

EVALUATION OF INTEGRATED PEST MANAGEMENT TOOLS USED TO CONTROL
SQUASH BUGS, *ANASA TRISTIS*, (HEMIPTERA: COREIDAE) IN SQUASH

by

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(Under the Direction of S. Kristine Braman)

ABSTRACT

Squash farmers in the Southeast seek alternative methods of managing squash bugs. Previous research has shown success with insecticides, however, there has been varying success utilizing cultural control methods. Through farmscaping or intercropping floral resources, beneficial insects will receive greater incentive to visit otherwise less enticing monocultures. The Griffin location during the 2014 season was the only positive result with lower abundance of squash bugs and greater yield in the treated than in the control plots ($F_{1,166} = 27.74, 30, 30.53, 18.18, \text{ and } 19.69$ respectively, $p \text{ value} < 0.0001$) and ($F_{1,117} = 1.77, p \text{ value} = 0.1862$). Fields treated with floral resources were expected to have greater abundance and diversity of beneficial insects (e.g. parasitic Hymenoptera and predatory Hemiptera). With the increased exposure to beneficial insects, damage done by the squash bug was expected to be less compared to plots without floral resources.

INDEX WORDS: *Anasa tristis*, farmscaping, biological control, beneficial insects, split-split plot design, and *Geocoris punctipes*

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DEDICATION

I dedicate this thesis to my friends and family. They believed in me, and gave me the support I needed when I had doubts.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

History and importance of squash production. In 2014, the United States harvested 38,530 acres (15,592.5 hectares) of squash, worth \$191,532,000. Georgia accounted for 3,200 (8.3%) acres (1,295.0 hectares) of squash harvested, and is the 5th largest producer in the United States behind: Florida with 6,800 acres (2,751.9 hectares) or 17.6%, California with 6,100 acres (2468.6 hectares) or 15.8%, Michigan with 6,000 acres (2,428.1 hectares) or 15.6%, and New York with 4,300 acres (1,740.1 hectares) or 11.2% (USDA 2015). Most of the large scale squash production occurs in the south central region of the state in Colquitt County with 1,150 acres (465.4 hectares), Echols County with 650 acres (263.0 hectares), and Tift County with 275 acres (111.3 hectares) (Wolfe and Shepard 2012). However, there is a growing market for small-scale commercial vegetable production, with the greatest number of growers in Georgia found in urban regions such as Atlanta, Athens, Savannah, Macon, and Columbus.

As the nation shifts its preference towards locally grown food, a substantial number of consumers are willing to pay premiums, especially for certain types of produce (Wolf et al. 2005). In 2011, Georgia had 23 organic certified vegetable farms that produced \$2,761,182 (2012 Certified Organic Production Survey). These estimates are typically considered to be conservative due to the fact that many farmers who use organic production methods are not certified organic. Local food and direct marketing opportunities, including farmers' markets, are one of the fastest growing segments of agriculture. As of 2014, there were 124 farms that were Certified Naturally Grown and 154 farmers' markets in Georgia. There has been a large increase

in the number of farmers' markets since 2003 when Georgia had a mere 9 farmers' markets (2012 Certified Organic Production Survey, 2014 Good Food Guide, Georgia Organics). Furthermore, these local farmers can reach more consumers with programs like Community Supported Agriculture (CSA), which provide a diverse share of their products to their customers on a weekly basis. According to the latest Census of Agriculture, direct sales of food products from farmers to individual consumers rose by nearly 50% between 2002 and 2007 (Farm Futures Aug 2013). The organic production market is growing, although production and harvesting expenses remain limiting (e.g., Biermacher et al. 2007). One of the pests for squash (especially non-conventional) production is the squash bug, *Anasa tristis* (DeGeer).

History of the squash bug. *Anasa tristis* (DeGeer) has long been considered a significant indigenous pest of squash and other members of the Cucurbitaceae family including pumpkin, watermelon, and zucchini. Adults and nymphs of *A. tristis* feed on the leaves, stems, and vines of squash plants by inserting their styletiform mouthparts into the phloem tissue. When squash bugs feeds, they cause damage to plants by consuming their nutrients and by reducing their photosynthetic capacity due to leaf chlorosis and necrosis (Beard 1935). Depending on the growth of the plant, seedling damage as well as plant damage and death can be caused by *A. tristis* (Woodson and Fargo 1991). The reduction in plant health and potential death of the plant caused by the squash bug can result in significant yield loss depending on the population density. Palumbo et al. (1993) reported over 50% yield loss in control plots due to large numbers of nymphs and adults resulting in high rates of plant mortality. Furthermore, squash farmers in parts of the eastern United States have experienced significant loss of yield due to cucurbit yellow vine disease caused by the bacterium, *Serratia marcescens* Bizio. The

disease was first observed in 1988 when farms in Texas and Oklahoma experienced significant yield loss due to the yellowing and wilting of their squash and pumpkin plants (Bruton et al. 2003). It was not until 2004 that *A. tristis* was conclusively shown as a competent vector of the pathogen (Pair et al. 2004).

The distribution of *A. tristis* extends from Canada into South America (Britton 1919, Beard 1940), and is more of a pest east of the Rocky Mountains in the United States (Chittenden 1908). The number of generations (voltinism) that the squash bug is able to have in one growing season varies, which further complicates monitoring pest populations to time control methods. In northern states like Connecticut and Massachusetts, *A. tristis* is only able to have one generation per growing season (Worthley 1923, Beard 1935). In the middle latitudes of the country, the squash bug is able to have 1.0 to 1.5 generations. Nechols (1987) completed a study in Kansas where one complete generation and one partial generation was observed. In Kentucky, researchers completed a study in 2005 and 2006 where they found 1.5 and 1.0 generations of squash bugs respectively (Decker and Yeargan 2008). In southern states such as Oklahoma, the squash bug has the potential to reach 2.5-3.0 generations during the growing season (Pair 1997). *Anasa tristis* overwinters as an adult, and will quickly move into developing squash fields in the spring. Decker and Yeargan 2008 first detected adults emerging from diapause on 9 June 2005, and 3 June 2006 in Kentucky. Further south, squash bugs have emerged as early as 17 April (Pair 1997). Adults have been found to initiate movement at a daylength of 13.4 hours and a soil temperature of 18.3° C (Eiben and Edelson, in prep). Overwintering adults took 28 days after exiting diapause to move into squash fields in Oklahoma (Fargo et al. 1988). Once in the field it took four to five weeks for the mean number of the first generation of insects to increase. When

the density of squash bugs reaches this exponential growth rate, the crop is in danger of major yield reduction. Farmers have been using the knowledge of squash bug voltinism and their population dynamics in hopes of controlling squash bug damage to crops and yield.

Methods of controlling squash bug populations. Chemical control methods have had varying success in controlling squash bug populations. Farmers encountered difficulties in reducing yield loss caused by squash bug damage with early insecticide application recommendations (Walton 1946, Roberts & Saluta 1985, Criswell 1987). More recent research has been able to point out some issues with the timing of the insecticide applications. Insecticides applied after the nymphs had become numerous or plant damage had become excessive were ineffective. Palumbo et al. (1993) sought to investigate the specific timing of insecticide applications for squash bug management. Their findings indicate that early spraying is more effective than spraying right before harvest. This allows the foliar spray to perform better as the plant is not overgrown and overlapping with neighboring plants and rows. Their data show that weekly applications and applications when egg mass densities reach one egg mass per plant provided suppression of nymphs and yielded the most fruit. However, the authors did note that the weekly insecticide applications are excessive and unnecessary as the results of weekly spraying are not significantly different than when the applications are made at the 1 egg mass per plant threshold. Scientists have also used systemically treated squash (<1% of total hectare) and semiochemical toxic baits to successfully control early squash bug populations (Pair 1997). While chemical control is a tool that conventional farmers can use, organic growers may not be able to use them to control their pest populations. Other pest management methods such as cultural control have been studied to examine their efficacy.

Beyond chemical control, researchers have continued to investigate other means of controlling squash bugs. Resistant cultivars have been developed in an attempt to limit damage done to the crops. Squash bugs reared in the lab on resistant and susceptible varieties of squash are able to overcome resistant cultivars, but the researchers believe that ecological and agricultural factors would prevent the development of resistance in the field (Margolies et al. 1998). Other approaches include various types of cloth or plastic row covers and different mulches including aluminum and different plastics. Row covers and mulches can reduce the abundance of pest species. Successful reduction of aphids and aphid-borne viruses in squash was achieved by using aluminum reflective mulches (Kring 1964, Chalfant et al. 1977). Natwick and Durazo (1985) reported data that show fabric row covers reduced incidence of viral diseases transmitted by the whitefly in summer squash. Cartwright et al. (1990) investigated the use of mulch and row covers to manage squash bug populations. They observed a strong preference for soils with mulch by squash bugs, and found no added benefit from row covers. Authors cite the protection that the bugs receive from the mulch from ground dwelling predators as a potential reason for their preference over bare ground. Mulch systems, while increasing soil moisture and reducing irrigation, may increase squash bug control costs, and therefore the benefits are likely nullified (Cartwright et al. 1990).

Given that early season control of squash bugs is recommended, delayed planting is one common cultural control method that has been shown to be effective in reducing the damage done to crops by overwintering adult squash bugs (Fargo et al. 1988, Palumbo et al. 1991). If growers removed the food source early in the season, squash bugs might have a more difficult time reaching damaging numbers, and control of the pest might be easier. Conversely, an early planting date can sometimes be useful. In Kentucky, the spring emergence of the squash bug is

early June. Therefore, farmers wishing to have a June harvest can transplant their crops after the first frost date (around the 10th of May), and the plants will have three to four weeks to grow without the risk of damage caused by the squash bugs (Decker and Yeargan 2008). The success of this method of control is based on the knowledge of voltinism and the degree days necessary for the squash bugs to go through their generational development time. This can change depending on previous year's pest populations, geographic location, and myriad other agricultural variables.

Another alternative control method includes the use of beneficial insects to control pest insects. Many beneficial insects act as predators or parasitoids of pest insects. There have been many studies to assess the natural predators of the squash bug. Studies have found the most prevalent predators to be spiders (Lycosidae and Linyphiidae), hemipteran predators *Geocoris punctipes* (Say), *Geocoris uliginosu* (Say), beetles *Coccinella septempunctata* (Linnaeus), *Coleomegilla maculata* (DeGeer), *Hippodamia convergens* (Guérin-Ménéville), and members of the Carabidae and Staphylinidae families (Decker and Yeargan 2008, Rondon et al. 2003, and Schmidt et al. 2014). While Derek and Yeargan (2008) only found predators to account for 2-7% of squash bug mortality, Schmidt et al. (2014) found that 11% of predators had preyed on squash bugs. Furthermore, squash bug nymphs and adults spend much of their time on the ground beneath the plants, and would therefore be subject to many ground dwelling predators (Britton 1919, and Palumbo et al. 1991). *Geocoris punctipes* has received particular consideration as a potential biological control agent, but furthering the knowledge can only help to inform growers.

