THESIS

THE IMPACTS OF INTROGRESSION ON THE BIOLOGY AND MANAGEMENT OF *HELICOVERPA ZEA*

Submitted by

Cooper Phillips

Department of Agricultural Biology

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Master's Committee:

Advisor: Micky Eubanks

Luke Tembrock
Seth Davis

ABSTRACT

THE IMPACTS OF INTROGRESSION ON THE BIOLOGY AND MANAGEMENT OF *HELICOVERPA ZEA*

This thesis examined the biological and pest-management consequences of introgression between *Helicoverpa zea* (corn earworm) and its invasive sister species, *Helicoverpa armigera*. Recent research has shown that *H. armigera* has introduced the *CYP337B3* allele into some North American populations of *H. zea*. This allele is associated with strong resistance to pyrethroid insecticides in *H. armigera*. Because *H. zea* is already a major agricultural pest and has evolved resistance to several Bt toxins, the spread of additional resistance traits could have important implications for crop protection and integrated pest management (IPM).

The first objective of the thesis was to determine whether introgression increases pyrethroid resistance in *H. zea*. Laboratory colonies representing susceptible, non-admixed, and admixed (B3+) genotypes were established and screened using molecular methods. Caterpillars were exposed to multiple concentrations of λ-cyhalothrin and Warrior II, and mortality was recorded over time. The results demonstrated that admixed *H. zea* carrying the *CYP337B3* allele were significantly more resistant to pyrethroid insecticides than non-admixed H. zea. Helicoverpa zea with the B3 allele were 7.86-fold more resistant to λ-cyhalothrin and a smaller increase in resistance to Warrior II. These findings provide the first direct evidence that introgression can increase pyrethroid resistance in *H. zea*. A second experiment evaluated whether the synergist piperonyl butoxide (PBO) could restore susceptibility by inhibiting cytochrome P450 enzymes. PBO produced only modest and inconsistent effects, suggesting that

it is unlikely to provide a practical solution for managing resistance associated with the *CYP337B3* allele.

The second objective was to determine whether introgression influences cold tolerance. Because overwintering ability affects geographic distribution and seasonal population dynamics, the study compared the supercooling points (SCPs) of admixed and non-admixed *H. zea* pupae. Supercooling point is a commonly used indicator of freeze tolerance and represents the temperature at which hemolymph begins to freeze. Contrary to expectations, no significant difference in SCP was detected between admixed and non-admixed populations. Both groups had average SCP values near –18.5°C. These results suggest that the *CYP337B3* allele does not alter cold tolerance, at least as measured by SCP. Additional analyses revealed significant differences between sexes, with male pupae exhibiting slightly lower SCP values than females. However, pupal weight did not differ significantly between sexes.

The third objective was to investigate whether introgression alters attraction to commercial pheromone lures used to monitor *H. zea* populations. Monitoring programs rely heavily on pheromone traps to estimate pest abundance and guide management decisions. The thesis tested whether admixed and non-admixed moths responded differently to commonly used lure types. The results indicated that introgression did not significantly affect pheromone attraction. These findings suggest that existing pheromone-based monitoring programs should remain effective for tracking both admixed and non-admixed *H. zea* populations.

Overall, this thesis demonstrates that introgression from *H. armigera* has already altered one important trait in *H. zea*: susceptibility to pyrethroid insecticides. In contrast, no evidence was found that introgression affects cold tolerance or attraction to pheromone lures. The study highlights the importance of continued monitoring of admixed populations and emphasizes the

need for diversified IPM programs that reduce reliance on a single control tactic. As resistance to both Bt crops and pyrethroid insecticides continues to evolve, integrated approaches combining cultural, biological, and chemical controls will be essential for sustainable management of *H. zea*.

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DEDICATION

To

My parents, Shanon and Jon

My grandparents, John, Norma, Janis and Fred

Your continued support of me is undeniable, the luck I have to be your family

is something I will never take for granted.

To my grandfather John,

The greatest man to ever live, my idol, my inspiration,

there will never be another you. I hope to be

half the man you were.

“You and I will meet again”

-Tom Petty

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Helicoverpa zea is a polyphagous moth in the family Noctuidae (Cunningham & Zalucki, 2014). As larvae, *H. zea* feeds on at least 123 different species of host plants, some of which are key crops in global agriculture (Cunningham & Zalucki, 2014; North et al., 2024). Host plants include corn, cotton, tomato, and tobacco (Kennedy & Storer, 2000). *Helicoverpa zea* is unique in that it has multiple official common names depending on its host plant. On corn, *H. zea* is known as the corn earworm; on cotton, *H. zea* is known as the cotton bollworm; on tomato, *H. zea* is known as the tomato fruitworm (Olmstead et al., 2016). Economic damage for *H. zea* on crops varies based on crop type and is typically estimated based on cost of control and the overall crop losses. The economic losses due to *H. zea* across corn, cotton, and soybean reach over $100/ha (Perera et al., 2020). *Helicoverpa zea* is considered a major pest of crops, because as a fruit feeder, even moderate infestations can result in large economic losses. *Helicoverpa zea* has been a major pest for decades and a variety of IPM strategies have been utilized in reducing economic losses in agroecosystems.

One of the main control tactics used to manage *H. zea* is Bt (*Bacillus thuringiensis*) crops (Vaeck et al., 1987; Benowitz et al., 2022; Legan et al., 2024). Transgenic crops such as Bt crops were first developed to reduce the reliance on insecticide-based pest management strategies, and to reduce the overall use of pesticides in agricultural operations (Vaeck et al., 1987; Tabashnik et al., 2013). These crops were bioengineered to produce insect-specific toxins and first entered the

market in the mid-1990s (Huesing & English, 2004). These plants, known as "plant incorporated protectants", or PIPs, all have toxins derived from the bacterium *Bacillus thuringiensis* (Vaeck et al., 1987; Huesing & English, 2004). *Bacillus thuringiensis* was chosen for its ability to produce large quantities of toxins that specifically target certain insect orders. Certain Bt crops, such as maize, cotton, and potatoes, have all been bioengineered to produce a variety of toxins (Tabashnik et al., 2013). The toxins themselves function by rupturing the insect gut membrane, causing death when ingested (Taylor et al., 2021). Different toxins in Bt crops are developed as insect resistance develops against previously successful Bt crop toxins (Reisig & Kurtz, 2018). In maize, specific toxins produced are: *cry1Ab, cry1Ac, cry1Fa2, cry3Bb1,* and *cry9* (Huesing & English, 2004).

Helicoverpa zea as a species has developed resistance to *cry1Ab and cry1Ac* toxins in maize (Reisig & Kurtz, 2018). Resistance against Bt crops occurs in agroecosystems over-reliant on Bt bio-control methods, as Bt resistance in insects is controlled by one gene and is often under spatial and temporal pressure (Groot & Dicke, 2001; Shelton et al., 2002; Sheikh et al., 2017). While initially, the majority of reports of Bt resistance were recorded in laboratory studies, researchers began documenting resistance in wild populations of pests (Ferré & Rie, 2002). One such study documented that across 77 different countries, 5 of 13 wild populations of major insect pests developed resistance to Bt crops in 2010, whereas only 1 species had documented Bt resistance in 2005 (Sheikh et al., 2017). Three of the five resistant pest species were found in the United States (Sheikh et al., 2017).

Where transgenic crops are not employed, farmers often rely on pyrethroids and similar chemical insecticides to manage *H. zea* populations. Pyrethroids are used due to the low-cost of the pesticide class, as well as the relatively low toxicity to humans (Vijverberg & Bercken,

2008). While pyrethroids are utilized to manage populations of *H. zea*, overreliance on this insecticide class has led to some instances of evolved resistance (Pietrantonio et al., 2007; Hopkins & Pietrantonio, 2010). For example, Patla et al. (2026) found that some populations of *H. zea* in the southern United States had >50-fold resistance to the widely used pyrethroid λ-Cyhalothrin. Thus, it is recommended to utilize a variety of mechanisms to control *H. zea* populations so that resistance traits cannot become fixed within populations.

Helicoverpa zea is highly migratory, with individual adults being able to migrate upwards of 600km (Westbrook et al., 1997). Through this high migratory rate, the rate of mating between physically separated populations is increased (Seymour et al., 2016). *Helicoverpa zea* females are attracted to the flowers or reproductive structures of host plants and typically oviposit one or a few eggs on the developing fruit (Hardwick, 1965). As a result, *H. zea* populations have high levels of gene flow and little to no geographic structure in their population (Seymour et al., 2016).

2. Biology of Helicoverpa armigera and Its Impacts on Global Agroecosystems

Similar to its sister species, *H. zea*, *Helicoverpa armigera* is a polyphagous moth in the family Noctuidae. As larvae, *H. armigera* is known to feed on around 300 species of crops, causing billions of dollars in profit loss to occur in global agroecosystems (Yadav et al., 2022). Legumes such as soybean appear to be the preferred host plant for *H. armigera* (Haile et al., 2021). In India and China, around half of insecticides used in agroecosystems are aimed at controlling populations of the pest (Yadav et al., 2022).

The geographic range of *H. armigera* spans across Asia, Africa, and Europe (Anderson et al., 2018). There are two subspecies within the species, and while subspecies *Helicoverpa armigera armigera* is present across Asia, Africa, and Europe; *Helicoverpa armigera conferta* is

present in Oceania and Australia (Valencia-Montoya et al., 2020; Anderson et al., 2018). Similar to *H. zea*, *H. armigera* is highly migratory, with adults able to travel 250km in a singular night and 1000km over a lifetime (Jones et al., 2015; Feng et al., 2004). Adults utilize wind currents in long distance dispersal. In Europe, *H. armigera* overwinters in Southern Europe and the Mediterranean before beginning a summer migration Northward, reaching the furthest limits of its geographic range near the United Kingdom (Haile et al., 2021).

Helicoverpa armigera has been geographically separated from *H. zea* for 1.5 million years, as genetic sequencing reveals the two species once shared a common ancestor (Behere et al., 2007). Due to the physical barrier of the Atlantic Ocean, the species was limited to geographic range expansion within the Eastern hemisphere. However, in 2013, the presence of *H. armigera* was confirmed in Brazil through genomic sequencing of *Helicoverpa* populations (Tay et al., 2013). It is believed that *H. armigera* made its way to the new world before its first identification in 2013. However, since *H. armigera* and *H. zea* are phenotypically identical, it was hard to determine the presence of *H. armigera* initially (Haile et al., 2021). It is unclear how *H. armigera* arrived in the New World; however, various theories exist. One theory suggests that wind currents may have carried *H. armigera* into the New World, as they are capable of long-distance dispersal and have been observed to utilize wind patterns in migration (Haile et al., 2021; Yadav et al., 2022). Another theory suggests that *H. armigera* arrived via international trade routes, potentially being carried on internationally imported crops. There had to have been numerous introductions of *H. armigera* to successfully establish the population in the New World. Genomic sequencing reveals ancestral DNA linking *H. armigera* in the New World to various populations in the Old World, mainly Asia and Africa (Tembrock et al., 2019).

Helicoverpa armigera has been a pest for decades, often with chemical control methods. Similarly to *H. zea*, one main chemical insecticide class used against *H. armigera* populations is pyrethroid insecticides. While *H. zea* is known to have some moderate-to-low insecticide resistance present in their genome, *H. armigera* have high levels of pyrethroid insecticide resistance within their genome (Joußen et al., 2012). This pyrethroid resistance in *H. armigera* has been determined to be caused by a specific mutation on a specific locus on chromosome 15 in the pests, where the cytochrome P450 gene *CYP337* is located.

However, *H. armigera* have a specific mutation at this locus, where an unequal crossover event between alleles *CYP337B1* and *CYP337B2* have produced a chimeric allele known as the *CYP337B3* allele (Joußen et al., 2012). This allele is known to confer an over 42-fold increased resistance to pyrethroid insecticides in *H. armigera* with it present in their DNA (Joußen et al., 2012). The allele produces enzymes that metabolize pyrethroid pesticides into non-toxic hydro-pyrethroids in *Helicoverpa armigera* (Joußen et al., 2012).

