4.8, 5.4)	6.3 ± 0.4	195.7	38	1.1*
Mariana, AR	NBT corn	128	4.0 (3.9, 4.2)	15.5 ± 1.7	88.5	38	0.9*[¥]
Taylor, TX	NBT corn	128	7.2 (6.9, 7.5)	7.7 ± 0.6	145.1	38	1.6*

[a] Total number of larvae assayed.

[b] Days post inoculation.

[c] Time ratios were calculated by dividing the LT_{50} value of the test population by the LT_{50} value of the SS laboratory strain.

* Indicates significant time ratios based on non-overlapping 95% CIs.

[¥] Indicates time ratio significantly lower to that of the SS susceptible laboratory strain based on non-overlapping 95% CIs.

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6. CONCLUSIONS

Due to continuous evolution of *Bt* resistance in *H. zea*, reliance on insecticides to manage the pest in *Bt* cotton has become common and widespread. However, *H. zea* control with insecticides has been inconsistent due to problems associated with insecticide resistance and action threshold timing. Considering the heavy reliance on chlorantraniliprole for the management of *H. zea* in cotton, as well as the insecticide having long residual activity and high selection pressure, there is great concern that resistance to chlorantraniliprole insecticides may rapidly develop. Thus, recommended action thresholds to manage *H. zea* in cotton using foliar insecticide were reassessed. Additionally, alternative *H. zea* management approaches were studied to potentially determine additional management tactics for a more comprehensive management strategy targeting *H. zea* in cotton.

Field experiments were conducted across the Mid-South and in Texas to devise economic thresholds for foliar insecticide applications targeting bollworm in cotton. A 6% fruiting forms injury threshold was chosen and compared with a preventive spray threshold. The utilization of 6% fruiting form injury threshold resulted in a 25 and 75% reduction in insecticide applications relative to preventive sprays for WS and BG2, respectively. Moreover, the current *H. zea* threshold recommendations for Texas, Mississippi, and South Carolina were also evaluated. For NBT cotton, all thresholds, including 20% oviposition, 6% fruiting forms injury, $\geq$3% large larvae, and preventive sprays, resulted in greater control of *H. zea* and higher yield and profitability relative to non-treated NBT cotton. The BG2 cotton sprayed based on 20% oviposition or preventively resulted in greater control of *H. zea*, and all treatments resulted in a yield or profitability equivalent to that of the non-treated BG2 cotton. For BG3 cotton, spraying based on any of the thresholds was not beneficial. For NBT and BG2 cotton, thresholds of 20% oviposition, 6% injury to fruiting forms, or ≥ 3% large larvae provided a reduction in insecticide applications relative to the preventive spray treatment.

Grain sorghum was evaluated as a trap crop of *H. zea* and a nursery crop for natural enemies of *H. zea* and *Hear*NPV dissemination into cotton. Growing cotton in an intercropping system did not result in consistent increase in *H. zea* control and beneficial arthropods relative to the cotton-only treatment. *Hear*NPV was detected in samples collected from all treatments which indicates that the virus is naturally occurring in the locations where this current study was conducted. Hence, there was no pertinent evidence that grain sorghum interplanted with cotton could serve as a source of *Hear*NPV for nearby cotton.

Several *H. zea* strains including laboratory and field-collected strains were evaluated for their susceptibility to *Hear*NPV. The field populations evaluated was consistently less susceptible to *Hear*NPV than the susceptible reference strain and the Cry-RR (resistant to Cry1 and Cry2 proteins) strains exhibited reduced susceptibility to *Hear*NPV than the susceptible reference strain in the first year of the study. The TRE-RR (resistant to Cry1, Cry2, and Vip3Aa proteins), VIP-RR-70 (resistant to Vip3Aa), and VIP-RR-15 (resistant to Vip3Aa) strains was similar to that of the SS strain.

The results of this research project suggest that the 20% oviposition, 6% fruiting forms injury, and the ≥ 3% large larvae thresholds are viable and can be implemented according to the *Bt* technology used or, in an alternative fashion, as conditions allow for *H. zea* management in cotton. Additionally, this study suggests that the effectiveness of using sorghum as a trap crop, and natural enemy and *Hear*NPV nursery will not consistently result in beneficial outcomes. Furthermore, our results indicate that the reduced susceptibility to *Hear*NPV is associated with the resistance to Cry1Ac and Cry2Ab2 *Bt* proteins in *H. zea* but not associated with *H. zea* genotypes resistant to Vip3Aa *Bt* protein or triple-resistant (Cry1, Cry2, and Vip3Aa proteins) *H. zea*.

The adoption of effective economic thresholds will hopefully reduce the unnecessary applications of chlorantraniliprole and reduce resistance selection pressure. In other words, incorporating a vetted economic threshold into an integrated pest management program targeting bollworm should improve the sustainability of cotton production. The availability of several alternative action thresholds to manage *H. zea* in cotton provides producers and/or pest managers with flexibility in choice of sampling technique to determine need for insecticide application targeting the pest. Future research should continuously evaluate the effectiveness of these thresholds for foliar insecticide treatments targeting *H. zea* in *Bt* cotton allowing to produce stronger conclusions favoring the most efficient management of *H. zea*. The characterization of the *H. zea* susceptibility to *Hear*NPV will allow for proactive *Hear*NPV resistance monitoring in *H. zea.*