Enhancing Biological Control by Native Stink Bug Parasitoids in Pecan Orchards through Cover Crop Implementation

A Thesis submitted
to the Graduate School
Valdosta State University

in partial fulfillment of requirements
for the degree of

MASTER OF SCIENCE

in Biology

in the Department of Biology
of the College of Science and Mathematics

April 2025

Emily B. Myers

B.S., Abraham Baldwin Agricultural College, 2022

This thesis, "*Enhancing Biological Control by Native Stink Bug Parasitoids in Pecan Orchards through Cover Crop Implementation*," by Emily B. Myers, is approved by:

Thesis Committee Chair

Erin E. Grabarczyk, Ph.D.
Assistant Professor of Biology

Committee Member

Eric Chambers, Ph.D.
Associate Professor of Biology

P. Glynn Tillman,
Ph.D.
Research Entomologist

Associate Provost for graduate studies and Research

Becky K. da Cruz,
Ph.D., J.D. Professor of
Criminal Justice

Defense date April 8, 2025

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ACKNOWLEDGEMENTS

The completion of this study would not have been possible without the expertise of my advisor, Dr. Erin Grabarczyk. I want to express how thankful that I am for her wonderful mentorship. The lessons and skills that she has taught me have been life changing. I never thought that I would be capable of this achievement, but Dr. G believed in me. She is a brilliant scientist and I look forward to conducting research with her in the future.

I also want to extend my thanks to committee members Drs Glynn Tillman and Eric Chambers. Dr. Tillman has taught me countless skills over the past few years, and I am inspired by her to become a great biologist and appreciate the joy of discovery. Dr. Chambers has provided unwavering mentorship and insightful advice to help refine my research and improve its quality.

I am extremely grateful for the technical support provided by Mikayla Barela. We spent countless hours in the pecan orchards together, and her positive attitude and strong work ethic highly contributed to the completion of my research.

This thesis would not have been possible without their dedication and generosity in sharing their knowledge and time. I am truly honored to have had the opportunity to learn from these distinguished scholars. Thank you all for your guidance and support.

CHAPTER 1

INTRODUCTION

Parasitoids are a diverse group of insects that contribute to several dimensions of biodiversity, including biological control of arthropod pests. Understanding parasitoid ecology, behavior, reproduction, habitat selection, and preferred pest host is crucial to increasing their populations for natural pest control. The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is a significant insect pest in agricultural systems, including pecan orchards. Natural enemies, such as stink bug egg parasitoids, may help control *H. halys* outbreaks in pecan. The addition of floral resources, such as crimson clover, which attract parasitoids, may also increase rates of parasitism as well as parasitoid abundance. For my thesis, I tested whether parasitoid rate of attack, abundance, and diversity differed with and without crimson clover in pecan tree canopies or on the ground next to trees. I monitored rate of attack with sentinel egg masses and abundance with yellow sticky cards. From *H. halys* sentinel egg masses, five native stink bug egg parasitoid species were detected, including one new species for the state of Georgia, *Anastatus pearsalli* (Ashmead), as well as *Trissolcus brochymenae* (Ashmead), *Trissolcus euschisti* (Ashmead), *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae), and *Anastatus reduvii* (Howard) (Hymenoptera: Eupelmidae). From sticky cards, I also detected five native stink bug egg parasitoid species, including *A. reduvii, T. brochymenae, T. euschisti, T. podisi,* and *Ooencyrtus* species (Hymenoptera: Encyrtidae). Overall, rate of attack in sentinel egg masses was higher in orchards with crimson clover compared to the control and was higher in the canopy compared to the ground. Parasitoid abundance was similar whether crimson clover was present or not. Despite similar abundance, increased rates of attack suggest that crimson clover improves biological control of *H. halys* in pecan orchards. Importantly, planting crimson clover in pecan orchards is a simple, sustainable approach for growers to control *H. halys*.

CHAPTER 2

LITERATURE REVIEW

Abstract

Parasitoids are a diverse group of insects that contribute to several dimensions of biodiversity, including biological control of arthropod pests. Understanding parasitoid ecology, behavior, reproduction, habitat selection, and preferred pest host is crucial to increasing their populations for natural pest control. Stink bugs (Hemiptera: Pentatomidae) are polyphagous agricultural pests that feed on and reproduce in a wide range of agricultural landscapes across the United States. In particular, the brown marmorated stink bug, *Halyomorpha halys* (Stål), is an invasive and nuisance pest that has become a significant threat to agriculture by causing significant crop losses. Natural enemies, such as parasitoids, may help control invasive and indigenous stink bug populations, including *H. halys*. The stink bug egg parasitoids *Trissolcus euschisti*, *Trissolcus brochymenae*, *Trissolcus edessae* (Fouts), *Trissolcus basalis* (Wollaston), *Telenomus podisi*, *Anastatus reduvii*, and *Ooencyrtus* species are beneficial parasitic wasps that successfully contribute to the biological control of stink bug populations in the southeastern United States. This review highlights the behavior and ecology of native stink bug egg parasitoids and the invasive *H. halys*, as well as how parasitoids may contribute to minimizing damage to plants in agroecosystems.