3. Adaptive Introgression in Insects with Emphasis on Interspecific Introgression Between Helicoverpa zea and Helicoverpa armigera

Genetic admixture refers to gene flow between two species, resulting in a new population of mixed ancestry. In some cases, where this gene flow and allele admixture between populations occurs regularly, rapid evolution can occur (Pélissié et al., 2018). Admixture events between closely related insect species can result in increased gene flow and an increased rate of genetic admixture between populations (Anderson et al., 2018; North et al., 2024). In some cases, insect admixture events have the potential to distribute advantageous alleles into new populations (Ma et al., 2024; North et al., 2024). For example, in African mosquitos, the introgression of a *kdr* gene conferring resistance to pyrethroid insecticides has been reported between closely related

species (Weill et al., 2000). In this example, a *kdr* mutation in one sibling taxa of *Anopheles gambiae* has moved to other sibling taxa via introgression (Weill et al., 2000; Abdoulaye et al., 2003). Introgression is the process by which novel genes become introduced into new gene pools (Aguillon et al., 2022). There are numerous examples of introgression between closely related insects including subspecies of honeybees (Pinto et al., 2005), species of *Heliconius* butterflies (Pardo-Diaz et al., 2012), and many species of *Drosophila* (Suvorov et al., 2022).

The frequency or rate of admixture events is directly correlated with the rate of successful allele introgression into new populations (van Boheemen et al., 2017). Many times, admixture events fail due to genetic differences between species, with successful mating events sometimes producing offspring of lesser fitness (Rios et al., 2022). However, the rate of admixture events is also dependent on a variety of factors, including climactic limitations and prezygotic/postzygotic mating barriers, to provide some examples (Emelianov et al., 2004; Mallet, 2018; Ma et al., 2024). If breeding populations are separated by some physical distance or barrier, the potential of successful admixture between populations is relatively low. Prezygotic barriers to mating like allopatry strongly reduce the probability of gene flow among populations (Mallet, 2018). This explains why there are often increased rates of admixture when two formerly allopatric populations become sympatric (Emelianov et al. 2004).

The potential of admixture has been studied between *H. armigera* and *H. zea* in previous studies. Laster & Sheng was the first study to document the potential for mating between these two species of *Helicoverpa* (Laster & Sheng, 1995). In their study, they attempted to determine if mating would cause male sterility at later generations, like other *Helicoverpa* study systems (Proshold, 1983; Laster & Sheng, 1995). When mating *H. zea* and *H. armigera*, and observing numerous generational backcrosses, they found no evidence of sterility due to admixture and

subsequent introgression (Laster & Sheng, 1995). They found that while early backcrosses and admixture events were somewhat difficult to obtain, there was no resulting evidence that long-term sterility was impacted. This suggests that in admixture events in wild populations, numerous mating events are required to ensure initial success in introgression. However, once that success in introgression has occurred, there is no postzygotic barrier blocking reproduction afterwards.

North et al. (2024) confirmed that the admixture between *H. zea* and *H. armigera* was occurring within the United States. They reported strong selection around the *CYP337B3* locus in the admixed *H. zea* samples collected. Of the 237 samples tested, only 2 individuals from Texas were positively identified as being admixed (North et al., 2024). This was the first major study to report introgression into the United States. Admixed *H. zea* are now established in various parts of North America, including Colorado (Nufer et al., 2024; USDA unpublished data). This is of importance when considering that *H. zea* in the United States had already gained resistance to Bt toxins used to control their populations. With the addition of the *CYP337B3* allele into their genome, these pests soon may have high levels of resistance to both Bt crops and pyrethroid insecticides, two main control methods used to reduce *H. zea* population sizes.

The goals of my thesis were to test 1) increases in insecticide resistance in admixed *H. zea* (*H. zea* x *H. armigera* backcrossed multiple times to *H. zea*) to non-admixed *H. zea*; 2) changes in the overwintering ability in admixed *H. zea* compared to non-admixed *H. zea* using supercooling point as a proxy; and 3) differences in attraction to the *H. zea* pheromone between admixed and non-admixed *H.* zea.

CHAPTER TWO: THE IMPACTS OF INTROGRESSION FROM *HELICOVERPA ARMIGERA* ON THE INSECTICIDE SUSCEPTIBILITY OF *HELICOVERPA ZEA*

4. Background on Cytochrome P450 Genes

Cytochrome P450 (CYP450) genes are known to perform a variety of essential biological functions within living organisms, such as the detoxification of harmful compounds (Scott, 1999). Cytochrome P450 genes are shown to directly impact the ability of *Tribolium castaneum* to adapt to new environments (Zhu et al., 2013). All cytochrome P450 genes currently present in genomes across Animalia evolved from one single common ancestor millions of years ago (Feyereisen, 2011; Nauen et al., 2022).

The number of CYP450 genes in any given insect varies across different species (Consortium, 1998; Zhu et al., 2013). In *H. zea*, only 9 CYP450 genes have been experimentally confirmed, while the best estimates based on sequence data predict approximately 100 CYP450 genes (Li et al., 2002; Gouin et al., 2017; Zhang et al., 2024). All currently known CYP genes are designated within four clans: *CYP2, CYP3, CYP4*, and the mitochondrial *CYP* clan (Feyereisen, 2011). The broad variety of CYP450 genes in any given lineage is sometimes collectively referred under the term CYPome (Nauen et al., 2022). Variation in the makeup of CYPomes does exist, as they can be composed of many gene families with few, single-copy allele variations, or be composed of a few gene families with abundant allele variation (Nauen et al., 2022). In every variation of a CYPome, there is at least one lineage of a CYP450 gene subfamily that appears abundant and over-expressed compared to others (Feyereisen, 2011). This abundance is referred to as a bloom, where gene duplication creates large clusters of CYP genes on chromosomes (Feyereisen, 2011; Nauen et al., 2022).

The CYP450 monooxygenases found within insect cells metabolize a variety of chemical compounds, from juvenile hormones, molting hormones, to the processing of toxic host plant chemicals (Bergé et al., 1998). In some insects, these monooxygenases can metabolize a chemical compound prior to the compound reaching its target (Bergé et al., 1998). In *Helicoverpa armigera*, CYP450 genes of the *CYP6AE* subfamily are known to be induced by insecticides and plant allelochemicals (Shi et al., 2018). Many examples exist demonstrating the importance of CYP450 enzymes in insect pesticide metabolism (Vandenhole et al., 2021; Vontas et al., 2020). In the event of mutation at specific loci, the effectiveness of a CYP450 gene can be altered (Feyereisen, 2011). In cases where mutation at the loci confers real fitness advantages, these CYP450 genes can sometimes become over expressed, increasing the toxic threshold of a pest (Feyereisen et al., 2011; Ye et al., 2022).

5. The Potential Impact of CYP337B3 on Helicoverpa zea

Real fitness advantages when describing CYP450s can be defined as those advantages that increase the catabolic activity of the enzyme (Scott, 1999; Nauen et al., 2022). For example, *CYP337* is a cytochrome P450 gene located on chromosome 15 in *H. armigera* and *H. zea* (Joußen et al., 2012; Anderson et al., 2018; Valencia-Montoya et al., 2020; North et al., 2024). While *CYP337* is known to regulate insecticide resistance in *H. armigera* and *H. zea*, the mutated *CYP337B3* allele confers a greatly increased resistance to insecticides compared to its non-mutated counterpart (Joußen et al., 2012). As we continue to quantify the potential effects of introgression in *H. zea*, it is important to document the effect of the *CYP337B3* allele on insecticide resistance in this serious pest.

If admixed *H. zea* continue to increase in frequency in the United States, it could have serious economic consequences for U.S. agriculture. If admixed *H. zea* individuals with the

CYP337B3 allele are more resistant to pyrethroid insecticides, farmers who utilize pyrethroid insecticides will suffer greater economic losses from this pest as seen on the Western Slope of Colorado in 2023 (Nufer et al., 2024). To determine if admixed *H. zea* caterpillars are more resistant to pyrethroid insecticides than their non-admixed counterparts, I conducted an experiment to monitor survival of *H. zea* caterpillars with and without the *CYP337B3* allele after treatment with pyrethroid insecticides. I conducted a second experiment to see if an insecticide synergist could override this resistance. Piperonyl butoxide (PBO) is an insecticide synergist that inhibits CYP enzyme activity (Jones, 1998). Piperonyl butoxide forms stable bonds with CYP enzymes, leading to a depletion of active CYPs (Adams et al., 1993; Jones, 1998).

6. Methods

Helicoverpa zea were reared from egg to adult in the laboratory. One colony was reared from eggs purchased from Benzon Research (Carlilse, PA). This colony was known to be susceptible to pyrethroid insecticides (e.g., Elkins et al., 2025). Two other colonies originated from caterpillars collected from sweetcorn on the Western Slope of Colorado (Montrose County, CO) in June 2024 and adult moths collected in June 2025 from the Colorado State University ARDEC research center on the Front Range of Colorado (Larimer County, CO). *Helicoverpa zea* from these two locations were tested for the B3 allele at the nearby USDA APHIS lab in Fort Collins (see methods below) and these field-collected individuals were used to start and supplement colonies, accordingly, of native *H. zea* with and without the B3 allele.

Caterpillars were kept in small plastic cups with a Multi-Species diet (Benzon Research, Carlisle, PA) through six instars of larval development. Diet was swapped twice, once at instar three, and once at instar five. Pupae were placed into clean plastic cups in an emergence chamber. The emergence chambers consisted of small plastic tents (2’x1’x4’) with humidifiers

and lights set to 25 ± 2°C, 14:10 L/D, 55% relative humidity. Pupal sex was recorded. Pupae were monitored over the course of two weeks until adult emergence. Adults were collected and placed into mating chambers in pairs. Mating chambers consisted of a clear 16oz plastic cup with cheesecloth bound to the upper rim of each cup with a rubber band. Inside each mating chamber was a sponge soaked in a honey-water artificial liquid diet (30% honey), and these sponges were swapped every two days. Adults were kept in mating chambers until viable egg production occurred. Adults were then frozen and subsequently sequenced via Real Time PCR for the presence of the B3 allele. Eggs were harvested and placed in marked plastic bags and new caterpillars were collected upon emergence.

When determining the presence of the B3 allele in individual moths or frozen *H. zea* pupae, my collaborators at the nearby USDA APHIS lab used Real Time PCR analysis (Frida Zink, Luke Tembrock). My collaborators at the local USDA APHIS lab only used Droplet Digital PCR (ddPCR) when grouping mass leg samples together, which typically only occurred when determining admixture from field caught samples (Zink et al., 2017).

My first experiment was a 2 x 3 x 6 completely randomized factorial design with two types of pyrethroids (λ-Cyhalothrin and Warrior II (microencapsulated λ-Cyhalothrin), three genotypes of caterpillars (Benzon, B3-, and B3+), and six insecticide concentrations (0, 0.0125, 0.125, 1.25, 2.5, and 5µg/µl). Microencapsulation has reduced pesticide phytotoxicity (Greene et al., 1992), improved heat and chemical stability (Demchak & Dybas, 1997), improved safety to handlers (Riley 1983), and when used in conjunction with PBO has been shown to restore pyrethroid efficacy in resistant insects (Bingham et al., 2008). I used third instar caterpillars from our three colonies (Benzon, B3-, and B3+) to control for background levels of pyrethroid resistance in North American *H. zea* (Benzon versus B3-) and to separate the effects of the B3

allele acquired via introgression versus previous levels of pyrethroid resistance in North American *H. zea* (B3- versus B3+). I scored mortality at five time points: 1, 4, 12, 24, and 32 hours. In addition, four live caterpillars (if available) were flash frozen four hours post treatment for *CYP337B3* expression analysis (to be conducted by USDA collaborators and data not included in this study). The experiment was replicated 32 times (1,152 caterpillars). Third-instar caterpillars were placed into individual wells in 128-cell bioassay trays. 1µl of each insecticide treatment was pipetted directly to the dorsal surface of each caterpillar utilizing a P10 micropipette. Mortality was determined by observing caterpillar response to stimulation with feather-tipped forceps. Moribund caterpillars were considered dead.

To test the hypothesis that PBO restores susceptibility of B3+ caterpillars to pyrethroid insecticides, I tested the effect of mixing PBO with λ-Cyhalothrin and Warrior II (microencapsulated λ-Cyhalothrin) on the survival of Benzon, B3-, and B3+ caterpillars in a second experiment. The four insecticide treatments were λ-Cyhalothrin, λ-Cyhalothrin mixed with PBO, Warrior II, and Warrior II mixed with PBO. The PBO was a 10% solution of 8-Exponent PBO (MGK, Minneapolis MN). The same concentrations of insecticides were used for this experiment, insecticides were applied in the same manner, and survival was observed at the same timeframes.