Big eyed bugs, *G. punctipes*, are common generalist omnivores found throughout the southern United States (Tamki and Weeks 1972). Previously, it has been shown to significantly

reduce fall armyworm numbers in turf grass (Joseph and Braman 2009, Braman et al. 2003), and prey on spider mites, plant bugs, leafhoppers, aphids, chinch bugs, and various lepidopteran larvae (Dunbar 1971). *Geocoris punctipes* is also known to feed on plant material, but Hunter (2009) assessed the tritrophic interaction, and determined that the net effect is usually in favor of the plant. The study by Rondon et al. (2003) determined that *G. punctipes* third instars and adults did consume *A. tristis* first instar nymphs, but at low levels. This current study seeks to expand on this finding by including data determining the difference in predation rates for both sexes of *G. punctipes*. We also seek to determine if the prey range of *G. punctipes* includes later instars of *A. tristis*, which would improve its efficiency as a biological control agent. If evidence can be brought forth that shows *G. punctipes* has greater potential as a biological control agent against *A. tristis* than previously realized, farmers can add another option for integrated pest management.

Additionally, the squash bug is known to have both a nymphal/adult parasitoid and many egg parasitoids. The nymph or adult parasitoid is the tachinid fly *Trichopoda pennipes* (Fabricius). Field samples collected in Kentucky yielded 20.0%, 23.7%, and 30.0% parasitism rates for the 4th, 5th, and adult stages respectively (Decker and Yeargan 2008). These parasitism rates are conservative estimates as the specimens collected were brought into a lab, and therefore no longer subject to future parasitism. The adult and late nymphal parasitoid provides important pressure on the number of adults and nymphs present in the population, which would help suppress the potential for the horizontal transmission of the bacteria *S. marcescens*. The egg parasitoids offer additional control of the squash bug populations. There are three parasitoid wasps that belong to the family Platygasteridae: *Gryon pennsylvanicum* (Ashmead), *Gryon anasae* (Ashmead), and *Gryon carinatifrons* (Ashmead) and three parasitoid wasps that belongs

to the family Encyrtidae: *Ooencyrtus anasae* (Ashmead) and two unidentified *Ooencyrtus* species *O. sp.* ‘light form’ and *O. sp.* ‘dark form’ (Ashmead 1886 Nechols et al. 1989). Most of the work that has been done with *A. tristis* egg parasitoids is on *G. pennsylvanicum*. Naturally occurring populations of these parasitoids are most abundant in the later months. Decker and Yeorgan (2008) first observed *G. pennsylvanicum* emerging from squash bug eggs in September, 2005 with 15.8% successful parasitism and in July, 2006, with 31.4% successful parasitism. While the egg parasitoid rates can match that of the adult/nymph parasitoid, the late arrival of the egg parasitoid might be too late for effective control needed early in the growing season. However, biocontrol parasitoids are often released to augment natural populations. If a release of these egg parasitoids could be properly timed with the early increase of egg masses, proper early control might be achieved. Additionally, the multiple releases of biological control agents can be very expensive (Olson et al. 1996). Further control can be achieved if additional cultural control practices are adopted by farmers. The cultural control method known as farmscaping has had mixed reviews in its efficacy in controlling pest populations.

Farmscaping and its potential in integrated pest management. The cultural control method known as farmscaping modifies the conventional farming habitat to improve ecosystem function and services while mitigating losses to production area and can include addition of floral resources (Smukler et al. 2010). A scarcity of data-supported guidelines exists to assist farmers in attempts to increase natural control of insects with beneficial insect habitat (Forehand 2004). Existing data are somewhat contradictory, inconclusive or present negative outcomes e.g., natural enemy activity in organic tomatoes was not enhanced, nor pests reduced by the addition of perimeters of a commercially available beneficial insect habitat (Forehand et al. 2006a). Moreover, evening observations indicated attraction of night-flying moths to “Border

Patrol” and “Good Bug Blend” pest control flower mixes which also harbored potential pests (Forehand et al. 2006b).

Farmscaping is a new area of research across many regions of the country and world, although what constitutes successful control in one area might not be duplicated in another. However, research has been completed that can help guide farmers through the implications of this method of control. Choice of floral resources is critical to meet the goal of attracting beneficial insects. First and foremost, the flowers must be able to establish themselves in companion with the crop plant of interest (Grasswitz 2013). Farmers that choose floral resources with the intention to sell them as cut or ornamental flowers can perhaps alleviate the cost of not planting the cash crop. Additional considerations include flower attractiveness, floral morphology, nectar accessibility, and parasitoid mouthpart morphology (Nfzinger and Fadamiro 2010). Short corolla flowers are known to be favored by parasitoid wasps (Campbell et al. 2012). Other beneficial insects can also be attracted when diversity of floral resources are increased (Smukler et al. 2010). Buckwheat (*Fagopyrum esculentum* Moench) has been shown to increase lifespan of female parasitic wasps by approximately 6 days as compared to a water only diet (Nfzinger and Fadamiro 2010). Dill (*Anethum graveolens* L.), Cosmos (*Cosmos bipinnatus* Cav.), Calendula (*Calendula officinalis* L.), and Marigolds (*Tagetes patula* L.) have been included in previous studies to attract beneficial insects (Grasswitz 2013, and Martinez-Uña et al. 2013). Planting specific flower species to attract beneficial insects to control pest species has been adopted to varying degrees in California (Smukler et al. 2010), New Mexico (Grasswitz 2013), Spain (Martinez-Uña 2013), and The United Kingdom (Campbell et al. 2012). Both organic and conventional farmers in the southeast United States may benefit from varied planting dates and farmscaping while avoiding expensive or less effective methods of control. However,

the integration of many or all of the discussed methods of control would be ideal for maximum control.

The squash bug is a significant pest of cucurbits throughout the eastern United States. Much research has been done on the biology and life history, which has helped give critical information to growers. Farmers have had some success in using conventional and cultural control methods. However, the growing number of organic farms in the Southeast and the rest of the United States need more research on effective cultural control methods. Farmscaping has been recently investigated, but has shown contradicting results from different locations and crop types. More research on farmscaping and varied planting dates is needed in the Southeast. The goal of this research is to investigate multiple integrated pest management methods to reduce squash bug impacts in squash plantings in the Southeast.

PROJECT OBJECTIVES AND METHODOLOGY

- 1. Assessment of Habitat Modification and Varied Planting Date to Enhance Biological Control of Squash Bugs, *Anasa tristis* (HEMIPTERA: COREIDAE) in Squash**
 - a. Select candidate floral resources based on their ecosystem function and attractiveness towards beneficial insects in the Southeast
 - b. Create field plots with a split-split plot design to test for both floral resource and planting date effects.
 - c. Determine the abundance of squash bugs found in floral treated plots as compared to control plots
 - d. Determine the reduction in yield loss of floral treated plots as compared to control plots
- 2. Assessment of Habitat Modification for Beneficial Insect Abundance in Squash**
 - a. Determine specifically what kinds of beneficial insects are found in the floral treated plots as compared to the control plots
 - i. Abundance of predators, parasitoids, and/or pollinators
 - b. Determine the measure of control on the squash bug populations done by the beneficial insects
 - i. Including but not limited to % parasitism by parasitoid wasps
- 3. Quantification of Predation Rates for *Geocoris punctipes* on *Anasa tristis***
 - a. Establish colonies *A. tristis* for laboratory studies
 - b. Collect wild caught *G. punctipes* for laboratory studies
 - c. Measure predation rates of adult male and female *G. punctipes* on squash bug eggs as well as 1st, and 2nd instar nymphs

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CHAPTER 2

ASSESSMENT OF HABITAT MODIFICATION AND VARIED PLANTING DATES TO
ENHANCE BIOLOGICAL CONTROL OF SQUASH BUGS, *Ansasa tristis* (HEMIPTERA:
COREIDAE) IN SQUASH

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Abstract

The squash bug *Anasa tristis* (Hemiptera: Coreidae) (DeGeer) is an indigenous pest to squash and other cucurbits. Pesticides can control squash bug populations although many small-scale growers in the Southeast seek alternative methods of management. Cultural control methods, including varying the planting date and farmscaping, have had limited research across the country especially in the Southeast. Farmscaping theoretically increases natural ecosystem functions to aid in the control of pest populations. In the summers of 2014 and 2015, field plots of squash were separated by a minimum of 150 meters and organized in a split-split plot design with floral resources at the whole-plot level and planting date at the sub-plot level. Data were collected on squash bug abundance and fruit yield (kg) for both years, and beneficial insect abundance in 2015. The Griffin location during the 2014 season was the only positive result with lower abundance of squash bugs and greater yield in the treated than in the control plots ($F_{1,166} = 27.74, 30, 30.53, 18.18, \text{ and } 19.69$ respectively, $p \text{ value} < 0.0001$) and ($F_{1,117} = 1.77, p \text{ value} = 0.1862$). With the increased exposure to beneficial insects, damage done to crops by pest insects such as the squash bug is expected to be less when compared to plots without floral resources. However, the floral resources chosen appeared to attract the squash vine borer, *Melittia curcurbitae*, among other pest insects. This multi-species farmscaping method requires further investigation. A change in the number and/or composition of floral resources is recommended for future studies.

Introduction

In 2014, squash production in the Southeast accounted for 30.7% of the United States total acreage harvested (USDA 2015). Georgia (at 8.3%) was the 5th largest producer in the United States, and 2nd largest in the Southeast. One major pest concern for squash producers is the squash bug, *Anasa tristis* (DeGeer). *A. tristis* is an indigenous pest of squash and other members of the Cucurbitaceae family including pumpkin, watermelon, and zucchini. *A. tristis* causes plant damage by feeding, and is a competent vector of the bacterium *Serratia marcescens* Bizio, the causal agent of cucurbit yellow vine disease (Beard 1935, Bruton et al. 2003, and Pair et al. 2004). Previously, chemical control methods have been shown to be successful in controlling squash bug populations with proper timing of insecticide applications (Palumbo et al. 1993). Scientists have also used systemically treated squash (<1% of total hectareage) and semiochemical toxic baits to successfully control early squash bug populations (Pair 1997). These methods of control have been tailored to aid the large commercial growers, and can be a great tool for much of the vegetable production occurring in the south central region of the state (Wolfe and Shepard 2012). However, the growing number of small-scale commercial vegetable growers who are producing for fresh markets in urban areas such as Atlanta, Athens, Savannah, Macon, and Columbus have different needs in terms of pest management.