Parasitoid Ecology

Biological control (hereafter; biocontrol) is a key component of a 'systems approach' to integrated pest management to counteract insecticide-resistant pests and minimize the usage of pesticides (Bale et al. 2008). Over time, the simplification of agricultural landscapes and their strong dependence on chemical inputs have negatively impacted environmental quality and increased the need for biodiversity conservation in agroecosystems (Evenson and Gollin 2003). Therefore, minimizing ecological disruption to enhance natural enemy populations (i.e. predators and parasitoids) is of critical importance. Understanding the relationships among farming systems, natural enemy diversity, and insect pest suppression is a key element in our understanding of natural pest control mechanisms (Rusch et al. 2010). Biocontrol is a safe approach for managing pests in agroecosystems, and parasitoid wasps are well-known biological control agents (Wang et al. 2019). Assessing biocontrol services to understand the impacts of indigenous parasitoids is crucial for pest management programs (Tillman et al. 2023a). Parasitoid wasps are important biocontrol agents for many crop pests (Pak et al. 2015), and it has been indicated that these natural enemies disperse between habitats in agroecosystems (Tillman 2011).

Unlike parasites, insect parasitoids ultimately kill their host, typically other insects, while parasites do not. Parasitoids are essentially parasites that have evolved to rely on killing their host for their own development and reproduction (Vinson 1976). There are two general categories of parasitoids: endoparasitoids, which hatch within the host from eggs or larvae laid there by an adult female and then feed and develop inside the host; and ectoparasitoids, which hatch on the outside of the host and feed through the host skin, sucking out body fluids. However, some parasitoids oviposit eggs on their hosts and then the larval parasitoids enter the body of the host for feeding.

In this study, I assessed parasitism by stink bug parasitoids which are endoparasitoids of stink bug eggs. Parasitoids have been found to locate their hosts within different feeding niches and rely on host-seeking behaviors to contribute to their reproductive performance (Grasswitz 1998). Female stink bug endoparasitoids locate their hosts by following vibrations with their antennae. The female uses her ovipositor to lay her egg directly into the body of her host. The parasitoid larval stage then emerges from the egg to feed and develop inside their host. Thus, essentially the larval stage of parasitoids is economically, the most important life stage, as this is what causes death to their host (Mi et al. 2021). Stink bug egg parasitoids have three larval stages then pupate inside the host. Adults eventually emerge from the host.

As adults, parasitoids require nectar or other sugar resources to cover their energetic needs (Bianchi and Wäckers 2008). Understanding their nutritional requirements is crucial for sustaining an ecologically balanced agricultural landscape (Lahiri et al. 2017). Recent studies show that starved parasitoids readily feed on sugar resources in the field, and floral resources improved their longevity and fecundity but did not increase parasitism rates (Lee 2024; Lee and Heimpel 2008). Further, buckwheat nectar was found to efficiently sustain *T. podisi* in a laboratory study (Lahiri et al. 2017). However, additional research is needed to identify food resources, such as wildflowers, which may enhance parasitoid abundance in the field.

Halyomorpha halys ecology

The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), has contributed to high economic losses in agriculture across the globe (Leskey et al. 2012; Leskey and Nielsen 2018; Rice et al. 2014). This polyphagous insect uses over one hundred different plant species for food and to lay egg masses (Bakken et al. 2015; Bergmann et al. 2016). In Georgia, there is a large variety of crops that serve as host plants for stink bug species, including pecan, peach, cotton, and soybean (Tillman et al. 2023ab; Tillman et al. 2022). Eliminating non-crop host plants could be beneficial to suppressing unwanted stink bug populations, however; removing stink bug host plants could also suppress beneficial parasitoids as well (Tougeron et al. 2020).

Insect pests, such as *H. halys*, are not only nuisances of agricultural habitats but invade human-built structures during cooler months as well (Leskey et al. 2012). Typically, this happens because certain insects enter diapause and overwinter or go through a life phase where they stay alive during the colder seasons. Both invasive and native stink bug populations go into diapause before overwintering (Musolin 2012). The brown marmorated stink bug has generally evolved to choose to overwinter in dry, quiet, and calm settings (Rice et al. 2014). Their behavior at the end of the warmer months encourages them to find overwintering sites in nature, but they will also choose to reside in buildings and homes if they are accessible (Gross 1993). Extreme winter seasons can decrease unwanted stinkbugs in buildings and domestic areas, as they are unlikely to survive in temperatures below ten degrees Fahrenheit. However, as spring temperatures increase, *H. halys* emerge from their overwintering habitats and move into crop fields and orchards (Grabarczyk et al. 2022).