I used a Generalized Linear Mixed Model (GLMM) to analyze the effects of caterpillar genotype (B3+, B3-, and Benzon), insecticides (unencapsulated and encapsulated λ-Cyhalothrin\), insecticide concentration, and time after exposure on caterpillar survival (binomial dependent variable scored as alive or dead) using the lme4 package of R (Bates et al., 2015). I calculated the Resistance Ratio (RR) between genotypes of caterpillars (RR = LC_{50} of the resistant genotype/LC_{50} of the susceptible genotype). I calculated the LC_{50} of pyrethroids for

each genotype of *H. zea* with the drc (Analysis of Dose-Response Curves) package of R (Ritz et al., 2015). LC_{50}'s were calculated using mortality of caterpillars 32 hours post treatment. All statistical analyses were done in R studio.

7. Results

I found evidence that the B3 allele increased the resistance of *H. zea* to λ-Cyhalothrin, especially at lower concentrations, but overall, the increase in resistance was low. For example, the survival of caterpillars with the B3 allele one hour after treatment with a 0.0125 concentration of λ-Cyhalothrin (91%) was significantly higher than caterpillars without the B3 allele treated at the same conditions (69%) ($\chi^2 = 4.73$, $p < 0.03$) (Figure 1). The Resistance Ratio of B3+ to B3- caterpillars for λ-Cyhalothrin was 7.86, indicating a 7.86-fold increase in resistance due to the B3 allele. There was evidence that B3- caterpillars were more resistant to λ-Cyhalothrin than the susceptible Benzon strain. Survival of B3- caterpillars one hour after treatment with a 0.0125 concentration of λ-Cyhalothrin (69%) was dramatically higher than similarly treated Benzon caterpillars (6%) ($\chi^2 = 26.67$, $p < 0.0001$) (Figure 1). Mortality of Benzon caterpillars was so high at the lowest concentrations of λ-Cyhalothrin that I could not calculate an LC_{50} for this genotype of *H. zea* and therefore could not calculate a Resistance Ratio comparing admixed and Benzon genotypes with my own colony. To do this, I compared published data on the Resistance Ratio of Benzon caterpillars compared to non-admixed *H. zea* and used this data to compare admixed the resistance of admixed *H. zea* compared to susceptible Benzon *H. zea* in my colony (Patla et al., 2026). Upon comparing, admixed *H. zea* caterpillars were 76.5-fold more resistant to pyrethroids compared to susceptible Benzon caterpillars.

Similar results were found with Warrior II, the encapsulated pyrethroid. The survival of caterpillars with the B3 allele one hour after treatment with a 0.0125 concentration of Warrior II (91%) was significantly higher than similarly treated caterpillars without the B3 allele (22%) ($\chi^2 = 30.72$, $p < 0.00001$) (Figure 2). The Resistance Ratio, however, of B3+ to B3- caterpillars for Warrior II was only 1.94. Mortality of Benzon caterpillars was very high at the lowest concentrations of Warrior II. I could not calculate an LC_{50} for this colony and therefore could not calculate a Resistance Ratio comparing Benzon caterpillars in my own colony.

Survival of caterpillars treated with PBO mixed with λ-Cyhalothrin was often lower than the survival of caterpillars treated with λ-Cyhalothrin without PBO (Figure 3), but these differences in survival were not statistically significant. For example, the survival of B3- caterpillars treated with λ-Cyhalothrin without PBO (69%) one hour after treatment was not significantly higher than the survival of B3- caterpillars treated with λ-Cyhalothrin with PBO (50%) (($\chi^2 = 2.32$, $p = 0.13$). Likewise, the survival of B3+ caterpillars treated with λ-Cyhalothrin without PBO (91%) one hour after treatment was not significantly higher than the survival of B3+ caterpillars treated with λ-Cyhalothrin with PBO (72%) ($\chi^2 = 3.70$, $p = 0.054$). I found similar non-significant results 32 hours post treatment. Survival of B3- caterpillars treated with λ-Cyhalothrin (36%) was not significantly higher than B3-caterpillars treated with λ-Cyhalothrin with PBO (25%) ($\chi^2 = 0.76$, $p = 0.38$). Similar results were found for B3+ caterpillars 32 hours post treatment (75% versus 64%; $\chi^2 = 0.76$, $p = 0.38$).

The survival of B3- caterpillars treated with Warrior II without PBO one hour after treatment (22%) was surprisingly lower than the survival of B3- caterpillars treated with Warrior II with PBO (47%) ($\chi^2 = 4.42$, $p = 0.035$) (Figure 4). At 32 hours post treatment: 0% survival of B3- caterpillars treated with Warrior II without PBO versus 32% survival of B3- caterpillars

treated with Warrior II with PBO ($\chi^2 = 10.72$, $p = 0.001$). The results for B3+ caterpillars were more consistent with my hypothesis. The survival of B3+ caterpillars one hour after treatment with Warrior II without PBO was (91%) versus the survival of B3+ caterpillars treated with Warrior II with PBO (66%) ($\chi^2 = 5.88$, $p = 0.015$). Survival differences at 32 hours post treatment were not significant, however (68% versus 46%; $\chi^2 = 2.62$, $p = 0.11$).

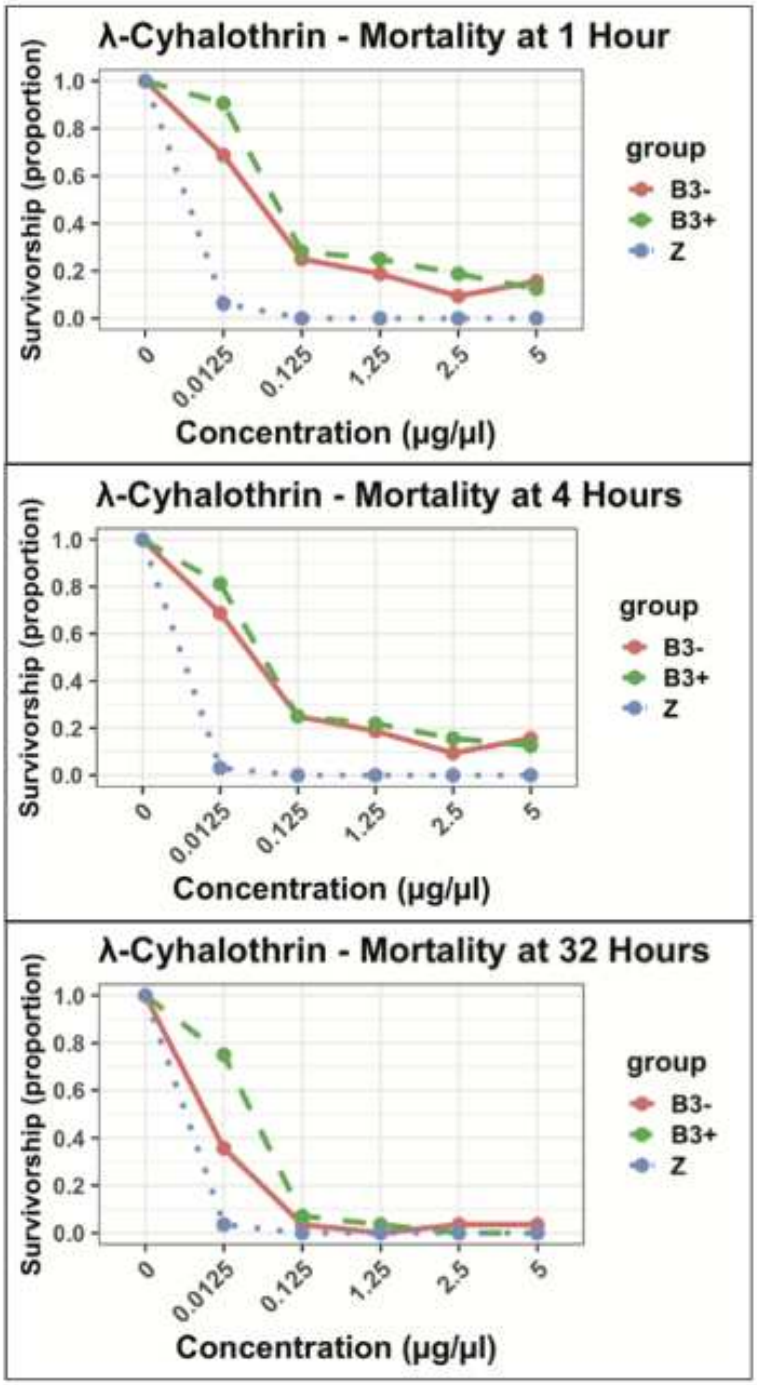

Figure 1. Proportion of survival in *H. zea* larvae (B3+, B3-, Z) at six different concentrations (0, 0.0125, 0.125, 1.25, 2.5, and 5µg/µl) of λ-Cyhalothrin across three separate timeframes post treatment (1, 4, 32 hours).

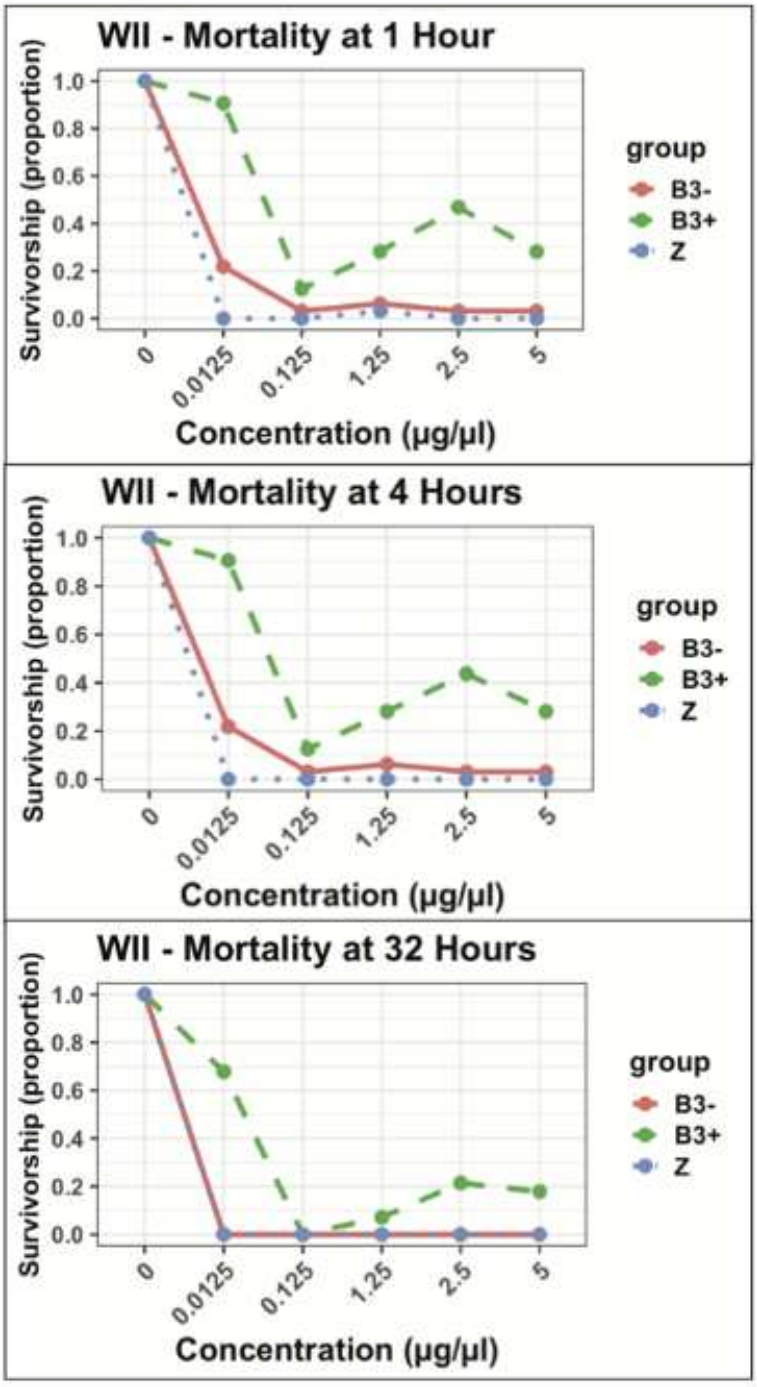

Figure 2. Proportion of survival in *H. zea* larvae (B3+, B3-, Z) at six different concentrations (0, 0.0125, 0.125, 1.25, 2.5, and 5µg/µl) of Warrior II across three separate timeframes post treatment (1, 4, 32 hours).