Cultural control methods are a common tool for small growing operations when pesticides are either not available (cost prohibitive) or not allowed (organic production). Cultivars with resistance to squash bug feeding have been developed in an attempts to limit damage done to the crop. Squash bugs reared in the lab on resistant and susceptible varieties of squash are able to overcome resistant cultivars, but the researchers believe that the ecological and agricultural factors would prevent the breakdown of resistance in the field (Margolies et al.

1998). Other studies that tested the use of row covers and mulches did not yield success in controlling squash bug damage (Cartwright et al. 1990). Researchers are constantly looking for potential methods to aid in the pest management strategies employed by the growers.

Recent studies have shown two methods of control with potential. Manipulating the planting date has shown some success in controlling squash bug damage (Fargo et al. 1988, Palumbo et al. 1991). The squash bug is an early season pest and, removing the food source early in the season limits the squash bug's ability to reach damaging numbers. Decker and Yeargan (2008) also showed that an early planting date would allow for squash plants to grow before overwintering adults emerge and begin feeding. This method of control is dependent on the squash growing season and the voltinism of the squash bug. Guidelines vary greatly depending on the region. Research conducted in the Southeast would greatly benefit growers in Georgia and the surrounding states.

Farmscaping has also gained a lot of interest in its potential as a cultural control method. Farmscaping refers to the addition of floral resources to support naturally occurring beneficial insects, which can aid in the management of pest species (Bugg and Pickett 1998). Adding floral resources to the agriculture system will also be beneficial in terms of reduced erosion and , runoff of agrochemicals, and the potential to be sold as cut flowers. The floral resources attract and act as a supplemental food source for many beneficial insects. However, there is also the potential for additional pest insects to be attracted by the added sources of vegetation and nectar. Floral resource characteristics, such as floral attraction, nectar accessibility, and nutritional suitability, must be considered to meet the needs of the beneficial insects and reduce the risk of potential pest outbreaks (Wackers and Van Rijn 2005, Wackers et al. 2007, Winkler et al. 2010-Philips et al. 2014). There has been some research investigating potential candidates for floral

resources to be used in farmscaping. Buckwheat (*Fagopyrum esculentum* Moench) has shown to increase lifespan of female parasitic wasps by approximately 6 days as compared to a water only diet (Nfziger and Fadamiro 2010). Dill (*Anethum graveolens* L.), Cosmos (*Cosmos bipinnatus* Cav., 1791), Calendula (*Calendula officinalis* L.), and Marigolds (*Tagetes patula* L.) have been included in previous studies to attract beneficial insects (Grasswitz 2013, and Martinez-Uña et al. 2013). Other floral resources can be considered for their varying flower morphology, and the timing of their maturity so they coincide with other floral resources chosen (Campbell et al. 2012). The objectives of this study are to determine how inter-planting floral resources that attract beneficial insects and varying the planting date of the squash crop impact subsequent squash bug populations and squash yield.

Materials and Methods

Study Area and Experimental Design

The study was conducted over two summers (April-August) in 2014 and 2015. In 2014, the two locations were the University of Georgia's Horticulture farm in Watkinsville GA, and the Research and Education Garden in Griffin GA. In 2015, the two locations were the University of Georgia's Research and Education Garden and the Dempsey Farm both in Griffin GA. Plots (7.2 m x 15.84 m) were set up as unrandomized strips in a one way design consisting of five rows (four rows utilized for planting squash, and the middle row and surrounding buffer for the floral resources) and a 0.6 meter buffer surrounding the plot with four contiguous blocks (Figure 2.1). The two treatments are 1) with floral resources and 2) without floral resources at the whole-plot level, and planting date at the sub-plot level. Treated and control plots at each location were separated by a minimum of 150 meters, thus creating a split-split plot design. The anticipated spillover effect of floral resources prevented our use of the more typical RCB design

where blocks with and without flowers would be adjacent. Significant separation of treatments was needed to better comprehend the value of beneficial insect habitat.

Experimental Plots

Plots were directly sown with the straightneck squash *Curcubita pepo* L. variety ‘Zephyr’ (Johnny’s Selected Seeds, Waterville, ME). Three seeds were planted in the center of the planting bed every 0.6 m and were thinned to one plant immediately following seedling emergence. Plots were lightly mulched with wheat straw to maintain moisture during germination. The treated plots had the middle row and buffers were hand-broadcast with a floral mix including species intended to attract beneficial insects: **Buckwheat** (*Fagopyrum esculentum*), **White Dill** (*Anethum graveolens*), **Cosmos** (*Cosmos bipinnatus*), **Calendula** (*Calendula officinalis*), **Centaurea** (*Centaurea cyanus*), **Hybrid Dwarf Sunflowers** (*Helianthus annuus*), **Nasturtiums** (*Tropaeolum majus*), **Zinnias** (*Zinnia elegans*), and **Baby’s Breath** (*Gypsophila elegans* var. Covent Market Garden) and Johnny’s Select Seeds Beneficial Insect Attractant Mix. The control plots were prepared in the same manner but did not have flowers planted in the middle or buffers. Planting dates were chosen based on soil temperature recommendations (above 17°C). In 2014, planting occurred on April 17th in Watkinsville, and April 10th in Griffin. In 2015, planting occurred on April 7th for both locations. A second planting occurred one month later for both years. Results from soil tests informed lime and fertilizer treatments. Overhead irrigation amounts were adjusted based on natural rainfall to prevent overwatering.

Data Collection

Squash plants in each treatment replication were visually evaluated weekly to count squash bug eggs, nymphs, and adults on all leaves, petioles, vines, fruits, and adjacent soil

surface (5 min/planting date/block). In 2015, additional weekly visual observations (5 min/per planting date/block) focused on *Geocoris* spp. and *Gryon* sp. that impact squash bugs was added (Figure 2.2). Squash yield (kg) was determined once or twice per week when fruit had reached the ideal size for harvest (Palumbo et al. 1991). Sampling of squash occurred more often during the summer of 2015 to better approximate typical harvest. Plants that were damaged by squash vine borer *Melittia cucurbitae* feeding (often found destroying basal vine) were counted throughout the field season (Figure 2.3). All sampling dates were the same for each planting date within the year.

Data Analysis

A generalized linear mixed model was applied to determine the influence of sampling date, the interaction between location and treatment, and varied planting date on squash bug abundance, beneficial insect abundance, and yield. The data collected were subjected to ANOVA using a generalized linear mixed model (PROC GLIMMIX, SAS Software) for count data. The categories of ‘All Mobile’ (Adults + Nymphs) and ‘All Bug’ (Adults + Nymphs + Eggs) were also added to the analysis. Differences in least square means were determined by pairwise t-tests ($\alpha = 0.05$) as the multiple comparisons post hoc test to determine significant differences between levels of all factors. The negative binomial distribution was used to model squash bug and beneficial insect abundance data, and a Gaussian distribution was used for the yield data.

Results

Seasonal Abundance

Data from the summer of 2014 indicates that there is strong evidence for two generations of squash bugs observed during the study as seen by the two distinct peaks occurring mid and

late during the study (Figures 2.4A, 2.4B, 2.5A, 2.5B, 2.6A, 2.6B, 2.7A, and 2.7B). Data from the summer of 2015 shows similar trends indicating two generations of squash bugs; however, the peaks are not as obvious (Figures 2.8A, 2.8B, 2.9A, 2.9B, 2.10A, 2.10A, 2.11A, and 2.11B). The squash yield peaked during the middle of the season for the first planting date (Figures 2.4C, 2.6C, 2.8C, and 2.10C), and later, although less, for the second planting date (Figures 2.5C, 2.7C, 2.9C, and 2.11C). There is an early decline in yield seen at sample date 9 in Figure 2.4C. This can easily be seen when compared to the increase in yield produced by the second planting date from the Watkinsville location (Figure 2.5C). The yield collected from the Research location during the summer of 2015 steadily declined after the third sampling date for the first planting date (Figure 2.8C). The yield collected from the second planting date peaked in the late season for both the Research and Dempsey locations, but the Research location yield was half as much than the Dempsey location (Figures 2.9C, and 2.11C). The visual observations of the beneficial insects completed during the summer of 2015 indicate that the *Gryon* spp. parasitoids are present at both locations and both planting dates. There were sightings of *Geocoris* spp., but never in high abundance (Figures 2.8D, 2.9D, 2.10D, and 2.11D).

Floral Resources

During the summer of 2014, the treated plot had greater abundance of squash bug adults, nymphs, all mobile, eggs, and all bug stages than found in control plots at the Watkinsville location, and the control plots had greater abundance of squash bugs adults, nymphs, all mobile, eggs, and all bugs stages than found in treated plots at the Griffin location for the first planting date ($F_{1,166} = 27.74, 30, 30.53, 18.18, \text{ and } 19.69$ respectively, $p \text{ value} < 0.0001$) (Figures 2.12A, and 2.12B). The yield collected from the first planting date was not significantly different between the treated and control plots at the Watkinsville location, but the yield was greater in the

treated plots than in the control plots at the Griffin location ($F_{1,117} = 1.77$, p value = 0.1862) (Figure 2.12C). The second planting date had more squash bug adults, nymphs, all mobile, eggs, and all bug stages in the treated plots than the control plots at the Watkinsville location, but at the Griffin location, the adult squash bugs were significantly more abundant in the control plot than the treated plot, and the nymphs, all mobile, eggs, and all bug stages were not significantly different between the treated and control plots ($F_{1,166} = 32.24, 12.82, 18.12, 2.98, \text{ and } 3.82$, p value < 0.0001, 0.0004, <.0001, 0.0863, and 0.0522 respectively) (Figures 2.13A, and 2.13B). The yield collected from the second planting date did not differ between the treated and control plots at the Watkinsville location, but was higher in the control plots than in the treated plots at the Griffin location ($F_{1,117} = 12.99$, p value = 0.0005) (Figure 2.13C).