Biocontrol of H. halys by stink bug egg parasitoids

To manage *H. halys* in agricultural systems, their natural enemies, such as parasitoids, can provide natural control of pests, thereby providing beneficial outcomes by decreasing crop damage. The stink bug egg parasitoids *Trissolcus basalis*, *Trissolcus euschisti*, *Trissolcus edessae*, *Trissolcus brochymenae*, *Telenomus podisi*, *Anastatus reduviid*, and *Ooencyrtus* species are important biological control agents of *H. halys* in the southeastern United States (Tillman et al. 2020).

Target-specific biological control measures reduce the use of conventional pesticides, are safe to use, and can be cost-effective. Most insecticides used for managing *H. halys* are toxic to native, natural enemies (Leskey and Nielsen 2018). These insecticides also disrupt integrated pest management systems and cause insect resistance while inhibiting beneficial stink bug predators and parasitoids. Over time, the constant use of pesticides and insecticides in integrated pest management systems has caused insect resistance to pesticides to increase. What would control unwanted insect populations from an agricultural setting, now does not provide the same effects in protecting crop damage. The overuse of these potentially harmful chemicals has caused higher insecticide resistance and suppression of beneficial insect parasitoids and predator populations globally (Mi et al. 2021). Excessive pesticide applications over time have been proven detrimental to naturally occurring parasitoid populations, and a world without them could be costly to agroecosystems. The behaviors of all species need to be studied further to preserve beneficial insect populations while suppressing insect pest populations.

Conclusions

Stink bug egg parasitoids are essential natural enemies in agroecosystems, including row crops, orchards, vineyards, woodlands, and even domestic habitats where insect pests overwinter and feed (Leskey et al. 2012; Rice et al. 2014). Protecting natural enemies while simultaneously suppressing pest populations without the use of unnecessary pesticide applications is crucial to conservation of beneficial insects. Importantly, an understanding of the parasitism of stink bug egg masses in agroecosystems is critical to developing effective biological control against the invasive *H. halys* (Cottrell et al. 2023). However, whether rates of parasitism by stink bug egg parasitoids increase due to the addition of nectar resources in orchards is poorly known. Moreover, parasitoids may show species-specific patterns of egg parasitism depending on the location of stink bug egg masses, such as in the tree canopy compared to on the ground near nectar resources (Cottrell et al. 2023). Therefore, in my thesis I seek to test if implementing cover crops improves herbivore suppression by increasing parasitoid abundance in a field setting. Overall, this study aims to demonstrate whether crimson clover increases stink bug egg parasitism rates as well as parasitoid species diversity in pecan orchards.

CHAPTER 3

CRIMSON CLOVER IMPROVES RATES OF EGG PARASITISM OF *HALYOMORPHA HALYS* (STÅL) IN PECAN ORCHARDS

Abstract

The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is a significant insect pest in agricultural systems, including pecan orchards. Natural enemies, such as stink bug egg parasitoids, may help control *H. halys* outbreaks in pecan. The addition of floral resources, such as crimson clover, which attract parasitoids, may also increase rates of parasitism as well as parasitoid abundance. For my thesis, I tested whether parasitoid rate of attack, abundance, and diversity differed with and without crimson clover in pecan tree canopies or on the ground next to trees. I monitored rate of attack with sentinel egg masses and abundance with yellow sticky cards. Overall, rate of attack in sentinel egg masses was higher in orchards with crimson clover compared to the control and was higher in the canopy compared to the ground. Parasitoid abundance was similar whether crimson clover was present or not. Despite similar abundance, increased rates of attack suggest that crimson clover improves biological control of *H. halys* in pecan orchards. Importantly, planting crimson clover in pecan orchards is a simple, sustainable approach for growers to control *H. halys*.

Introduction

In agroecosystems, natural enemies, such as parasitoids, can provide control of insect pests, therefore providing beneficial outcomes by decreasing crop damage (Bale, van Lenteren, and Bigler 2008). Establishing cover crops in agricultural landscapes may provide supplementary resources for natural enemies (Beaumelle et al. 2021). For example, cover crops may provide shelter for natural enemies by protecting parasitoids from insecticide spray applications, which are typically lethal to both pests and parasitoids (Bryan et al. 2021). In addition, floral resources provide a source of nectar, which may enhance parasitoid fecundity and longevity (Lee and Heimpel 2008). Moreover, planting multiple species of flowering plants in one landscape can lengthen the bloom period, therefore attracting beneficial insects to areas with high pest populations (Mcintosh et al. 2020). However, for cover crops to be effective for biological control, the timing of bloom and parasitoid activity should co-occur with pest outbreaks.