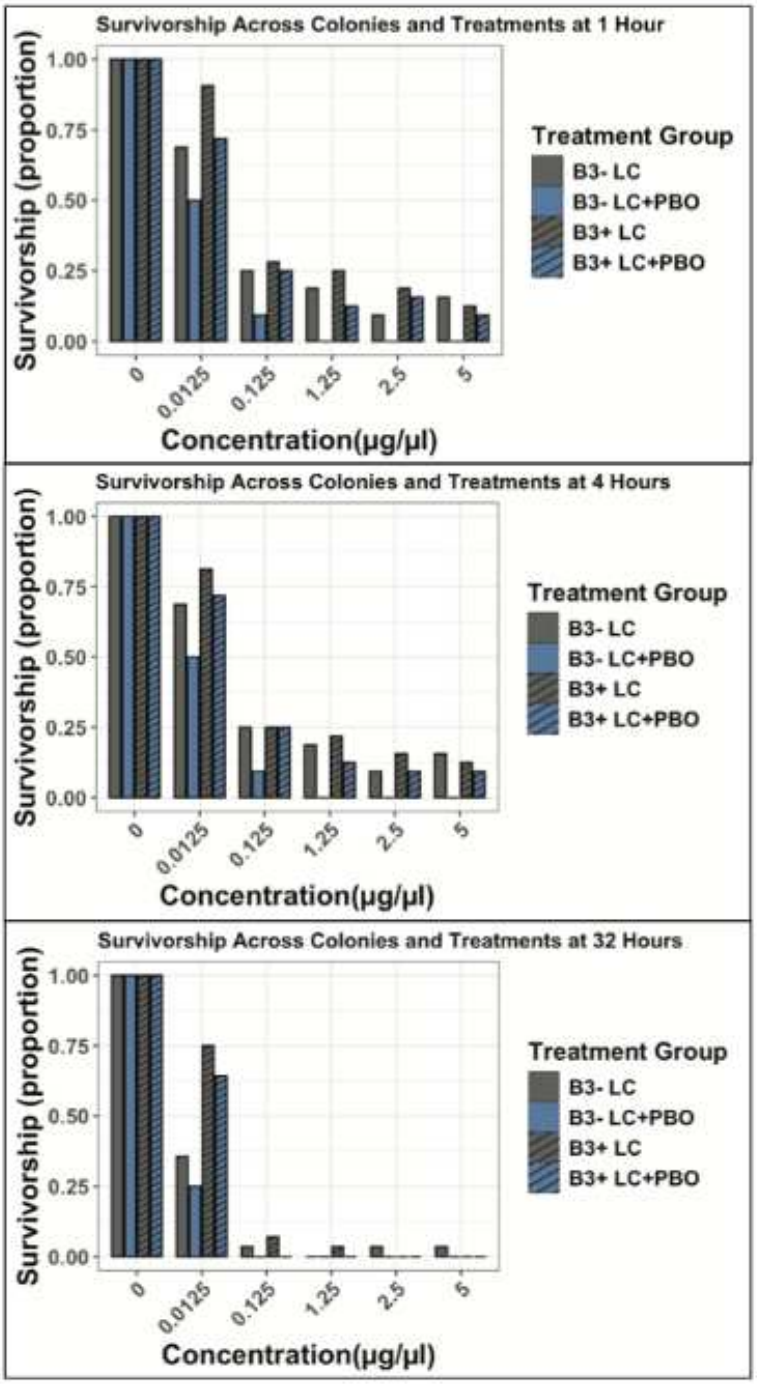

Figure 3. Proportion of survival in *H. zea* larvae (B3+, B3-) at six different concentrations (0, 0.0125, 0.125, 1.25, 2.5, and 5µg/µl) of λ-Cyhalothrin and λ-Cyhalothrin with PBO synergist across three separate timeframes post treatment (1, 4, 32 hours).

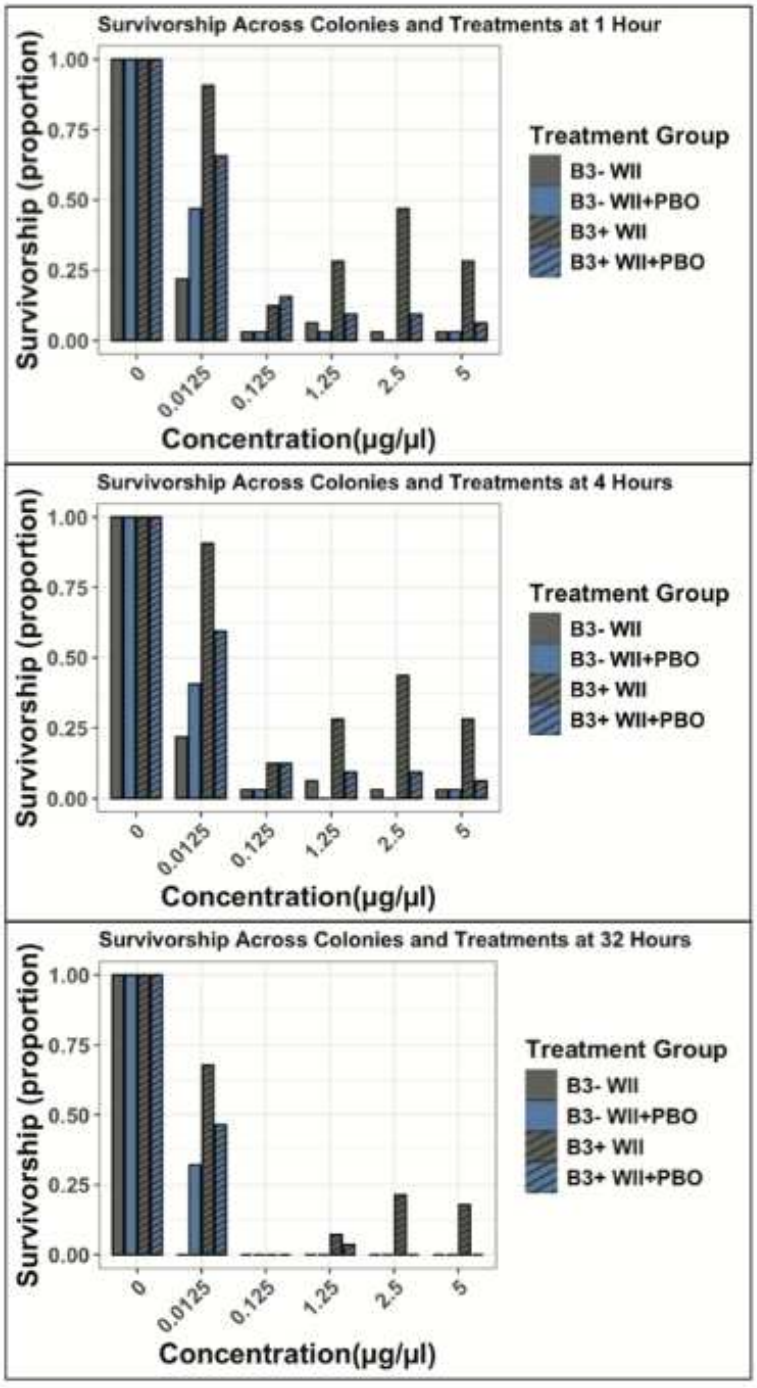

Figure 4. Proportion of survival in *H. zea* larvae (B3+, B3-) at six different concentrations (0, 0.0125, 0.125, 1.25, 2.5, and 5µg/µl) of Warrior II and Warrior II with PBO synergist across three separate timeframes post treatment (1, 4, 32 hours).

8. Discussion

While there have been studies documenting pyrethroid insecticide resistance in *H. armigera* with the B3 allele (Joußen et al., 2012; Anderson et al., 2018), this was the first study to document the effect of the B3 allele on insecticide resistance in admixed *H. zea*. Results show that *H. zea* with the B3 allele had 7.86-fold greater resistance to λ-Cyhalothrin compared to *H. zea* without the B3 allele. The magnitude of this resistance was far lower than what has been found associated with the B3 allele in *H. armigera*. For example, Joußen et al. (2012) found that *H. armigera* with the B3 allele had 42-fold resistance to fenvalerate than *H. armigera* without the B3 allele. It is important to note that studies like Joußen et al. (2012) compared the resistance of resistant genotypes of *H. armigera* to very susceptible genotypes. I compared the resistance of the descendants of wild-caught *H. zea* with the B3 allele to the resistance of the descendants of wild-caught *H. zea* without the B3 allele. Non-admixed *H. zea* in North America already have relatively high levels of pyrethroid resistance (up to 50-fold to λ-Cyhalothrin) (Patla, 2024). When comparing my results for admixed *H. zea* with the results for susceptible *H. zea* found in previous studies (Patla et al., 2026), I found admixed *H. zea* to be 76.5-fold more resistant to pyrethroids. This number suggested that admixed *H. zea* are far more resistant to pyrethroids than their susceptible counterparts. In my study, there was very high mortality of the Benzon colony even at the lowest concentration of λ-Cyhalothrin, so future studies would need to use much lower concentrations of λ-Cyhalothrin to generate an LC_{50} estimate for this susceptible colony.

The B3 allele also increased the resistance of *H. zea* caterpillars to Warrior II, but the magnitude of this effect (1.94-fold resistance compared to *H. zea* without the B3 allele) was smaller than for λ-Cyhalothrin (7.86-fold). This result, however, was consistent with recent

studies of *H. zea* resistance to Warrior II. Patla (2024) found that *H. zea* populations in the southeastern United States were, on average, 5.3-fold resistant to Warrior II versus <50-fold resistant to λ-Cyhalothrin.

Comparing insecticide applications in laboratory studies of resistance to real-world applications of insecticides was challenging. The label rate for both λ-Cyhalothrin and Warrior II insecticides are between .25-.35µg/µl, roughly corresponding to the 0.125µg/µl and 1.25µg/µl concentrations used in my study (US EPA, 2014; US EPA, 2024). When focusing on survivorship in this range, admixed larvae exposed to Warrior II insecticides had a 3.6-15.8% greater chance of survival compared to non-admixed larvae. Similarly, admixed larvae subjected to λ-Cyhalothrin insecticides in this dosage range had a 2.8-5.6% greater chance of survival compared to non-admixed larvae.

When discussing how the *CYP337B3* allele functions in *H. zea*, it is important to note how much resistance it confers in its origin species, *H. armigera*. There are numerous studies documenting the resistance benefits of the *CYP337B3* allele against pyrethroids in *H. armigera*. Durigan et al. (2017) found that, even at the highest dosages (10µg/µl), less than 50% mortality occurred in 3rd instar *H. armigera* larvae subjected to pyrethroid insecticides. This study utilized different pyrethroids such as fenvalerate and deltamethrin and observed mortality after 48 hours. When these results compare to my results, at the latest time period (32 hours post inoculation) I documented 100% mortality at 0.0125µg/µl treatments and 93% mortality at 0.125µg/µl treatments of Warrior II. With λ-Cyhalothrin trials, I documented 93% mortality in 0.0125µg/µl treatments and 96% mortality at 0.125µg/µl treatments at 32 hours.

I wanted to see if the addition of a 10% 8-Exponent PBO synergist would potentially override the capability of the *CYP337B3* allele to detoxify pyrethroid insecticides (Hadaway et

al., 1963). Mixing PBO with λ-Cyhalothrin, however, did not significantly increase the lethality of λ-Cyhalothrin. Likewise, mixing PBO with Warrior II had modest effects on the lethality of Warrior II. These results were somewhat surprising since PBO had been shown to increase the efficacy of a number of pyrethroids used against a variety of insect pests (e.g., Syme et al., 2022). It is interesting to note that a study of the effects of PBO on the resistance of H. armigera to fenvalerate found only partial inhibition of resistance (Gunning et al., 1999). It also interesting to note that some insects have recently evolved resistance to PBO (Zhou et al., 2022). My data suggests that mixing PBO with pyrethroid insecticides is unlikely to recover enough efficacy to warrant its use.