During the summer of 2015, the treated plot had a greater abundance of squash bug at all stages than in the control plot for the Research location, but at the Dempsey location the adult squash bugs were significantly more abundant in the control plot than the treated plot, and the other life stages were not significantly different between the treated and control plots for the first planting date ($F_{1,152} = 14.64, 11.94, 16.42, 2.74, \text{ and } 7.84$, p value = 0.0002, 0.0007, <.0001, 0.0999, and 0.0058 respectively) (Figure 2.14A, and 2.15B). The yield collected from the first planting date was significantly higher in the control plot than the treated plot for the Research and the Dempsey location ($F_{1,152} = 0.09$, p value = 0.7611) (Figure 2.14C). There was no significant difference in the number of *Gryon* sp, and *Geocoris* spp. between the treated and the control plots at both locations for the first planting date ($F_{1,152} = 2.36, \text{ and } 0.02$, p value = 0.1265, and 0.8921 respectively) (Figure 2.14D). The second planting date had more squash bug eggs, and all bugs in the control plot than in the treated plot, but no significant difference in the number of adults, nymphs, and all mobile squash bugs between the treated and control plots at

the Research location. The Dempsey location had more squash bug eggs, adults, and all bugs in the control plot than in the treated plot, but no significant difference in the number of nymphs, and all mobile squash bugs between the treated and control plots ($F_{1,152} = 3.35, 2.29, 3.45, 0.65,$ and 0.16 , p value = $0.069, 0.1327, 0.0651, 0.4213,$ and 0.6918 respectively) (Figure 2.15A, and 2.15B). The yield collected from the second planting date was significantly higher in the control plot than the treated plot for the Research and the Dempsey location ($F_{1,152} = 1.77$, p value = 0.1856) (Figure 2.15C). There was no significant difference in the number of *Gryon* sp, and *Geocoris* spp. between the treated and the control plots at both locations for the second planting date ($F_{1,152} = 0.01$, and 0 , p value = $0.9338,$ and 0.993 respectively) (Figure 2.15D). Complete positive, negative and non-significant effects can be seen in Table 2.1.

Squash Vine Borer

The total number of plants damaged by the squash vine borer *M. cucurbitae* for each year, location, and treatment were totaled (Table 2.2). The treated plot at the Research and Education garden had the greatest damage during the 2015 field season. *M. cucurbitae* was present in 2014, but caused less damage than in 2015.

Discussion

The seasonal abundance data from the summer of 2014 and 2015 indicate that there are two generations of *A. tristis* occurring in Georgia from May to early August. If data were collected after the study completed in August, it is possible that a third generation here in Georgia may occur. The seasonal abundance data also showed how the two different planting dates affected squash bug numbers. Squash bugs are found in greater abundance in the first planting date than in the second planting date. Growers need this information to inform their decisions regarding squash bug management. Decker and Yeargan 2008 posit the idea of

modifying the planting date to avoid growing plants at times when squash bug pressure would likely affect yield. For an early season harvest, transplanted plants could be planted as soon as the frost threat is gone, and the plants would be able to grow in the absence of harmful levels of squash bugs. A later season harvest could benefit from the added floral resources as the *Gryon* sp. parasitoids have had enough time to increase in abundance to provide the necessary control.

Much of the floral resource data is inconsistent from year to year, location to location, and for the two planting dates. There were few instances in which plots with the added floral resources had a lower abundance of squash bugs, and the yield was subsequently higher in the treated plots. This was seen as a positive or successful treatment (Table 2.1). The majority of the data indicate either a non-significant or negative result. Some data show a decrease in squash bug abundance, but a reduction in the yield in the treated plots. This continues the narrative published by previous researchers investigating this method of control (Forehand et al. 2006a, Forehand et al. 2006b). In some instances, there was a successful reduction in squash bug abundance but there was no reduction in the yield. This could be explained by the natural variation in the microhabitats of each individual plot, or more likely the unanticipated attraction of other pest insects. The Squash Vine Borer *Melittia cucurbitae* (Figure 2) was first seen during the summer of 2014, and can likely be attributed to the decline in the yield at sampling date 9 for both planting dates in Griffin (Figure 5C, and 6C). *M. cucurbitae* was seen much earlier during the summer of 2015, and damaged many plants at the Research location, where yield values declined after reaching an early peak (Figure 7C, and 8C). *M. cucurbitae* was present in the Dempsey location, but damage to plants was not as severe as seen in the Research location (Figure 9C, and 10C).

The addition of multi-species of floral resources, theoretically will attract a wide range of beneficial insects and natural predators of the squash pest, *Anasa tristis*, but in this research had the unintended consequence of attracting additional insect pests into the system. This method of cultural control appears to require a very specific mixture and dosage of floral resources so as to attract just enough beneficial insects and natural enemies but not to attract additional pest insects. The land area allocated to floral resources in this study is likely larger than a grower would be comfortable with devoting to a single method of control. Further investigations regarding how much space is required to attract beneficial insects would be useful for farmers so that they do not limit the production of their cash crop. The types of floral resources used in this study could also have caused the attraction of additional pests. The addition of single species floral resources might provide sufficient attraction of beneficial insects (Phillips et al. 2014). Regardless of flower species or area set aside for floral resources, the characteristics of the flower species and the beneficial insects desired should be taken into consideration (Nfzinger and Fadamiro 2010, Grasswitz 2013, Campbell et al. 2012). While a diversity of floral resources is thought to attract a wide range of beneficial insects and natural enemies (Smukler et al. 2010), there seems to be a threshold at which the attraction of additional pest insects overcomes the benefits of the beneficial insects.

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Tables

Table 2.1. Effect of floral resources on squash bug and beneficial insect populations, and yield. Treatment outcome for each response variable: positive (+) negative (-), and non-significant (0) effect for each year, planting date, and locations.

2014		Adults	Nymphs	All Mobile	Eggs	All Bug	Yield		
Planting Date 1	Location 1	-	-	-	-	-	0		
	Location 2	+	+	+	+	+	+		
Planting Date 2	Location 1	-	-	-	-	-	0		
	Location 2	+	0	0	0	0	-		
2015		Adults	Nymphs	All Mobile	Eggs	All Bug	Yield	<i>Gryon</i> sp.	<i>Geocoris</i> spp.
Planting Date 1	Location 1	-	-	-	-	-	-	0	0
	Location 2	+	0	0	0	0	-	0	0
Planting Date 2	Location 1	0	0	0	+	+	-	0	0
	Location 2	+	0	0	+	+	-	0	0

Table 2.2. Squash Vine Borer Damage. Table showing number of plants damaged by squash vine borer for each year location and treatment across all sampling dates.

2014		1	2	3	4	5	6	7	8	9	10	11	12	Total
Watkinsville	Treated	0	0	0	0	0	0	0	0	1	0	0	0	1
	Control	0	0	0	0	1	2	3	1	1	0	0	0	8
Griffin	Treated	0	0	0	0	0	0	0	1	1	0	0	0	2
	Control	0	0	0	0	0	1	2	2	1	0	0	0	6
2015														
Research	Treated	0	1	1	3	4	4	6	1	0	0	0		20
	Control	0	0	0	0	1	2	1	0	0	0	0		4
Dempsey	Treated	0	0	0	0	0	1	2	0	0	0	0		3
	Control	0	0	1	0	0	1	1	0	0	0	0		3

Figures

Figure 2.1. Experimental Plot Design. Image shows arrangement of floral resources within the treated plot. Floral resources border the rows of squash in addition to a row of flowers in the middle of the four rows of squash.



Figure 2. 2. Images of *Anasa tristis* Life Stages, and *Gryon* sp Parasitoid. (Left) Image of adult *Anasa tristis* laying eggs on a squash leaf. (Middle) Image showing recently hatched nymphs found during visual observations on squash. (Right) Image showing *A. tristis* eggs parasitized by one of the egg parasitoids *Gryon* sp.



Figure 2.3. Images of the Squash Vine Borer *Melittia cucurbitae*. The left image is of the larvae consuming the vine tissue. The image on the right is an adult on the leaf of a squash plant.

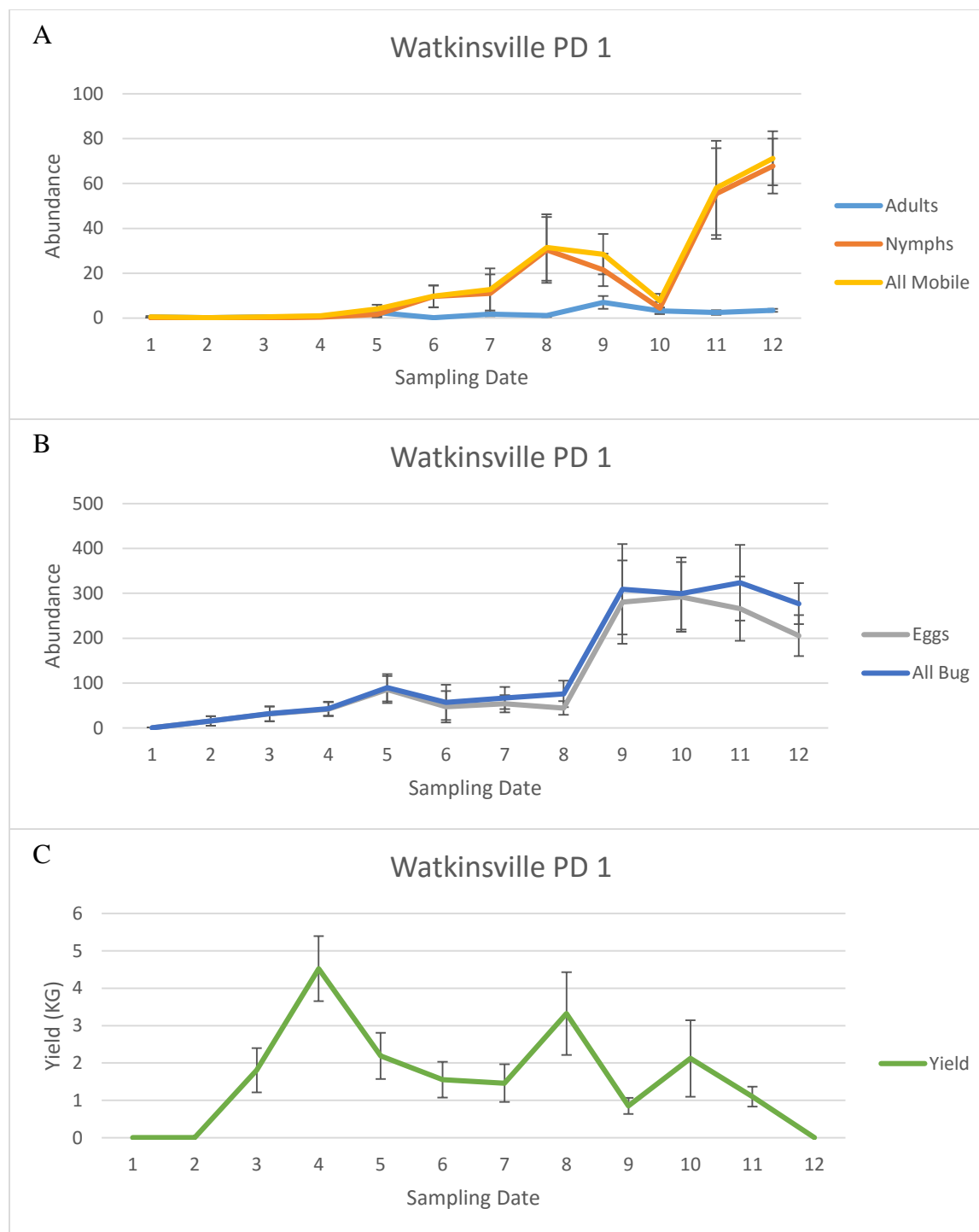


Figure 2.4. Watkinsville First Planting Date Seasonal Abundance. Graph showing the mean (\pm SE) number of squash bugs observed and yield collected during the summer of 2014 at the Watkinsville location for the first planting date at each sampling date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield.