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive pest that contributes to high economic losses in crops (Leskey et al. 2012; Leskey and Nielsen 2018; Rice et al. 2014). In the southeastern United States, pecan trees (*Carya illinoinensis* (Wangenh.) K. Koch) are known host plants for *H. halys*, where adults consume early-season nuts, which results in significant kernel damage that may go unnoticed until harvest (Ree 2018). Native egg parasitoids of *H. halys* including *Trissolcus euschisti* (Ashmead), *Trissolcus brochymenae* (Ashmead), *Trissolcus edessae* Fouts, *Telenomus podisi* (Ashmead), and *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), and *Anastatus reduvii* (Howard) (Hymenoptera: Eupelmidae), and *Ooencyrtus* species (Hymenoptera: Encyrtidae) are known biological control agents in the southeastern United States (Tillman et al. 2020). Most *Trissolcus, Anastatus,* and *Ooencyrtus* species successfully parasitize naturally occurring *H. halys* egg masses and successfully emerge from the eggs in the field (Tillman et al. 2023ab). Therefore, an understanding of the parasitism of stink bug egg masses in pecan critical to developing effective biological control against the invasive *H. halys* in agroecosystems across the southeastern United States (Cottrell et al. 2023).

Because many parasitoid species rely on nectar as a food source, identifying cover crops that flower could potentially enhance pest control in pecan orchards. Crimson clover (*Trifolium incarnatum* L.) is a cool season legume that provides a variety of beneficial characteristics to pecan trees through nitrogen fixation, reducing weed competition, and preventing soil erosion (Wells, 2024). And parasitoids may be more active when crimson clover is planted as a cover crop (McCutcheon et al. 1995). If crimson clover also increases rates of stink bug egg parasitism in pecan, this will add to the list of benefits provided to pecan growers.

Stink bug egg parasitoids are proven to be successful biological control agents and conserving their population may be beneficial to pecan growers in the southeastern United States. While extensive research supports the view that parasitoids should live longer and have greater fecundity in the field with floral resources (Lee and Heimpel 2008), additional research is needed to determine which plants will help facilitate successful biocontrol (Mcintosh et al. 2020). Therefore, the objective of my study is to identify whether crimson clover may contribute to the suppression of *H. halys* by increasing the rate of parasitism of egg masses as well as parasitoid abundance and species diversity in pecan orchards. For five weeks, sentinel *H. halys* egg masses and yellow sticky cards were deployed in pecan orchards either planted with crimson clover or that were routinely mowed (i.e. control). Both *H. halys* and native stink bug egg parasitoids are found at varying heights within the pecan tree canopy (Cottrell et al. 2023).

Therefore, in addition to the treatments, sentinel *H. halys* egg masses and sticky cards were deployed at two heights: in the pecan canopy as well as on the ground near the base of the tree.

Methods

Field Site

I conducted my study at the USDA-ARS, Southeastern Fruit and Tree Nut Laboratory experimental station in Byron, Georgia, United States (Peach County). In total, 48 pecan trees were monitored over a five-week period from April – May 2024. In preparation for my study, crimson clover was planted in two 100 m X 100 m plots of pecan during Fall 2023. For my control, an additional two plots, approximately the same size, were routinely mowed but otherwise unmanaged. Within the areas planted with crimson clover, a total of 24 mature pecan trees reaching heights up to 20-22 m were selected for my study, with 12 trees per plot. An additional 24 mature pecan trees (12 per plot) were selected for the control.

Monitoring Egg Parasitoids in Pecan

To monitor egg parasitism rates, species richness, and abundance in pecan, refrigerated *H. halys* sentinel egg masses and yellow sticky cards were deployed once per week for five weeks. The sentinel egg masses were acquired from a *H. halys* colony maintained at the USDA-ARS, Southeast Watershed Research Laboratory, Tifton, GA, United States (Tift County).

Sentinel egg masses allow for assessment of egg parasitism rates with similar outcomes to natural egg masses (Tillman et al. 2023a). Moreover, refrigerated sentinel egg masses generally remain in good condition as they maintain the same shape as natural egg masses (Mcintosh et al. 2020). During Fall 2023 and Spring 2024, adult *H. halys* were collected from the field and brought into the rearing facility. Adults were housed in cages (27.9 cm long x 26.7 cm wide x 20.3 cm tall) and fed whole bean pods, apple slices, and raw peanuts ad libitum. Knit cloth (97% cotton, 3% spandex (Jo-Ann Stores, LLC, Hudson, OH) was placed in the cages for adults to lay eggs. Cages were checked every one to three days to provide fresh food and water as well as to remove egg masses from the knit cloth. After egg masses were retrieved from the cages, each egg mass was refrigerated at 2.8 – 3.3 °C for at least 36 hours but no longer than 120 hours to ensure that egg masses would not hatch in the field. This also eliminated the possibility of first instars *H. halys* feeding on parasitized eggs. Before setting sentinel *H. halys* egg masses in the field, all eggs were counted, and any damaged eggs were carefully removed.