CHAPTER THREE: THE EFFECTS OF INTROGRESSION FROM *HELICOVERPA ARMIGERA* ON THE COLD TOLERANCE THRESHOLD OF *HELICOVERPA ZEA*

Some important aspects of biology to monitor in admixed *H. zea* are traits that influence the potential for geographic range expansion. Some traits involved in geographic range expansion include wing size, cold tolerance, and migratory fitness (Verhoeven et al., 2011). There are significant differences between the geographic ranges of *H. armigera* and *H. zea*. The furthest extent of *H. armigera*'s geographic range in the summer is 59°N latitude (Macleod, 2007) compared to *H. zea's* northern limit during summer of 52°N latitude (Hardwick, 1965; Lawton et al., 2022). In North America, that would roughly equal a 500-mile range expansion. Similar to *H. zea*, *H. armigera* is highly migratory, with adults able to travel 40km in a night and 1000km over a lifetime (Feng et al., 2004; Jones et al., 2015). In any highly migratory insect species, a key fitness benefit linked to survival is the ability to adapt to differing cold tolerance thresholds and temperature gradients (Andersen et al., 2015). It is known that *H. armigera* is more well suited for tropical and sub-tropical climates whereas *H. zea* is often more suited for temperate climates (Kriticos et al., 2015; EFSA Panel, 2020). For example, Liang et al. (2007) found that the super cooling point of non-diapausing pupae of *H. armigera* was -18.1°C whereas Morey et al. (2012) found that the super cooling point of non-diapausing pupae of *H. zea* was -16.4°C. If introgression in *H. zea* increases the cold tolerance of this pest, then that would result in *H. zea* overwintering farther north which could result in earlier colonization of crops during the growing season and ultimately larger pest populations (Lawton et al., 2022).

Cold tolerance refers to the ability of an organism to sustain itself through periods of colder temperatures (Clark & Worland, 2008; Lee, 1991; Morey et al., 2012). There are many

factors that influence insect cold hardening, such as cryoprotectants, water content, and ice nucleators (Clark & Worland, 2008; Liu et al., 2007). Insects were initially divided into freeze susceptible and freeze tolerant specifications based on their supercooling points, which is the temperature that the internal hemolymph freezes (Bale, 1993; Clark & Worland, 2008; Morey et al., 2012; Lawton et al., 2022). In freeze tolerant insects, insects typically freeze at around –5°C to -10°C and can survive temperatures as low as -50°C (Bale, 1993; Zachariassen, 1985). After thawing, these freeze-tolerant insects can develop and reproduce normally (Bale, 1993). Some insects, however, die from chilling stress prior to total freezing of the hemolymph. This is referred to as chill susceptible (Bale, 1993; Clark & Worland, 2008).

Since insects are ectothermic, they depend on their environment to regulate their internal body temperature. In diapausing insects, the production of cryoprotectants such as trehalose can help aid in the resistance to cold temperatures near the super cooling point (Liu et al., 2007). Cryoprotectants in insects' function by increasing the osmolarity of biological solutions, which limits the potential for ice to form within the body (Meryman, 1971; Mazur, 1984). This is especially beneficial in freeze-intolerant insects, where the formation of ice within the body can be lethal (Bale, 1993). However, the production of cryoprotectants varies between insect species, with some species producing greater amounts of these compounds than others (Bale, 1993; Liu et al., 2007).

Studies have shown that the production of the cryoprotectant compound trehalose in insects can vary based on the insects' host plant. For instance, *H. armigera* feeding on corn produced significantly more trehalose compared to *H. armigera* feeding on other crops (Liu et al., 2007). Studies documenting the cold tolerance threshold of *H. armigera* suggest that the pest is freeze-intolerant, meaning the majority of deaths occur near the SCP (Liang et al., 2007;

Kurban et al., 2008). Compare this to *H. zea,* where the species was believed to be freeze-intolerant (Hardwick, 1965) but has since been redefined as chill-intolerant (Morey et al., 2012). Chill-intolerant insects often die at temperatures above the mean super cooling point of the insect, whereas freeze-intolerant insects often die at temperatures at the mean super cooling point (Lee, 2010; Morey et al., 2012).

In 2023, sweet corn farmers on the Western slope of Colorado reported significantly earlier arrival of *H. zea* in sweet corn fields (M. Schreiner, Personal Communication). There are varying explanations as to why this may happen. For instance, the increase in atmospheric temperatures over the past few decades has resulted in some geographic range expansion for *H. zea* (Lawton et al., 2022). By 2099, researchers expect the geographic range of *H. zea* to double (Lawton et al., 2022). However, 2023 is when the first admixed *H. zea* were documented in Colorado after there was large outbreak of *H. zea* in Colorado in 2023 (Nufer et al., 2024). If these admixed individuals gained increased cold tolerance resistance from *H. armigera*, then they may be able to overwinter at latitudes that normally would kill them.

If admixed *H. zea* gained fitness benefits associated with increased cold tolerance resistance, farmers in the Northern U.S. could see earlier arrivals of the pest, and as a result, larger populations of the pest later in the growing season. While *H. zea* is present in North and South America, the species is limited in its overwintering range in the United States.

The impact of introgression on climactic tolerance has been documented in several insect species (Galludo et al., 2018), such as *Thrips palmi,* a small Thysanopteran insect pest in China (Ma et al., 2024). In this example, admixed *T. palmi* had an increased geographic range when compared with their non-admixed conspecifics (Ma et al., 2024). Studies have long documented the impacts of introgression on increasing fitness in insects (Verhoeven et al., 2011). For

example, introgression can eliminate the impacts of inbreeding depression, potentially conferring a broad variety of fitness benefits. In some cases, introgression can impact fitness benefits relating to increased environmental tolerance (Verhoeven et al., 2011).

Super cooling point can be a reliable link between lab experiments and real-world scenarios when assessing an insect's freeze tolerance (Atapour & Moharramipour, 2009; Ditrich et al., 2018). In this study, I quantified the supercooling point of admixed and non-admixed *H. zea* to estimate the effects of introgression on the cold tolerance of this pest.

9. Methods

Helicoverpa zea were reared from egg to adult in the laboratory. One colony was reared from eggs purchased from Benzon Research (Carlilse, PA). This colony was known to be susceptible to pyrethroid insecticides (Elkins et al., 2025). One colony originated from caterpillars collected from sweetcorn on the Western Slope of Colorado (Montrose County, CO) in June 2024 and reared for 20 continuous generations in the laboratory. I believed this colony to have some insecticide resistance since multiple studies have identified U.S. *H. zea* populations as having some level of resistance (North et al., 2024). A third colony was collected from the Colorado State University ARDEC research center on the Front Range, where adult males were collected via bucket trapping. This third colony was confirmed to have the B3 allele present within its genome through genomic sequencing done at the nearby USDA APHIS lab in Fort Collins.

I had three colonies for this experiment. The 1st colony were descendants from moths from Colorado without the B3 allele, the 2nd colony were descendants of moths with the B3 allele, and the 3rdcolony is a Benzon group used as a control. Caterpillars were kept in small plastic cups with a Multi-Species diet (Benzon Research, Carlisle, PA) through six instars of

larval development. Diet was swapped twice, once at instar three, and once at instar five. Upon pupation, pupae were swapped into clean plastic cups and placed in an emergence chamber. The emergence chambers consisted of small plastic tents (2'x1'x4') with humidifiers and lights set to 25 ± 2° C, 14:10 L/D, 55% relative humidity. Pupal sex was recorded. Pupae were monitored over the course of two weeks past pupation until adult emergence. Adults were collected and placed into mating chambers in pairs. Mating chambers consisted of a clear 16oz plastic cup with cheesecloth bound to the upper rim of each cup with a rubber band. Inside each mating chamber was a sponge soaked in a honey-water artificial liquid diet (30% honey water), and these sponges were swapped every two days. Adults were kept in mating chambers inside emergence chambers until viable egg production occurred. Adults would be frozen then sequenced via Real Time PCR for the presence of the B3 allele. Collected eggs were then harvested and placed in marked plastic bags, to which new caterpillars were collected from upon emergence. Three separate colonies were tested; (1) Wild Admixed, (2) Wild Non-admixed, and (3) Benzon Susceptible.

When determining the presence of the B3 allele in individual moths or frozen *H. zea* pupae, my collaborators at the nearby USDA APHIS lab used Real Time PCR analysis (Zink et al., 2017).

Supercooling point (SCP) trials involved attaching subject *H. zea* individuals to thermocouples with high vacuum grease and placing them inside Styrofoam cubes in -80° freezers. Individual larvae or pupae were subjected to cooling at rates of around -0.5°C/min. Temperatures were recorded every second utilizing a thermocouple data logger (Liu et al., 2009). At a certain temperature prior to mortality, latent heat fusion was released within the pupae, marking an abrupt spike in the temperature reading on the thermocouple record. The lowest temperature recorded prior to this spike was the SCP (Mohammadzadeh & Izadi, 2018). Prior to

recording SCP values, pupal weight and sex were recorded for each individual pupa. Three colonies were tested: admixed, non-admixed, and Benzon (Carlilse, PA). Mortality was confirmed by the reaction of individual *H. zea* to touch and disturbance after trials (Morey et al., 2012).

SCP values were compared using a t-test of means. Mean calculations were made per tested colony and compared in Microsoft Excel. Standard deviations were calculated along with standard errors across each colony. SCP values were also compared between sexes of pupae.

Pupal weight values were compared using a t-test of means. Mean calculations were made per tested colony and compared in Microsoft Excel. Standard deviations and standard errors were calculated between colonies and sexes.

10. Results

Admixed pupae had an average SCP of –18.568°C, (SD = 1.32, SE = 0.152). Non-admixed pupae had an average SCP of –18.512°C, (SD = 1.35, SE = 0.089) (Figure 5). Upon completing a t-test to compare means, I found no statistical significance between the two values ($p = 0.754$).

To compare how pupal weight and SCP were related to each other between sexes, I plotted all recorded data points of the variables to determine if any relationships existed. There is an inverse relationship, whereas pupal weight decreases, the SCP of a pupae increases towards 0°C (Figure 6).

Male pupae had a lower mean SCP value than female pupae, and upon conducting a t-test, these results were deemed statistically significant ($p = 0.0064$, $t = 2.749$, 95% CI = -0.728, -0.121) (Figure 7).

There was no statistically significant difference in pupal weight between male and female pupae ($p = 0.7055$, $t = 0.2783$, 95%CI = -0.013, 0.009) (Figure 8). Strong variation was recorded in the pupal weights between both sexes.

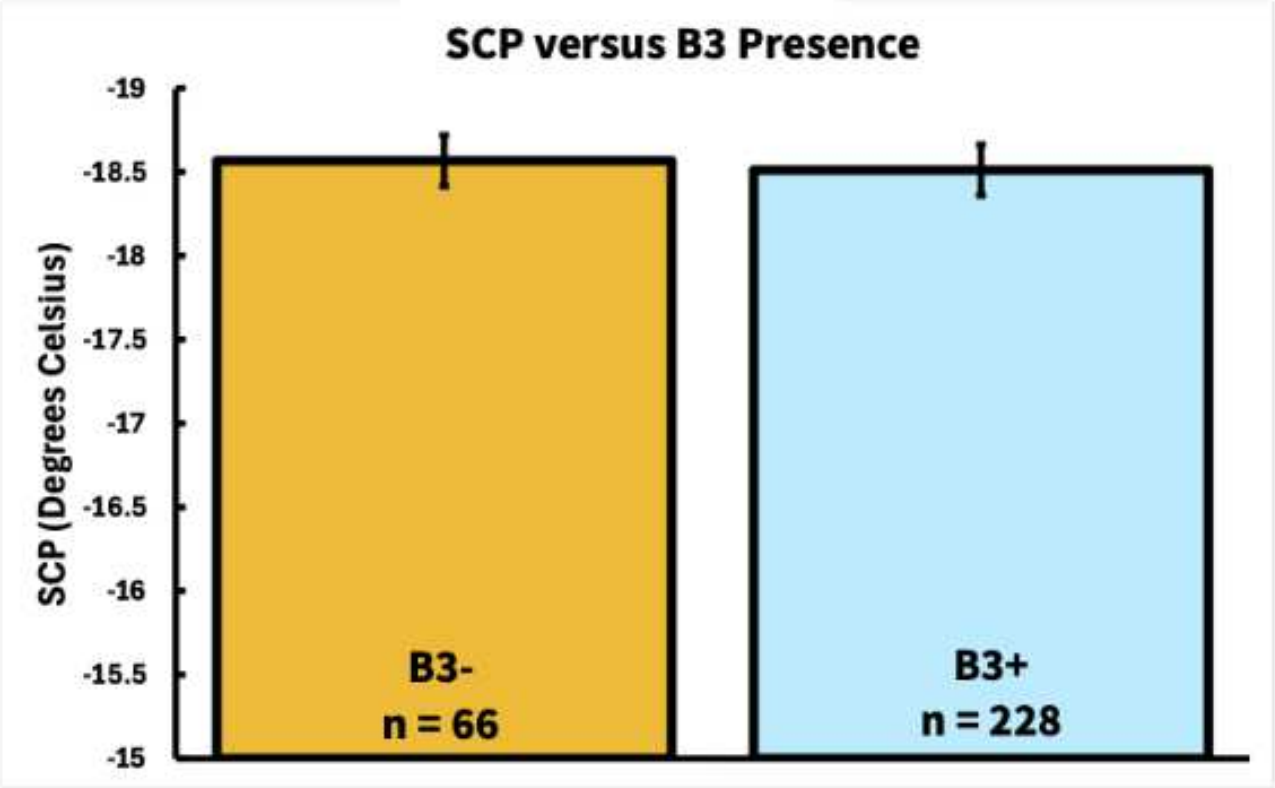

Figure 5. Average (±SE) SCP of non-diapausing of both admixed (B3+) and non-admixed (B3-) *H. zea* pupae by exposure to low temperatures ($p = 0.754$, $t = 0.314$, $n = 294$).