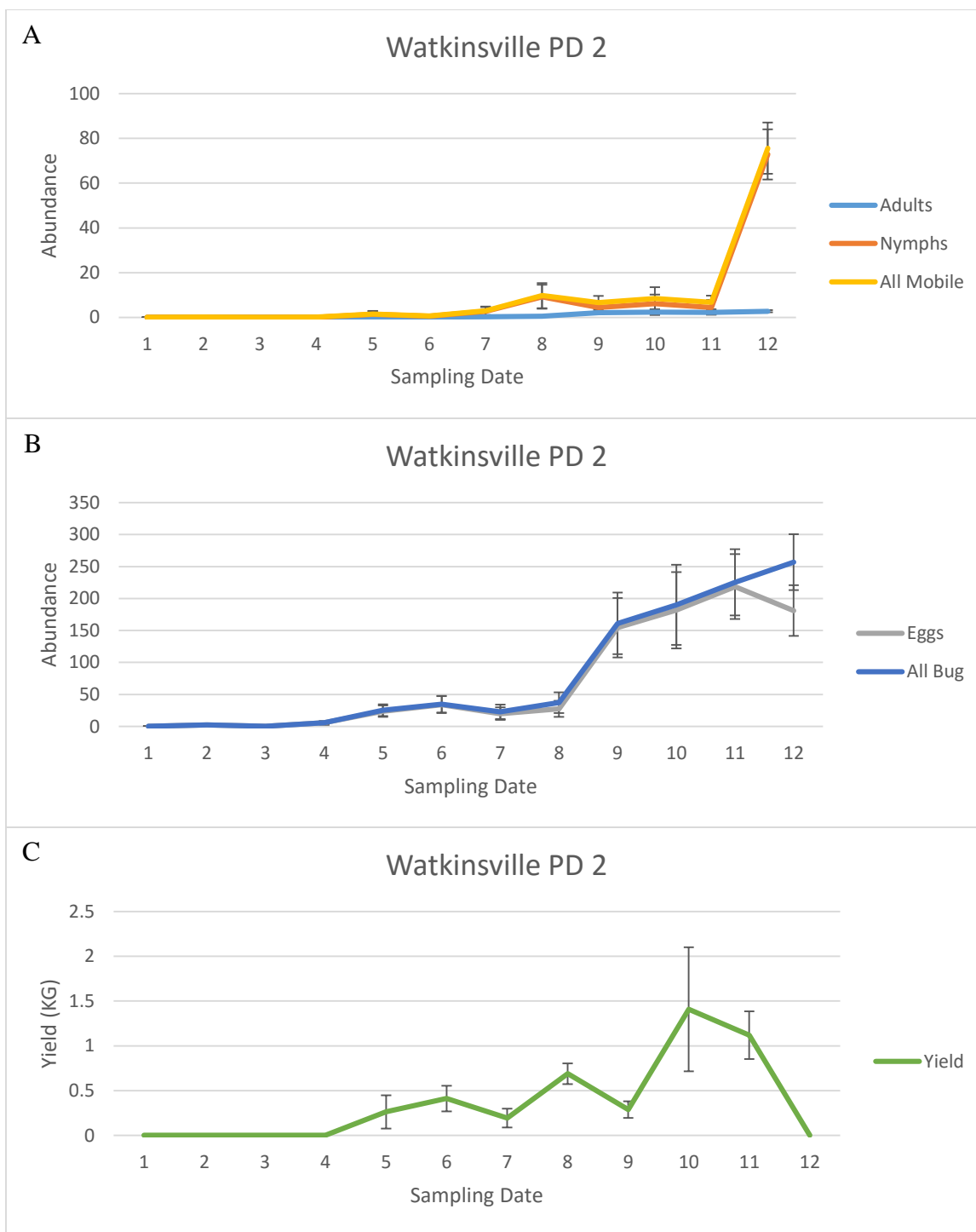


Figure 2.5. Watkinsville Second Planting Date Seasonal Abundance. Graph showing the mean (\pm SE) of squash bugs observed and yield collected during the summer of 2014 at the Watkinsville location for the second planting date at each sampling date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield.

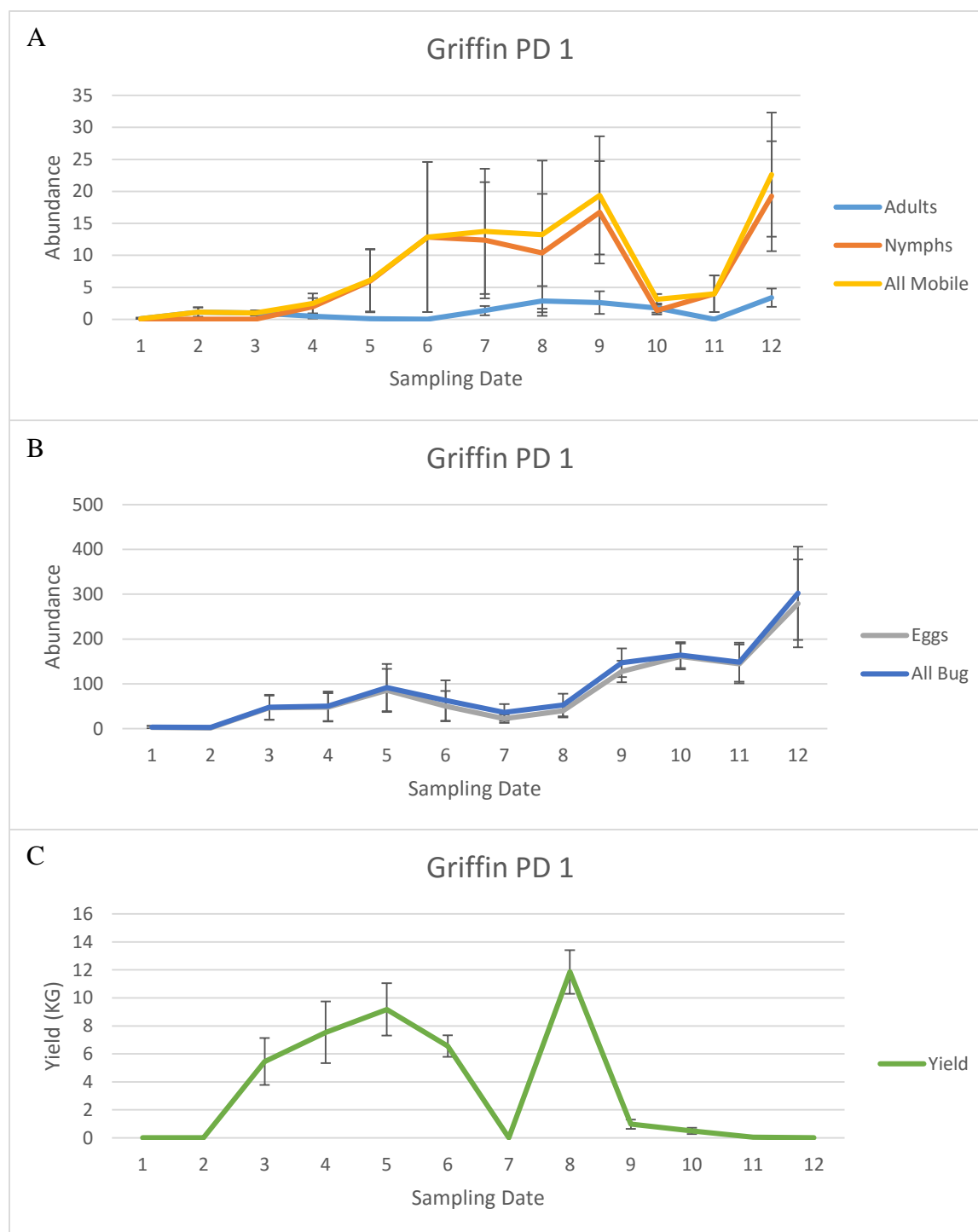


Figure 2.6 Griffin First Planting Date Seasonal Abundance. Graph showing the mean (\pm SE) of squash bugs observed and yield collected during the summer of 2014 at the Griffin location for the first planting date at each sampling date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield.