Sentinel egg masses were deployed at two locations on each tree, on the ground (0.76 m) and in the lower canopy (4.5 m) to assess parasitism by location. The ground treatment was slightly elevated to reduce predation by fire ants and for visibility during routine mowing. Egg masses deployed at ground heights were attached to 1.82 m bamboo poles anchored 2.43 m away from the base of each tree. Egg masses deployed in the canopy were attached to metal hangers and placed on tree limbs in the lower canopy, with heights ranging between 6.09 and 7.62 m. A total of 48 egg masses were deployed at the same trees per plot in April-May of 2024. Back folding yellow sticky cards (23x28 cm, Alpha Scents, West Linn, OR) were deployed at separate trees than egg masses to monitor parasitoid species richness and abundance. A total of 16 sticky cards were deployed weekly in each treatment and control plot on the same dates as egg deployment. Eight sticky cards were attached to anchored rebar at 0.06 m above the ground located 1.82 m away from tree bases. An additional eight sticky cards were suspended with paracord at a height of 6.7 m within the pecan tree canopies.

All egg masses and sticky cards were deployed and collected by using 7.07 m extension poles. All egg masses and sticky cards were retrieved after 4 days (96 hours) in the field following Tillman et al. (2022) for optimal parasitism rates and returned to USDA-ARS, Southeast Watershed Research Laboratory in Tifton, GA. Egg masses were stored in environmental chambers at (12: 12 h [L:D]; 25 ± 2.0 °C; 50 ± 10% RH). Each egg mass was evaluated daily for parasitism *Trissolcus* parasitoids were identified using the Talamas et al. (2015) key. *Anastatus* species were identified using the Burks (1967) key. *Telenomus podisi* was identified using the Johnson (1984) key.

Sticky cards were stored in freezers at -20 °C prior to examination for parasitoid detection. Each sticky card was examined under a dissecting microscope, and parasitoids were removed for identification following Bergh et al. (2023). Each specimen of interest was counted and identified as egg parasitoids of *H. halys* by using the same keys used for egg mass evaluation. Voucher specimens of *Anastatus pearsalli* Ashmead will be placed in the archive collection located at the Florida Department of Agriculture and Consumer Services in Gainesville, FL.

Statistical Analysis

For my analysis, I used R program software v. 4.3.3 (R Core Team, 2024) to fit a series of generalized mixed effects models, testing whether treatment and location affect egg parasitism rates. To determine the percentage of parasitism, the number of parasitized eggs was divided by the total number of eggs in each egg mass. First, I fit a general linear mixed effects model (package: lme4) (Bates et al 2015) to analyze whether treatment and location affected parasitism percentages. I included treatment (clover or control) and location (canopy or ground) as fixed effects. I included deployment date as a random effect. Percentages were log-transformed to improve residual fit. For parasitoid abundance data on sticky cards, I used a general linear mixed effect model to assess the differences between treatment, location, and parasitoid species. My fixed effects included an interaction term between treatment and parasitoid species as well as an interaction between location and parasitoid species with date of collection as a random effect. Abundance data was also log transformed to improve residual fit. For all models, I estimated marginal means (package: emmeans, function: pairs) (Lenth 2016) to assess differences between treatments. I considered means to differ significantly when $P < 0.05$. In my study, I define the successful parasitism of *H. halys* egg masses and increased parasitoid species richness in pecan orchards where crimson clover is present. To calculate parasitoid diversity, I estimated the Shannon diversity index (package: vegan) (Oksanen et al. 2024) for each treatment by location. To calculate species richness, I summed the number of different parasitoid species that emerged from egg masses as well as the number of species that were captured on sticky cards.

Results

Rate of H. halys egg parasitism and parasitoid abundance

Based on mixed effect models, I found that treatment ($F_{1, 232} = 10.2$, $P = 0.002$) and location ($F_{1, 232} = 19.0$, $P = <0.0001$) both had a significant effect of rates of *H. halys* egg parasitism (Fig. 1). A higher percentage of sentinel egg masses were parasitized on the ground, and more were parasitized in pecan orchards planted with crimson clover (Fig. 1). However, the interaction between treatment and location was not a significant predictor of the rate of egg parasitism ($F_{1, 232} = 0.06$, $P = 0.8$). I found that the interaction between parasitoid species and location was a significant predictor of abundance on yellow sticky cards ($F_{1, 272} = 25.8$, $P < 0.0001$). More *T. podisi* were detected than any other species and were more common on the ground than in the canopy. Treatment was not a significant predictor of parasitoid abundance ($F_{1, 272} = 2.6$, $P = 0.1$) (Fig. 2).