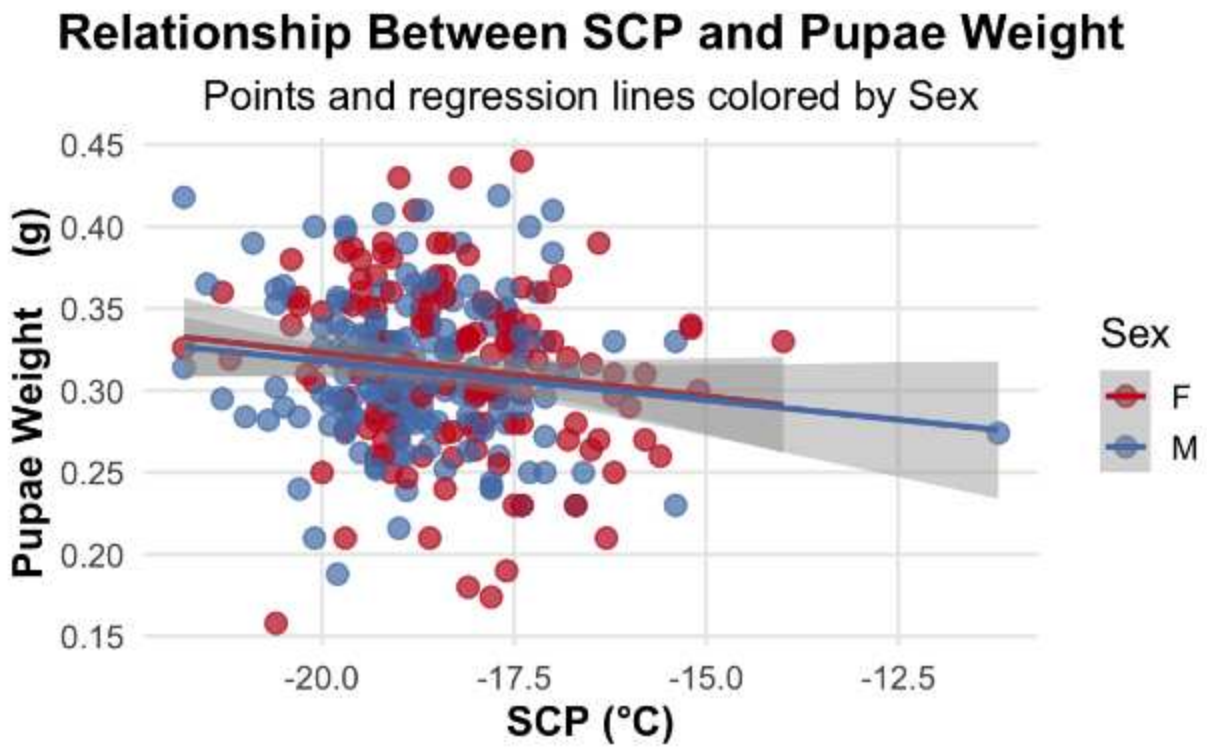

Figure 6. Regression between SCP (°C) and Pupae Weight (g) between non-diapausing male and female *H. zea* pupae.

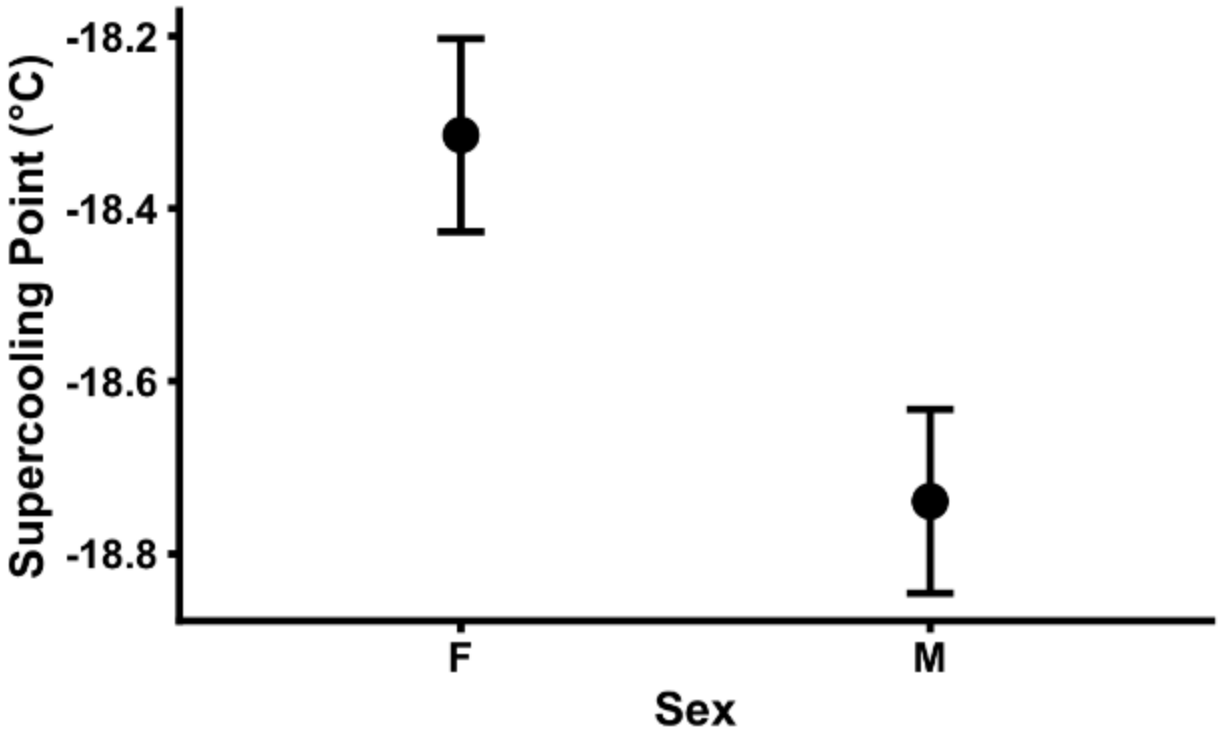

Figure 7. Mean SCP (±SE) between female and male *H. zea* pupae across all groups.

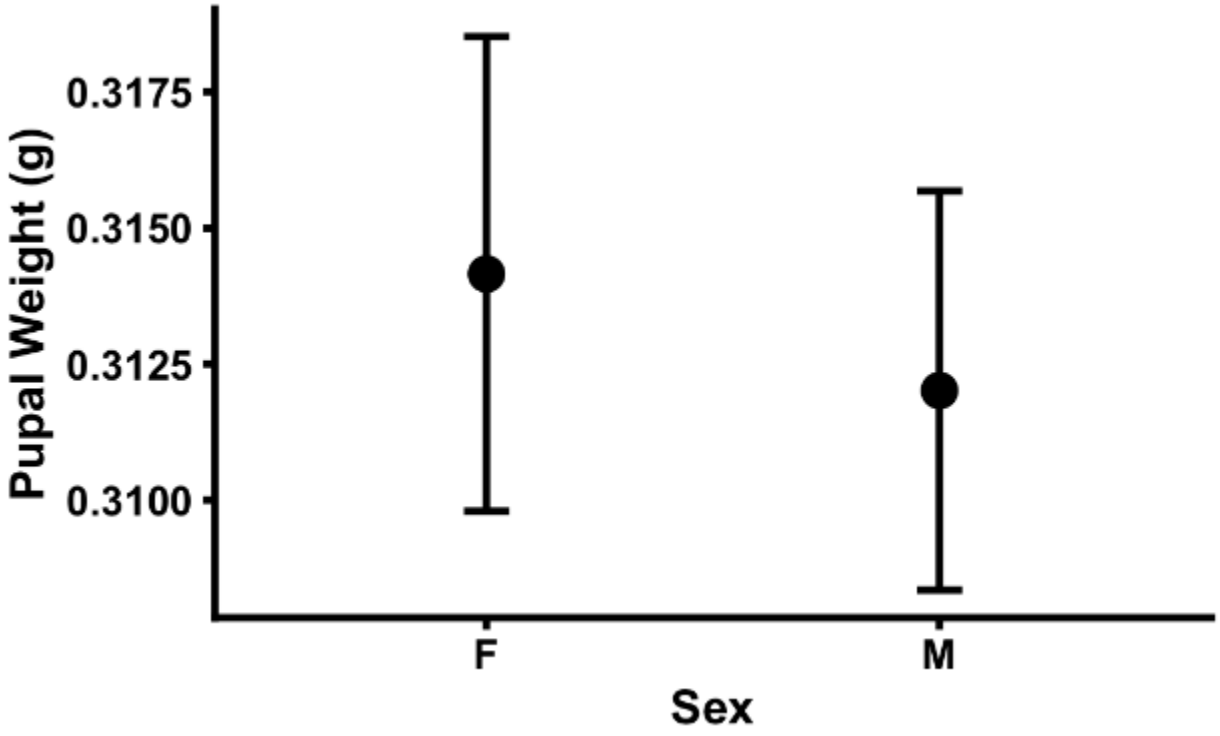

Figure 8. Mean pupal weight (±SE) between female and male *H. zea* across all groups.

11. Discussion

I found no statistically significant difference in SCP between admixed and non-admixed *H. zea*. The mean SCP (B3+ = -18.568°C, B3- = -18.512°C) for non-diapausing *H. zea* was also much lower compared to what other studies previously reported (-16.4°C) (Morey et al., 2012). My data suggests that non-diapausing *H. zea* had a lower mean SCP than previous studies report, and this could impact early spring populations that experience temporary freezes. If populations in these timeframes are pupating in soil without diapausing, they may be able to sustain colder temperatures prior to freezing than previously believed. If this genotype began to spread to other parts of the globe, not only would they be more resistant to pyrethroids, but they also would be able to survive colder temperatures than previously reported. Farmers may need to readjust IPM strategies for *H. zea* on the basis that admixture is creating more cold tolerant individuals. In my study, male *H. zea* had lower SCPs compared to female *H. zea*, which is inconsistent with what previous studies have reported (Morey et al., 2012). Since I determined that an increased pupal weight was correlated to a lower SCP, I examined to see if the difference between sexes was due to pupal weight. However, I found no significant evidence that a difference in pupal weight occurred in my colony. One explanation for this may be found in how my colony was bred. Since my colony was randomly selected for mating, often fitness benefits that may benefit wild organisms were not intentionally selected for, such as size of adults. Sexual dimorphism has been documented in wild *H. zea,* consistent with the general pattern of female-biased size dimorphism in insects (Mikac et al., 2026).

If admixed *H. zea* do have cold tolerance resistance benefits gained from *H. armigera*, they may be able to survive lower temperatures around their SCP. Proper genome sequencing on the synthesis of cryoprotectants such as trehalose in admixed *H. zea* has yet to be done, and so a

baseline for comparison cannot be determined at this time. Future studies should examine the differences between trehalose synthesis in non-admixed *H. zea* and admixed *H. zea* when exposed to temperatures around their mean SCP to determine if admixed *H. zea* more closely resemble *H. armigera* in response to colder temperatures.

CHAPTER FOUR: IMPACTS OF INTROGRESSION FROM *HELICOVERPA ARMIGERA* ON THE ATTRACTIVENESS OF COMMERCIAL PHEROMONE LURE TYPES TOWARDS *HELICOVERPA ZEA*

12. Helicoverpa zea and Its Impact on Sweet Corn Agriculture

A major pest to sweet corn, *H. zea* females are attracted to ethylene volatile plant pheromones released during the silking stage of corn development (Olmstead et al., 2016; Raina et al., 1992). The ethylene compound produced by silking sweet corn stimulate the production of sex pheromones in *H. zea* females, which thus attract male *H. zea* (Raina et al., 1991). After mating, female *H. zea* lay eggs on the silks of corn ears, and after about two to three days, these eggs hatch into larval *H. zea* (Olmstead et al., 2016). It is paramount that larval *H. zea* be sprayed prior to moving downward into the corn husk, otherwise they can damage corn ears at a level where they cannot be sold to market (Hardwick, 1965). For consumers, the ideal sweet corn product has no damage to the ear, and if damage has occurred, it must be on the most minute scale.