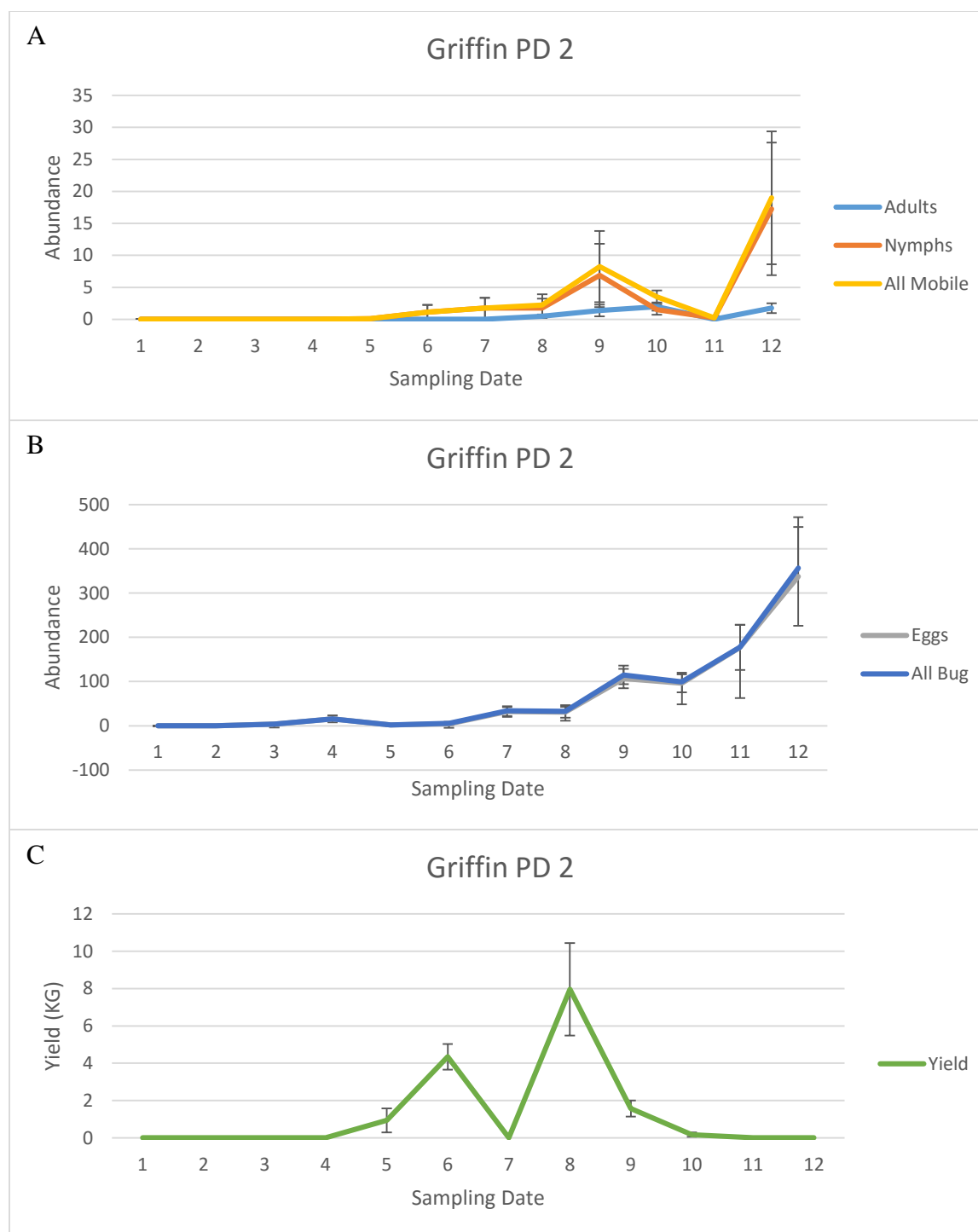


Figure 2.7. Griffin Second Planting Date Seasonal Abundance. Graph showing the mean (\pm SE) of squash bugs observed and yield collected during the summer of 2014 at the Griffin location for the second planting date at each sampling date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield.

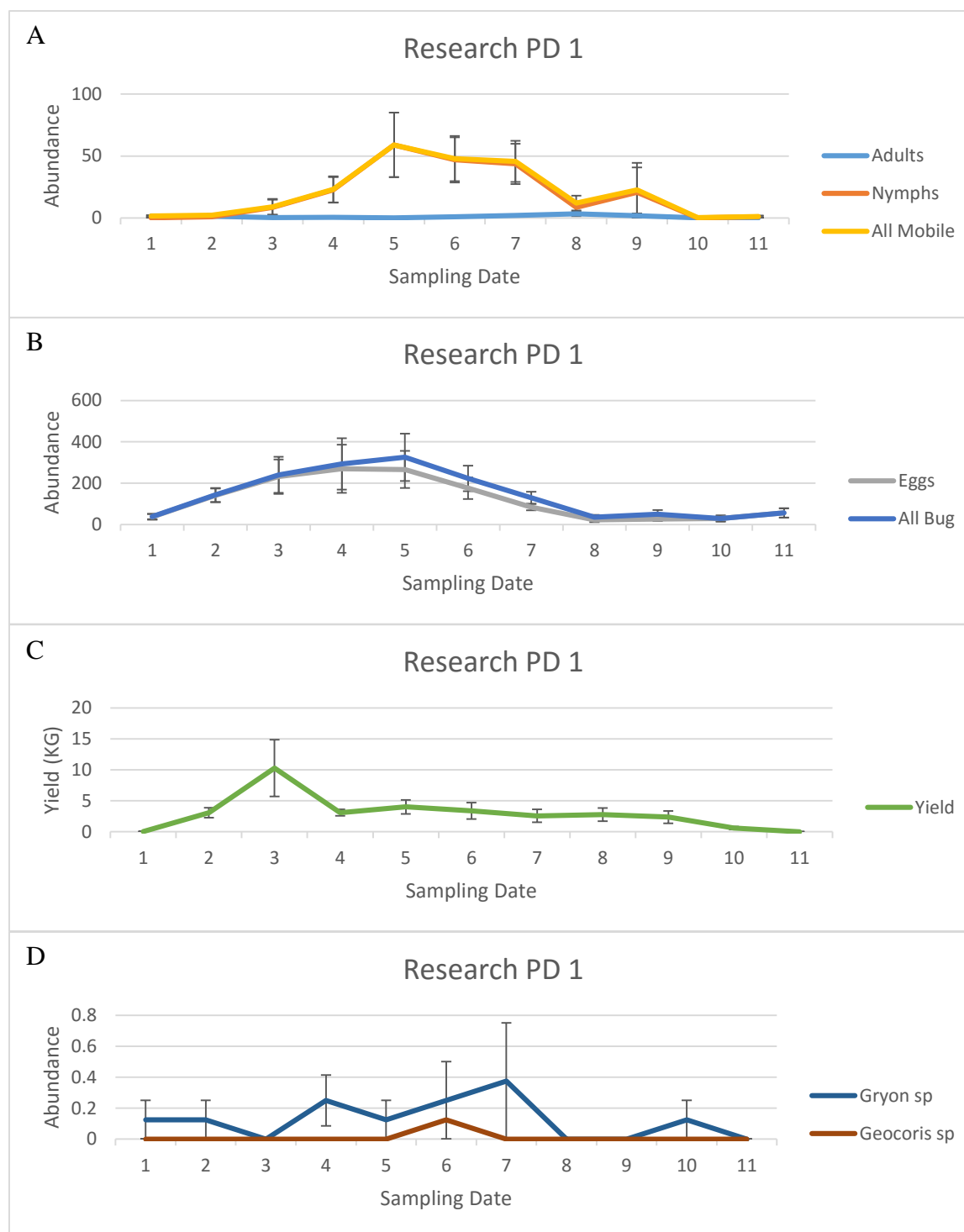


Figure 2.8. Research First Planting Date Seasonal Abundance. Graph showing the mean (\pm SE) of squash bugs observed and yield collected during the summer of 2014 at the Griffin location for the second planting date at each sampling date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield.

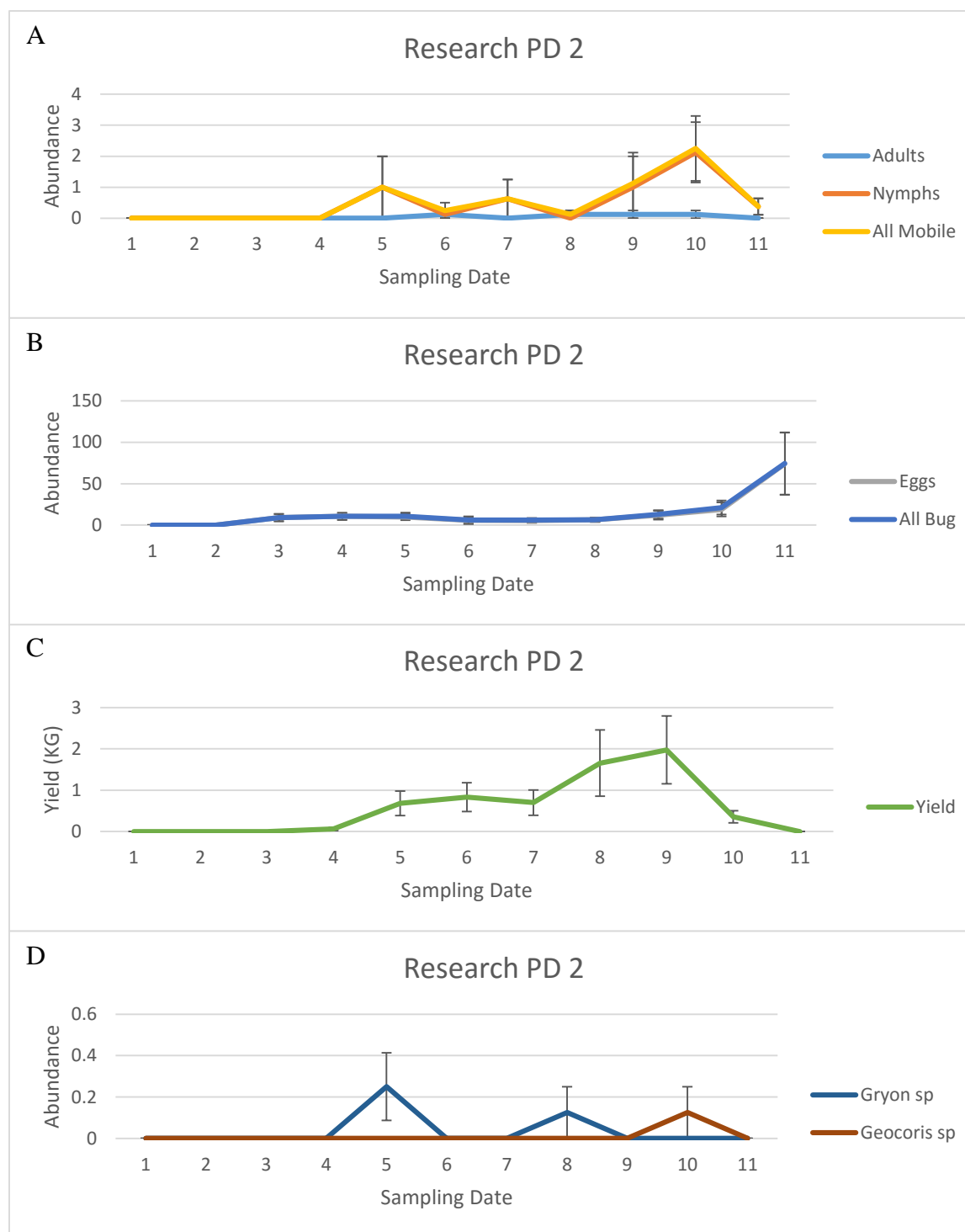


Figure 2.9. Research Second Planting Date Seasonal Abundance. Graph showing the mean (\pm SE) of squash bugs, and beneficial insects observed and yield collected during the summer of 2015 at the Research location for the second planting date at each sampling date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield (D) *Gryon* sp., and *Geocoris* spp.