Parasitoid diversity

From *H. halys* sentinel egg masses, five native stink bug egg parasitoid species were detected, including *A. pearsalli, A. reduvii, T. brochymenae, T. euschisti,* and *T. podisi.* From sticky cards, I also detected five native stink bug egg parasitoid species, including *A. reduvii, T. brochymenae, T. euschisti, T. podisi,* and *Ooencyrtus.* Although *Ooencyrtus* spp. parasitoids were found to be the most abundant on sticky cards, they were not included in abundance and diversity calculations as they are a generalist parasitoid species. Furthermore, no *Ooencyrtus* spp. emerged from *H. halys* sentinel egg masses. Therefore, I could not confirm with confidence that the *Ooencyrtus* spp. Captured on yellow sticky cards were independently searching for *H. halys* egg masses.

Total parasitoid species richness was numerically higher in *H. halys* egg masses deployed at both locations (ground and canopy) in orchards with crimson clover than orchards that were mowed (Table 1). Accordingly, diversity was the highest in egg masses deployed in the canopy of clover plots, species richness and diversity was highest in the canopy of mowed plots, followed by in the canopy of clover plots (Table 1).

Discussion

Within pecan orchards, crimson clover had a positive impact on the rate of *H. halys* egg parasitism and parasitoid species' richness. More native parasitoids attacked *H. halys* sentinel egg masses in orchards planted with crimson clover than in control plots where the ground cover was managed with regular mowing. Flowering plants can provide parasitoids with food resources and shelter from adverse environmental conditions (Landis, Wratten, and Gurr 2000). And nectar-producing cover crops show promise for natural enemy enhancement as a result of access to floral resources (Irvin et al. 2014). Although prior research suggests that crimson clover serves as an excellent food source for parasitoids (McCutcheon et al. 1995), little research has focused on whether crimson clover may also improve stink bug egg parasitism in pecan systems. Including accessible floral resources in agricultural systems is a simple practice to ensure that beneficial parasitoids are not food-limited and are more likely to be effective biocontrol agents (Russell 2015). However, identifying floral resources with shorter corollas is important to ensure that nectar is accessible by parasitoids' short mouthparts (Patt, Hamilton, and Lashomb 1997). Moreover, temporal continuity of flowering plants that provide suitable nectar resources should improve the survival and conservation of parasitoid species in agroecosystems (Mcintosh et al. 2020).

The vertical distribution of stink bugs, their egg masses, and parasitoid activity may overlap. For example, in a non-crop host, the tree of heaven (*Ailanthus altissima* (Mill.) Swingle) *H. halys* activity was higher in the upper canopy and the egg parasitoid *Trissolcus japonicus* (Ashmead) was found to parasitize *H. halys* at the mid-tree canopy, but not the lower canopy (Quinn et al. 2019). Therefore, for host plants that are large fruit or nut-bearing trees, location on or near the tree may affect parasitoid activity (Cottrell et al. 2023; Quinn et al. 2019). In this study, I found that egg masses deployed in the canopy had lower parasitism rates than eggs deployed closer to the ground. Within pecan canopies, rates of *H. halys* egg parasitism have been shown to be higher at the tallest points of pecan tree canopies compared to the lower canopy (Cottrell et al. 2023). Thus, my finding that rates of parasitism were higher on the ground than the canopy adds to our understanding of parasitoid ecology in tree nut systems.

Deploying stink bug sentinel egg masses is essential to analyzing parasitoid preferences, their locations, and behaviors. Undoubtedly, natural stink bug egg masses are the best for monitoring parasitism in *H. halys* eggs. Natural egg masses allow researchers to assess the natural patterns of parasitism in the field. Utilizing natural egg masses reduces the effort involved in rearing, transporting, and deploying egg masses. However, searching for and finding natural stink bug egg masses in tree canopies, such as mature pecan, can be challenging (Tillman et al. 2022). Therefore, much of what we know about *H. halys* parasitoids comes from studies that rely on sentinel egg masses (Tillman et al. 2022).