Due to the high risk associated with *H. zea* outbreaks on the profit margin of sweet corn, accurate monitoring of the pest must be routinely conducted. One main method, being pheromone lure trapping, is routinely conducted in areas known to be impacted by *H. zea* (Rivera et al., 2021; Ivey & Hillier, 2023). Pheromone lure traps targeting *H. zea* function by mimicking the sex pheromone compounds produced by female *H. zea*, thus luring male *H. zea* into the traps. Each trap has a lure positioned within it, and once male *H. zea* enter the trap, a lethal agent is present within the trap to ensure no moths escape sampling. There are a number of

trap types used when sampling *H. zea*, such as Hartstack, Heliothis, and Bucket traps (Mahas et al., 2025).

Hartstack traps are designed as a wire funnel, where moths enter through a large opening in the bottom and are filtered to the top of the apparatus, becoming trapped (Hartstack et al., 1979). Hartstack traps are typically very large and require metal poles to support the weight of the apparatus itself, making them difficult to move around with ease (Kehat et al., 1991). Heliothis traps function similarly, where moths are funneled upwards and trapped (Kehat et al., 1991). Heliothis traps are made of mesh, making this trap lighter and easier to transport within field sites. However, the mesh structure makes the traps require high maintenance, particularly in windy areas (Guerrero et al., 2014). Bucket traps are made of hard plastic, and function by luring moths down into the bowl of the trap, in which moths cannot escape from. Bucket traps are light, cost efficient, and effective at sampling *H. zea* populations, and have been used regularly to do so (Kehat et al., 1991; Rivera et al., 2021; Ivey & Hillier, 2023).

While some studies state that Heliothis traps prove to be the most effective means of capturing moth pests, other studies sampling *H. zea* had high success rates when Bucket trapping (Rivera et al., 2021; Guerrero et al., 2014). In routine monitoring of fields during a growing season, traps cannot be placed and left throughout the entire duration of the growing season. As farmers harvest crops, these traps must be moved to other unharvested corn fields to continue to effectively sample the population of pests. Ideally, when sampling sweet corn pests, traps are placed in fields where the corn crop has just begun silking, attracting a larger sample compared to a corn field with no attractant volatiles.

I decided to utilize Bucket traps due to their efficiency in capturing *H. zea* and the ability to move the traps between sites with relative ease. There are a variety of companies that produce

specific pheromone lures, and there are also a variety of colors of traps available. Previous studies have found that differences in attraction occur between lure types and trap colors (Mahas et al., 2015). In this example, researchers found that green bucket traps have very low efficacy in capturing *H. zea*, and that Scentry lure types are a very effective lure in attracting *H. zea*. However, reports vary between studies, as one study found that green bucket traps are useful in areas of low population densities, which you would see in early pest arrivals (Guerrero et al., 2014). This difference in results confirms that the topic still needs to be fully understood.

It is known that differing species of *Helicoverpa* respond to pheromone combinations differently than others, and females often utilize antagonistic compounds to signal to males when they can mate (Chang et al., 2017). The majority of *Heliothis* moths utilize two main compounds in their respective pheromone blends, with most *Helicoverpa* moths utilizing (Z)11-hexadecenal (Z11-16:Ald) as their primary component (Choi et al., 2002). While the major component is the Z11-16:Ald compound, the minor components produced by female *H. zea* moths are (Z)7-hexadecenal (Z7-16:Ald), Z9-16:Ald, and hexadecenal (16:Ald) (Choi et al., 2002). All components are synthesized and regulated by a pheromone biosynthesis activating neuropeptide (PBAN) (Choi et al., 1998). Upon release of the PBAN into the hemolymph, the pheromone gland begins producing pheromones (Choi et al., 1998). While pheromone blend composition is constant across certain species, there have been reported instances of these compositions being altered due to admixture between sympatric species (Löfstedt et al., 1989; Jurenka et al., 1994; Baker, 2002).

While we know the *CYP337B3* allele is an example of an alteration within the DNA of admixed *H. zea* that may confer insecticide resistance, we do not know the full extent of changes within the genome of *H. zea*. For example, it is known that the compounds of mating

pheromones between certain *Helicoverpa* species differ and can act as antagonists to one another (Baker, 2008; Ivey & Hillier, 2023). If admixture potentially impacts the compounds within the sex pheromones of admixed female *H. zea*, it could impact the sampling of *H. zea* via pheromone trapping. If admixed male *H. zea* began to recognize a sex pheromone compound that differs from non-admixed *H. zea* pheromone lures available on a commercial market, then Bucket trapping methodology would become ineffective until a new pheromone lure could be created to match.

Since pheromone lure trapping is utilized to make assessments of population genomics and density, the potential impact of admixture on the sampling methodology could be detrimental to farmers who rely on their efficacy (Ivey & Hillier, 2023). In this study, I utilized two commercially available *H. zea* lures (Scentry, Trece), as well as two separate trap colors (Tricolor, Green) to see if either had a greater impact on attracting admixed *H. zea* males.

13. Methods

8 separate sweet corn fields were sampled in and around the town of Brighton, CO, using bucket traps. Traps were positioned no less than 30 feet away from one another to limit cross attraction. Traps were placed on metal poles roughly 3 feet above ground in an upwind position next to the chosen crop fields. Two separate pheromone lures were used (Scentry, Trece). Lures were swapped every two weeks based on the listed longevity of each lure. Two separate color types were utilized in traps as well (Tricolor, Green). In total, I had four variations of a bucket trap per block: Yellow with Scentry, tricolor with Scentry, yellow with Trece, and tricolor with Trece. Trap variations were randomized per block to ensure variation within each sampled field. In each sampled field, there were two blocks consisting of four traps each, for a total of eight traps per block. Traps were collected weekly from July to September in 2025.

After collection, two whole legs were removed from each moth collected per trap per collection date and ran through ddPCR genome analysis to determine the presence of B3 in the sampled population. My collaborators at the local USDA APHIS lab used ddPCR when grouping mass leg samples together, which occurred when determining admixture from field caught samples (Zink et al., 2022).

When comparing counts between traps on admixed and non-admixed groups, I fit negative binomial GLM models to track variation among the counts of admixed and non-admixed *H. zea.* All results were analyzed in R studio.

14. Results

Regarding B3 status, green traps with Trece lures brought in the largest number of admixed moths (133). However, tricolor traps with Trece lures brought in the greatest mean percentage of admixed moths through a collected sample (68%) (Figure 9). Traps with Scentry lures brought in the greatest mean number of moths across all collection dates, where Green traps with Scentry lures brought in the greatest mean amount (Figure 10). Across all trap and lure combinations, a total of 466 *H. zea* were collected, of which 302 were positive for possessing the B3 allele (Figure 11). This means that 64.8% of the sampled population were positive for retaining the *CYP337B3* allele after interspecific introgression, higher than other previously reported rates of admixture in *H. zea* in the state of Colorado (Nufer et al., 2024).

When examining effectiveness of trap colors to attract B3 positive *H. zea*, I found that of the samples collected from green bucket traps, 63% of *H. zea* were positive for B3. For all samples collected from tricolor traps, roughly 67% were admixed. I found there to be no statistically significant difference between trap colors in their ability to attract admixed *H. zea* ($\beta = -0.080 \pm 0.323$ SE, $z = -0.246$, $p = 0.806$) (Figure 12). I found no significant evidence of a

difference in *H. zea* male attraction to trap color alone, where green bucket traps captured 249 male *H. zea* moths, and tricolor traps caught 217 male *H. zea* moths ($\beta = -0.138 \pm 0.295$ SE, $z = -0.467$, $p = 0.641$).

I compared the results of pheromone lures alone based on their attractiveness towards admixed and non-admixed *H.* zea. I found there to be a statistically significant difference in admixed *H. zea* attraction between lure types, where Trece lures attracted 397 moths and Scentry lures attracted 69 moths ($\beta = 1.742 \pm 0.287$ SE, $z = 6.061$, $p < 0.001$) (Figure 13). I found strong evidence of a difference in total *H. zea* attraction between lure types, with Trece being far more attractive ($\beta = 1.750 \pm 0.255$ SE, $z = 6.853$, $p < 0.001$).

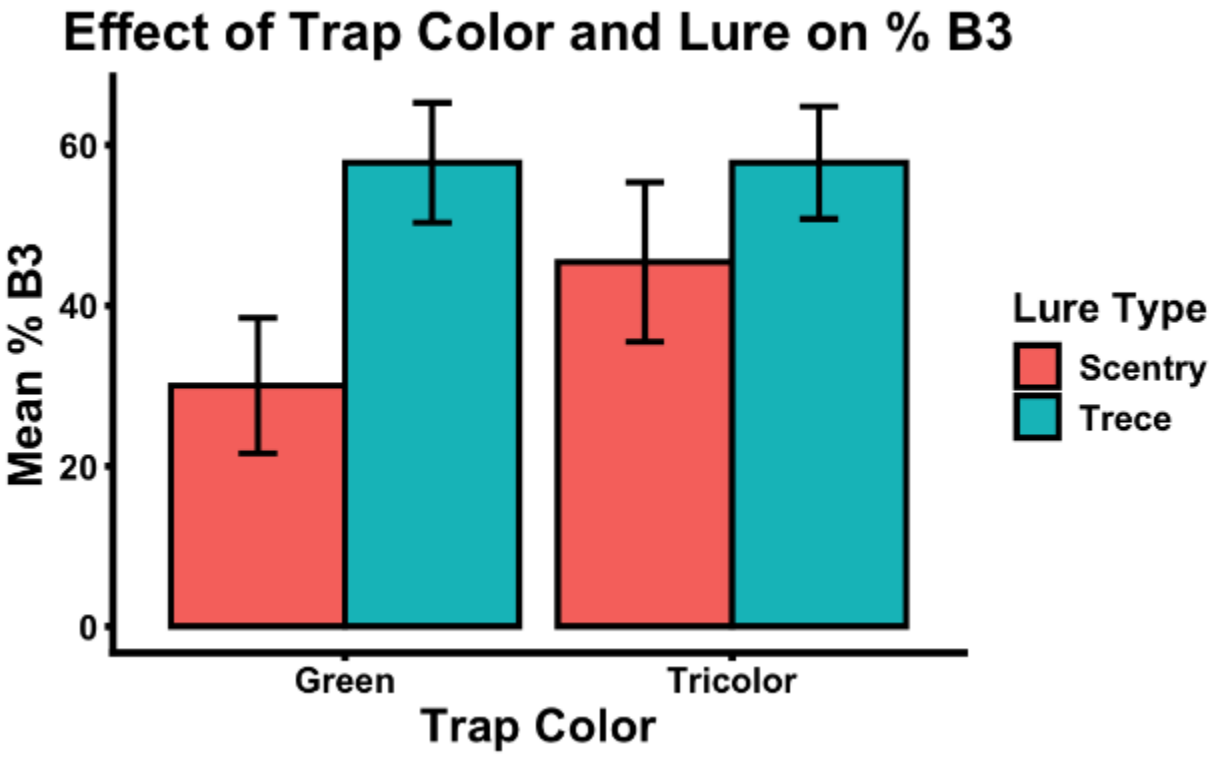

Figure 9. Average % admixture (±SE) of sampled *H. zea* moths collected between green and tricolor bucket traps with Scentry and Trece pheromone lures.