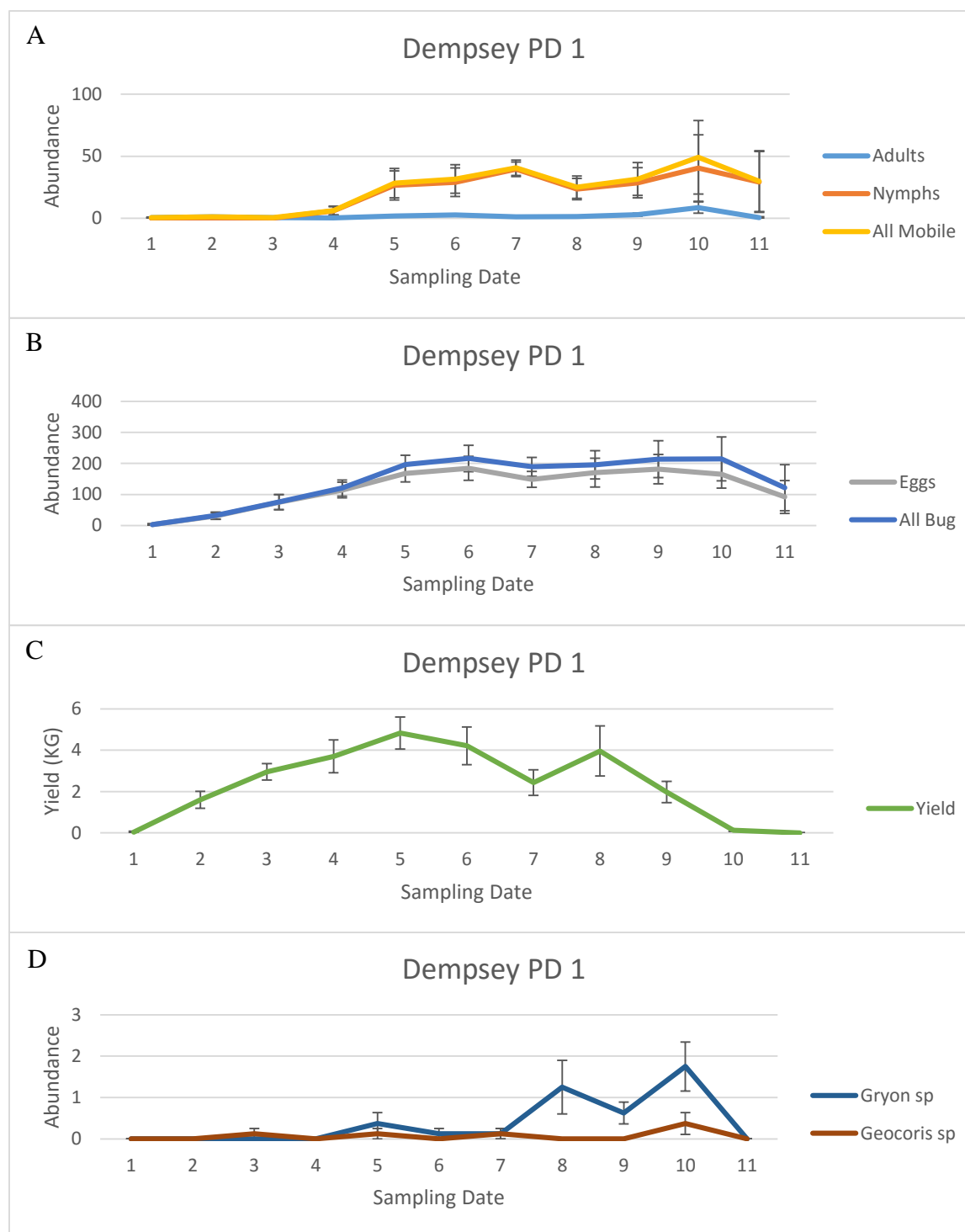


Figure 2.10. Dempsey First Planting Date Seasonal Abundance. Graph showing the mean (\pm SE) of squash bugs, and beneficial insects observed and yield collected during the summer of 2015 at the Dempsey location for the first planting date at each sampling date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield (D) *Gryon* sp., and *Geocoris* spp.

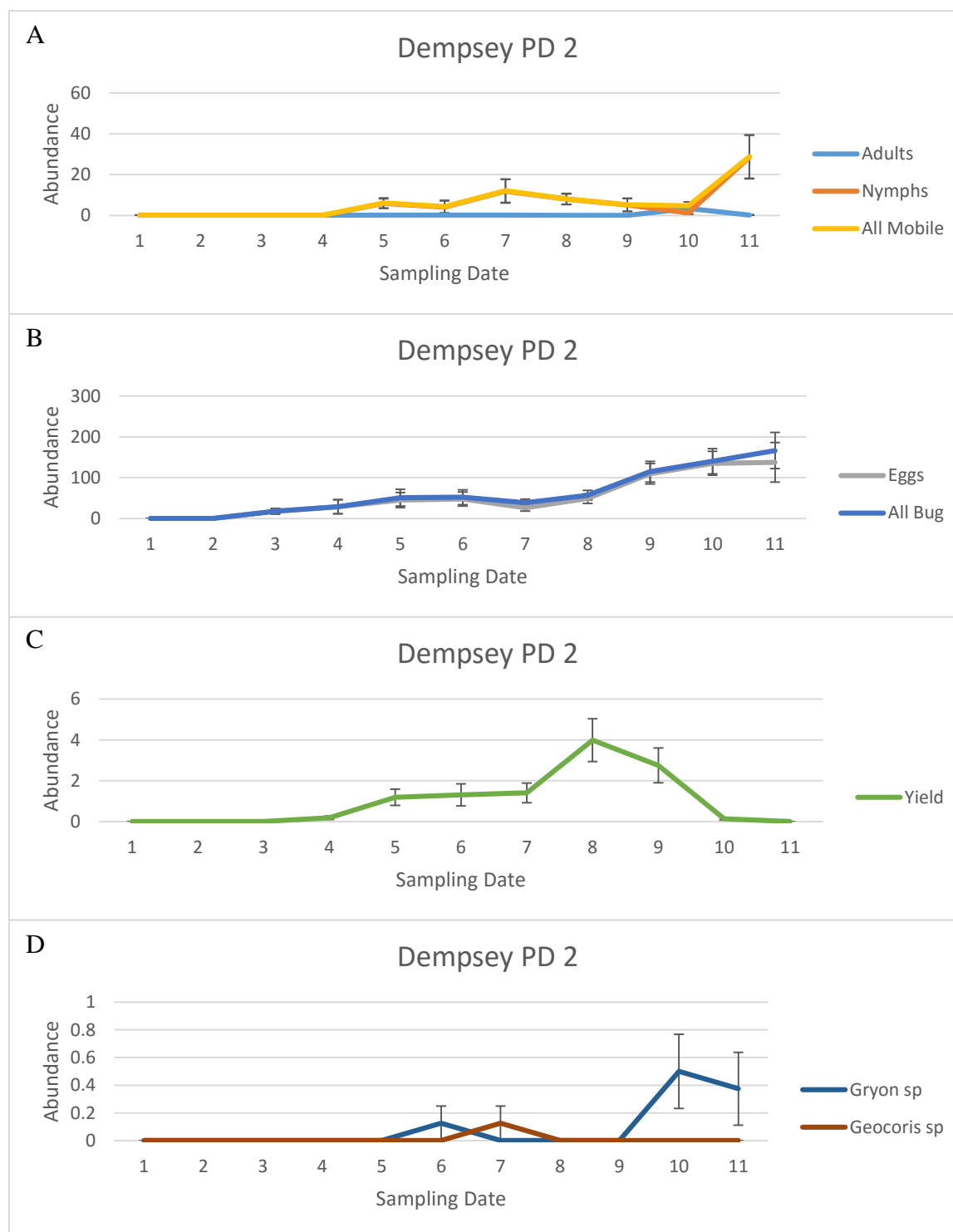


Figure 2.11. Dempsey Second Planting Date Seasonal Abundance. Graph showing the mean (\pm SE) of squash bugs, and beneficial insects observed and yield collected during the summer of 2015 at the Dempsey location for the Second planting date at each sampling date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield (D) *Gryon* sp., and *Geocoris* spp.