An understanding of parasitism of *H. halys* egg masses is essential to develop effective biocontrol tactics for this pest in agroecosystems across the United States. Nearctic hymenopteran endoparasitoids in the genera *Anastatus* Motshculsky (Eupelmidae), *Trissoclus* Ashmead, *Telenomus* Haliday, and *Telenomus* Haliday, and *Gyron* Haliday (Scelionidae), and *Ooencyrtus* spp (Ashmead) (Encyrtidae) have been reported to parasitize eggs of *H. halys* in the US (Tillman et al. 2020). In this study, *Telenomus podisi* was the most prevalent species found to parasitize egg masses and was also detected in high numbers on sticky cards. Recent laboratory and field studies reveal that *T. podisi* has a high capacity for parasitism (de Freitas Bueno et al. 2020), and has been found to attack several stink bug species including *Nezara viridula* (L.), *Eushistus servus* (Say), and *H. halys* (Lahiri et al. 2017; Ogburn et al. 2016; Tillman 2011). In row crop systems, *T. podisi* often attacks *H. halys* if native stink bugs, including *N. viridula* and *E. servus* are also present (Tillman et al. 2022). Thus, supporting populations of *T. podisi* not only results in *H. halys* egg parasitism, but control of native stink bug pests as well.

In addition, *Anastatus* spp. have been reported as a strong candidate for targeting *H. halys* in augmentative biological control programs (Jones et al. 2017). To my knowledge, this is the first reported detection of *Anastatus pearsalli* in the state of Georgia (Jones et al. 2017; Tillman et al. 2020). *Ooencyrtus* species Ashmead (Encrytidae) have also been reported to parasitize *H. halys* in the United States (Ogburn et al. 2016; Tillman et al. 2020). Physiological host range tests indicate that *Ooencyrtus* spp. are generalist egg parasitoids with innate host preferences, including heteropterans and lepidopterans (Power, Ganjisaffar, and Perring 2020). More *Ooencytrus* spp. were detected from sticky cards than any other parasitoid species, however none were found to parasitize any egg masses during this study. Therefore, I cannot confirm that the species of *Ooencyrtus* detected on yellow sticky cards were specialist species that attack *H. halys* or other native stink bug species egg masses. However, their prevalence in pecan orchards should encourage further investigation of *Ooencyrtus* species, including their ability to discriminate between target and non-target hosts.

CHAPTER 4

CONCLUSIONS

The brown marmorated stink bug, *Halyomorpha halys*, is a global agricultural pest, and innovative approaches to the biological control of their populations should be considered (Conti et al. 2021). Native, natural enemies of stink bug pests, such as egg parasitoids, are proven to be successful biological control agents and conserving them could be beneficial to agriculture across the globe (Jones et al. 2017). However, the simplification of agricultural landscapes and a strong dependence on chemical inputs negatively impact the environment, with downstream negative impacts on natural enemies. As a result, there is an increased need for biodiversity conservation in agroecosystems (Evenson and Gollin 2003). For stink bug egg parasitoids, one way to protect their populations from insecticides in orchards is to provide additional flowering plants as refuges (Landis, Wratten, and Gurr 2000; Lee and Heimpel 2008; Lee 2024). Moreover, parasitoids are attracted to a wide variety of flowers, which may increase their longevity in agricultural plant communities (Russell 2015). Because stink bug egg parasitoids rely on nectar as a food source, nectar-producing cover crops, such as crimson clover, which attract parasitoids could be influential to stink bug pest control measures in orchards, including *H. halys*. My thesis research shows that stink bug egg parasitoid rate of attack on *H. halys* egg masses was higher in pecan orchards planted with crimson clover compared to the control. However, I found that stink bug egg parasitoid abundance and diversity did not vary significantly between orchards with and without crimson clover in pecan tree canopies or on the ground next to trees. Thus, additional field research is needed to determine methods that may contribute to the behavioral manipulation of parasitoids for positive impacts on biocontrol in conservation techniques.