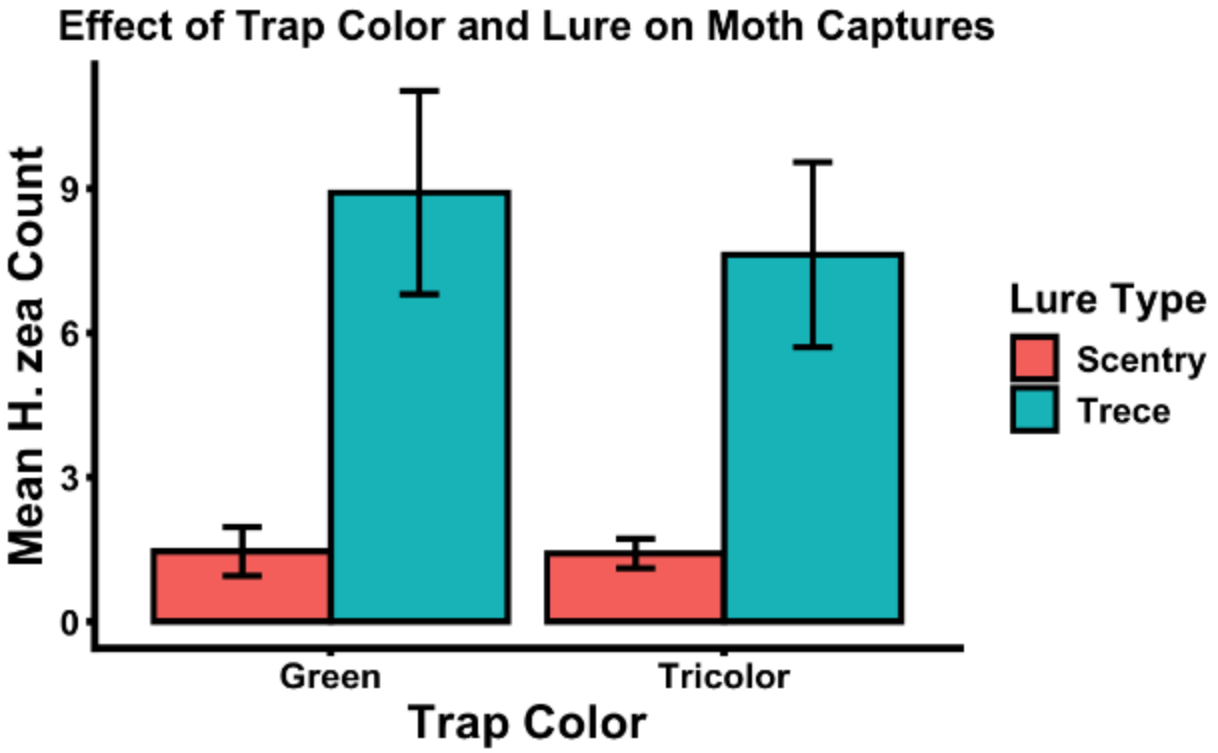

Figure 10. Average count (±SE) of *H. zea* moths collected from green and tricolor bucket traps with Scentry and Trece pheromone lures.

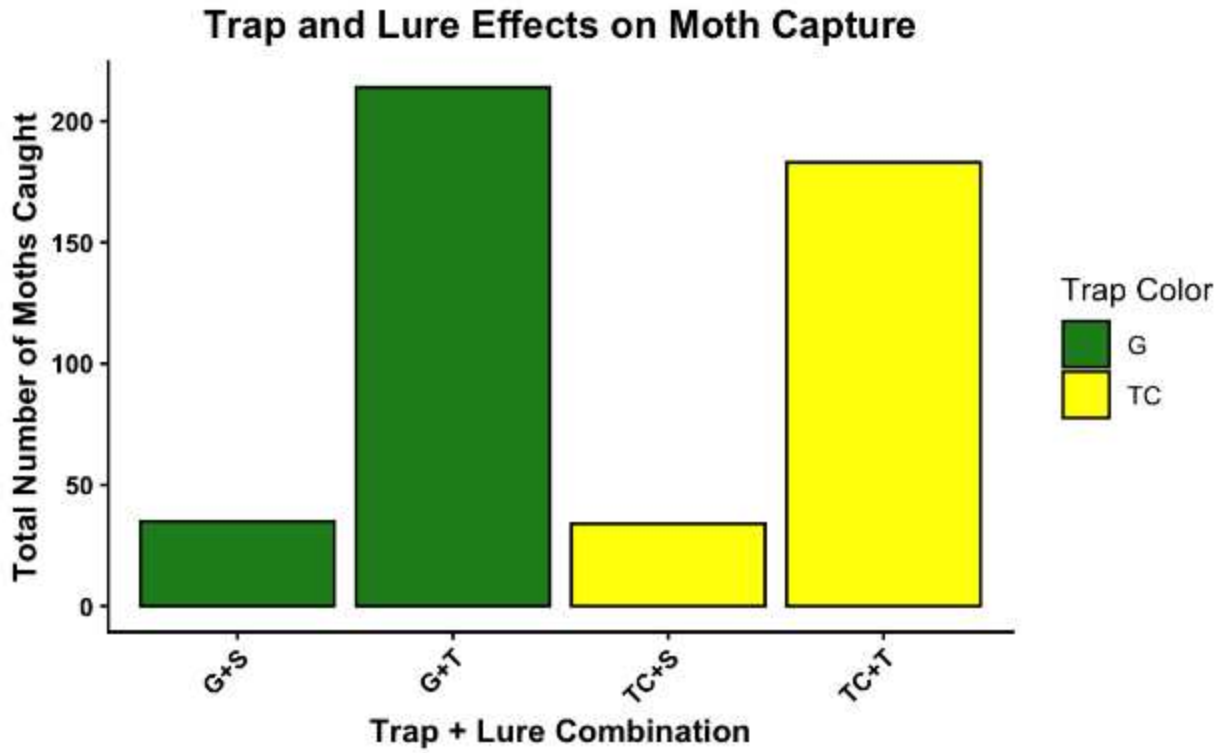

Figure 11. Total captures of *H. zea* between green (G) and tricolor (TC) bucket traps with both Trece (T) and Scentry (S) lure types.

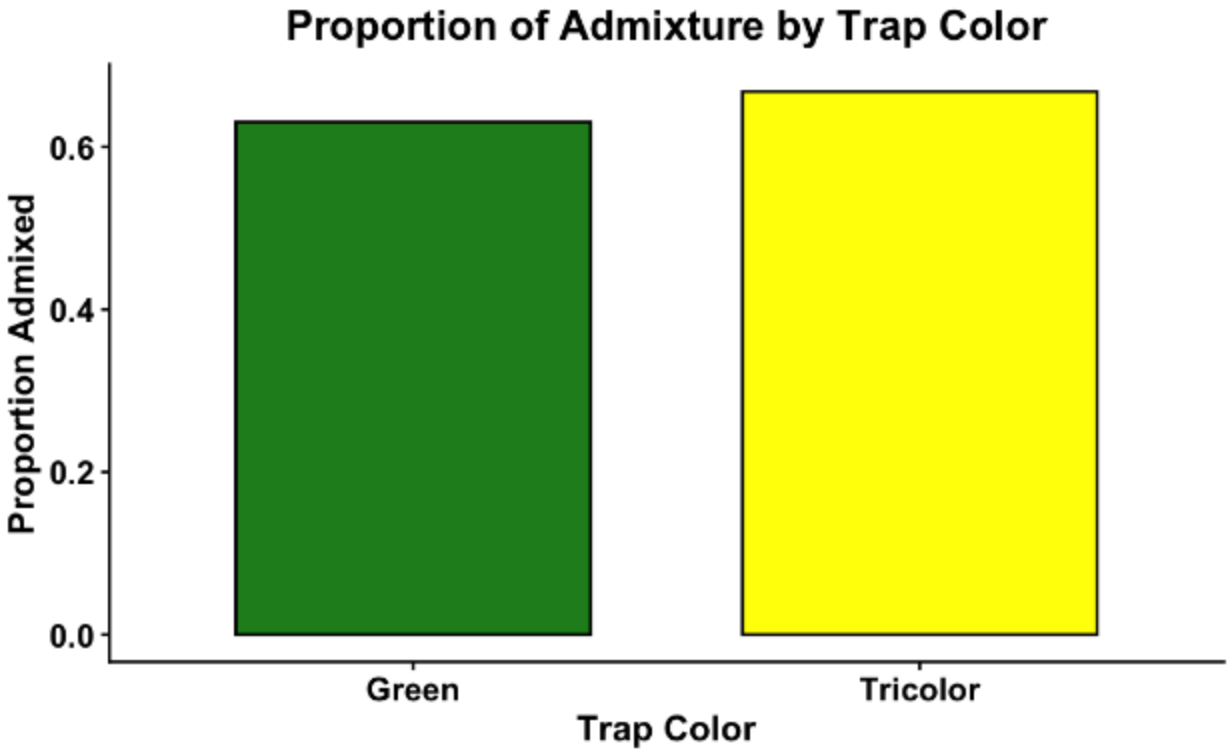

Figure 12. Percentage of admixed *H. zea* moths caught between both green and tricolor Bucket traps.

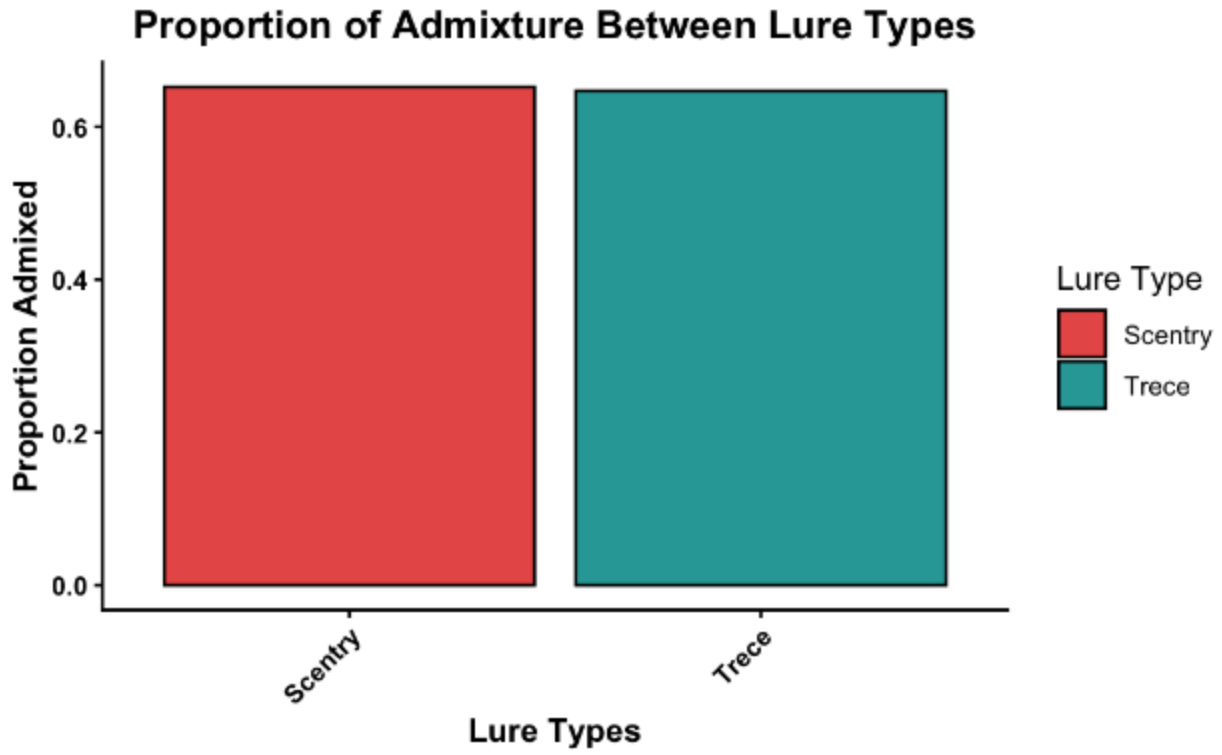

Figure 13. Percentage of admixed *H. zea* moths caught between both Scentry and Trece lure types.

15. Discussion

The results of my sampling indicated that the rate of admixture in wild populations of *H. zea* is significantly higher than previously reported (Nufer et al., 2024). This increase indicates that the B3 allele is present in greater percentages of the population's gene pool. This difference suggests that the B3 allele does confer some fitness benefits for admixed *H. zea*, further supporting my data from our insecticide susceptibility trials that shows fitness is conferred through the B3 allele.

Previous studies found Scentry lures to be far more attractive towards moths compared to Trece lures (Mahas et al., 2025). My results directly contradict this, where Scentry lures captured only 69 total moths compared to the 397 caught by Trece lures. Previous studies raised concern on the potential of introgression to impact the attraction of *Heliothis* moths to differing

pheromone lures (Ivey & Hillier, 2023). However, I did not find evidence suggesting that either lure type was more attractive towards admixed *H. zea* individuals. Studies suggest that *H. zea* may choose trap choices based on color, suggesting that tricolor traps are much more attractive under nocturnal conditions due to their visual contrast at night compared to green traps (Kwadha et al., 2025). However, our results suggest otherwise, where we found no statistically significant difference between trap color and the ability to attract *H. zea* moths.

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