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Appendix A: Figures

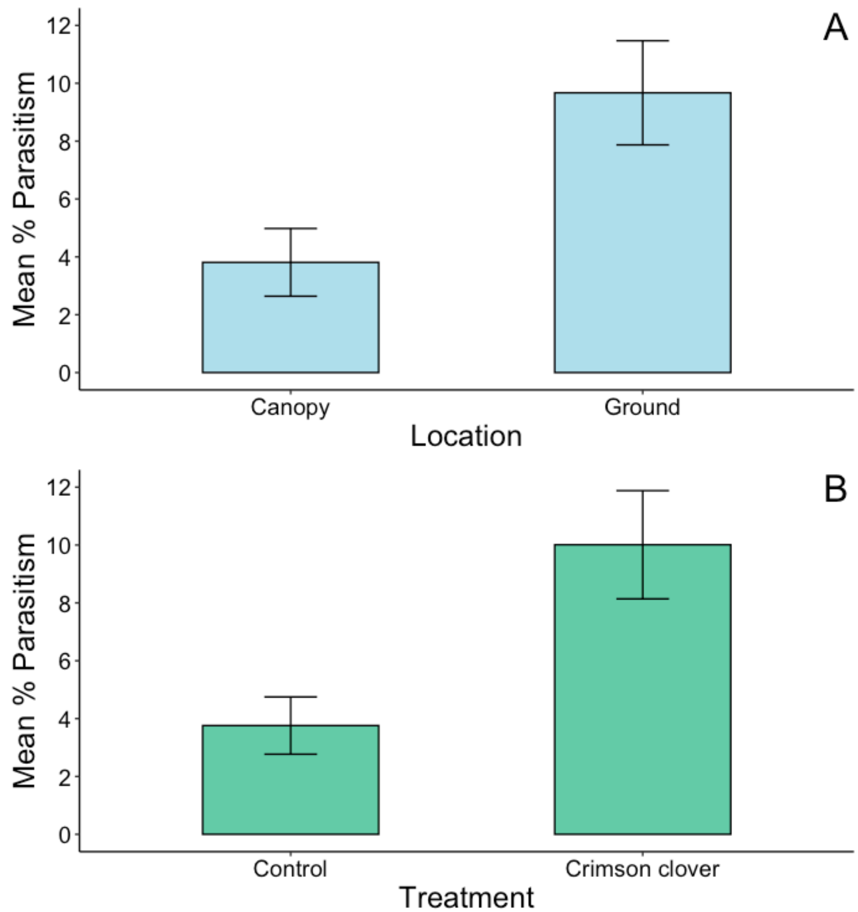

Figure 1: Mean ± SE for the percentage of stink bug egg parasitism of *H. halys* egg masses deployed in (A) pecan canopies and on the ground next to trees deployed in (B) control plots as well as plots planted with crimson clover.

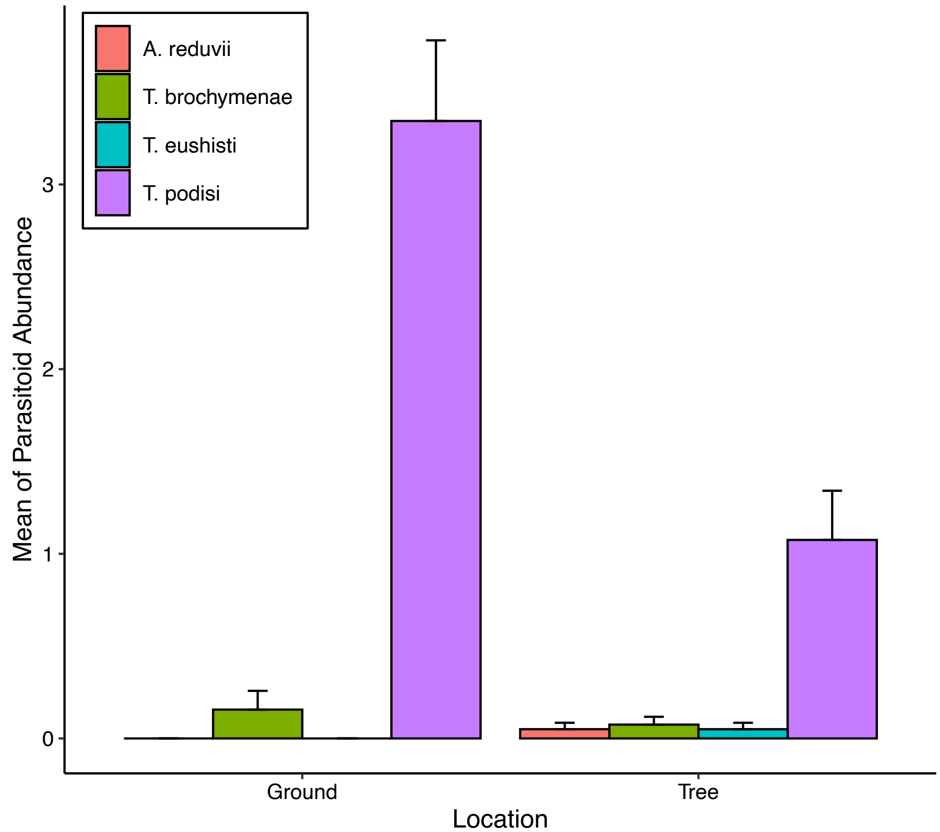

Figure 2: Mean ± SE abundance of stink bug egg parasitoids detected on yellow sticky cards deployed in pecan canopies and on the ground next to pecan trees.

Appendix B: Table

Table 1: Stink bug egg parasitoid species richness and diversity from *H. halys* sentinel egg masses and yellow sticky cards. Diversity was calculated with the Shannon Diversity Index (SHDI).

	Egg masses		**Sticky cards**	
Treatment	**Richness**	**SHDI**	**Richness**	**SHDI**
Ground + Clover	3	0.80	2	0.27
Canopy + Clover	3	1.04	3	0.47
Ground + Mowed	1	0	1	0
Canopy + Mowed	0	0	4	0.57