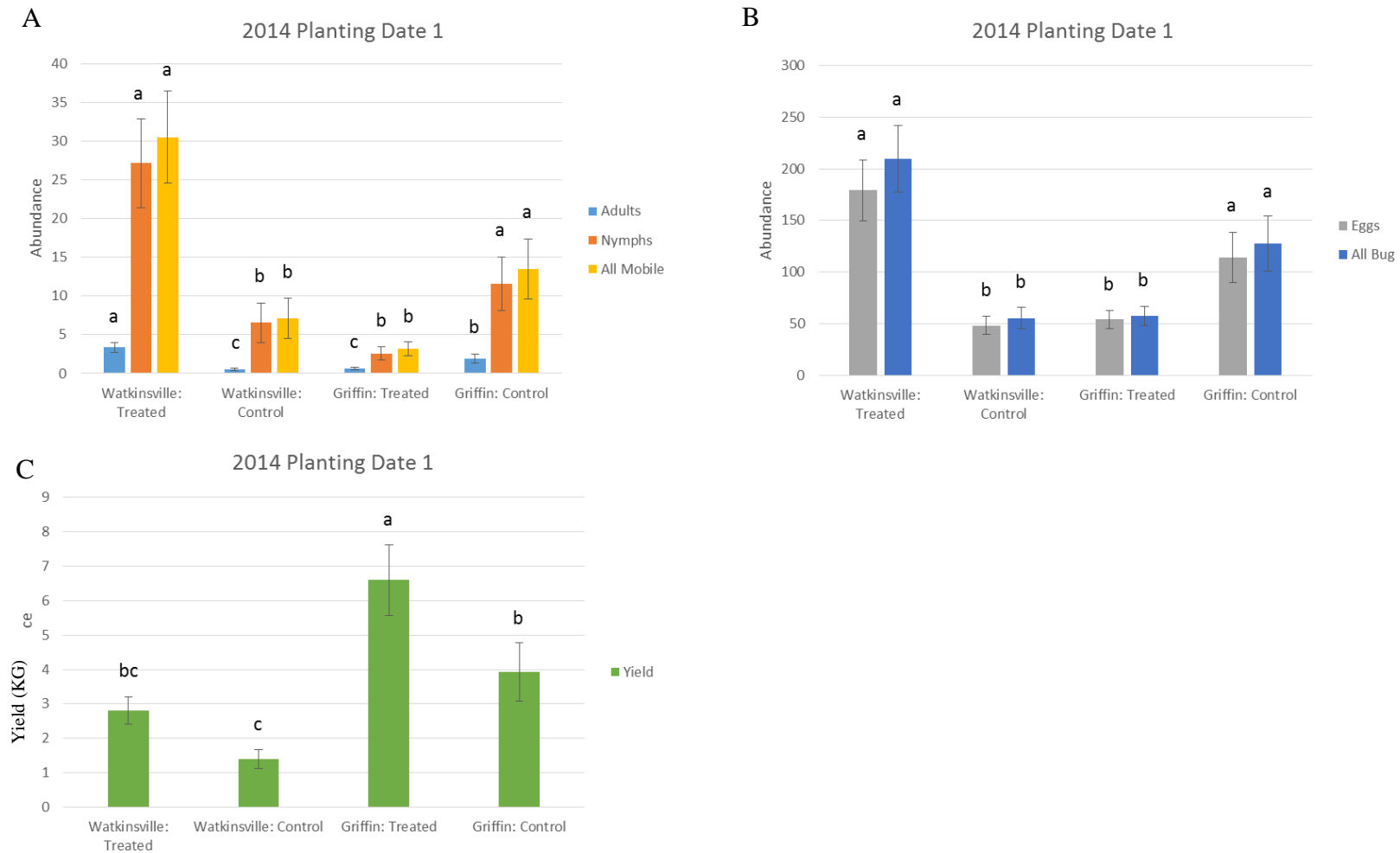


Figure 2.12. 2014 Data First Planting Date Floral Resources. Graphs showing the mean (\pm SE) number of squash bugs observed and yield collected during the summer of 2014 for treated (with floral resources) and control (without floral resources) plots at both locations for the first planting date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield. Different letters indicate significant differences ($\alpha = 0.05$).

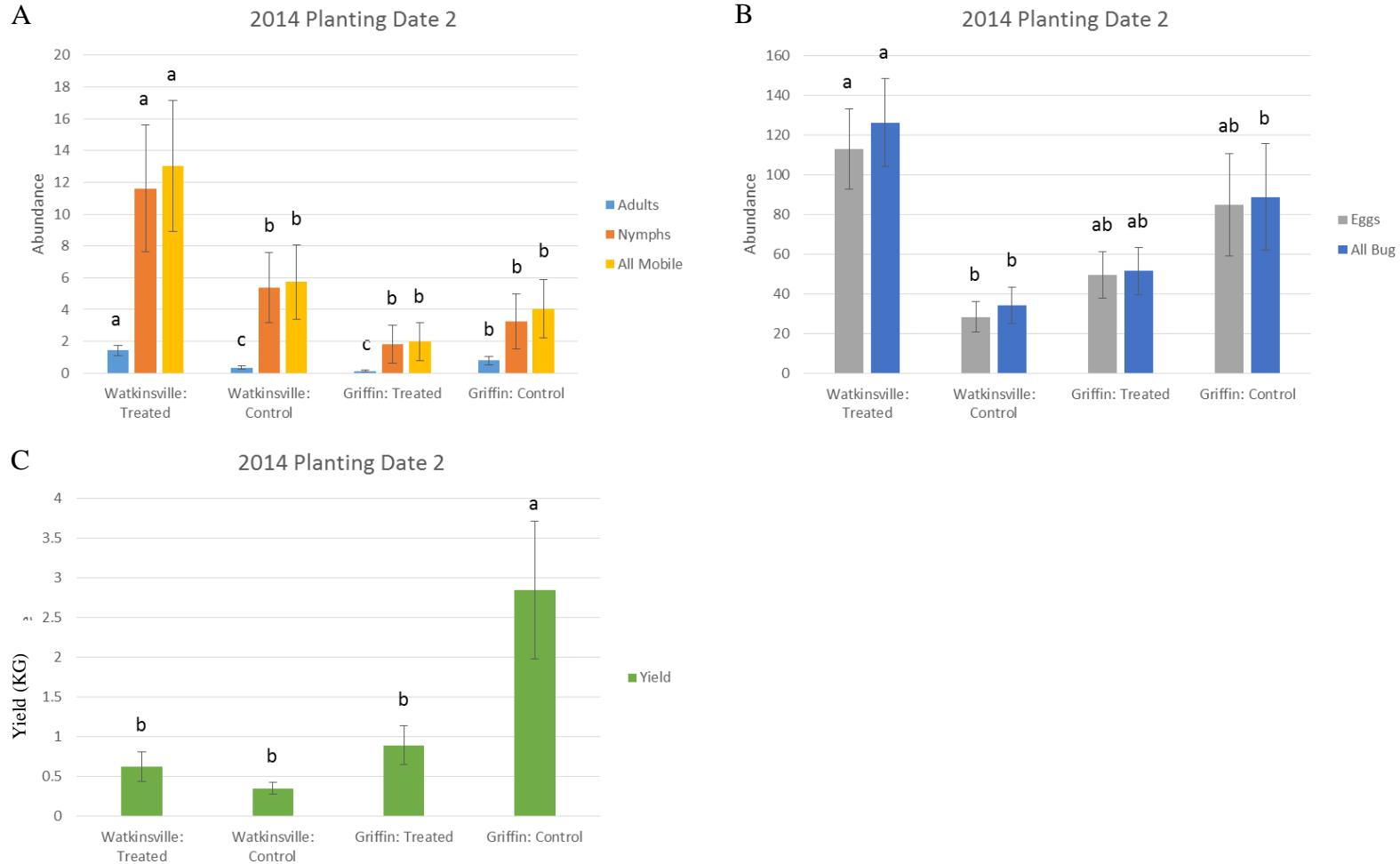


Figure 2.13. 2014 Data Second Planting Date Floral Resources. Graphs showing the mean (\pm SE) number of squash bugs observed and yield collected during the summer of 2014 for treated (with floral resources) and control (without floral resources) plots at both locations for the second planting date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield. Different letters indicate significant differences ($\alpha = 0.05$).

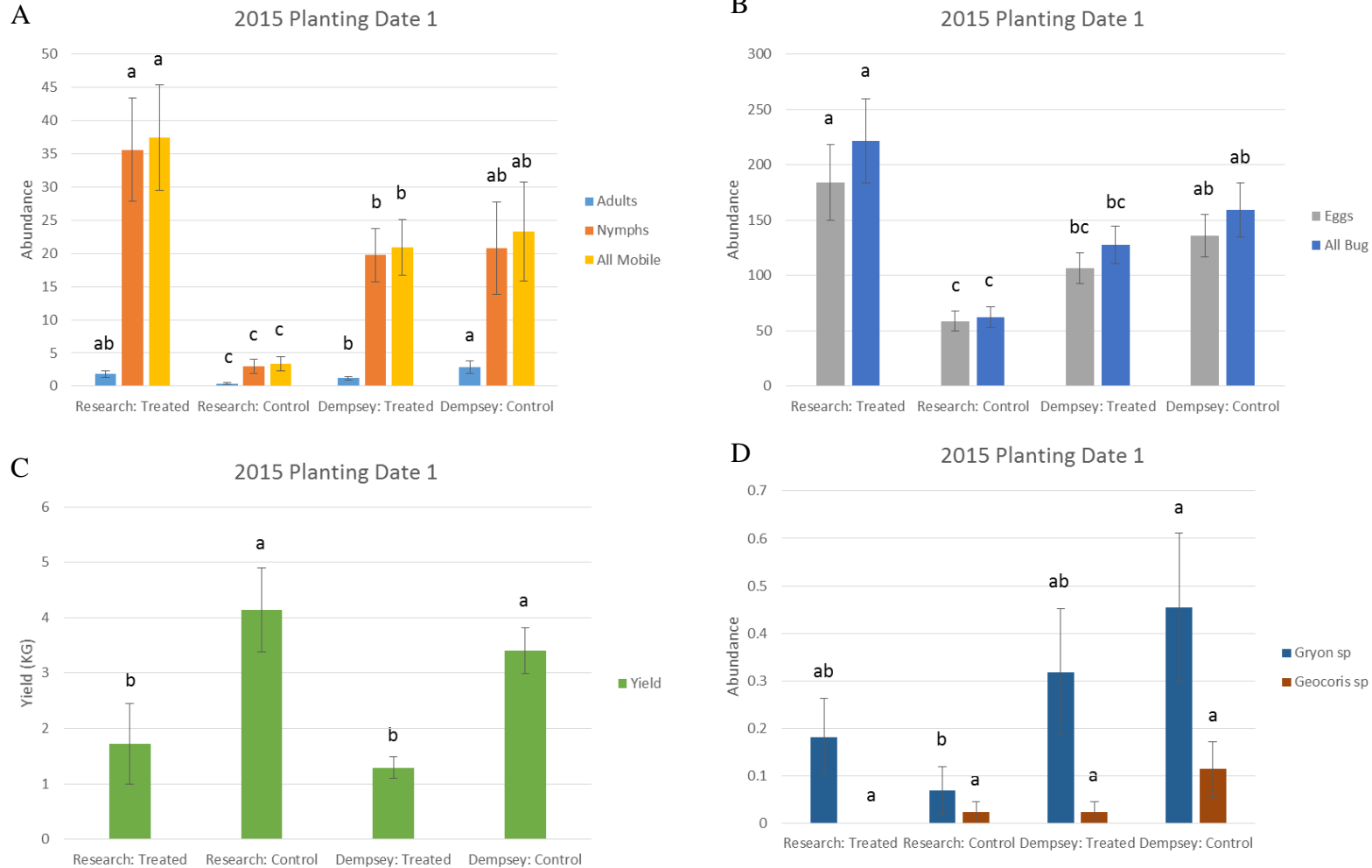


Figure 2.14. 2015 Data First Planting Date Floral Resources. Graphs showing the mean (\pm SE) number of squash bugs, and beneficial insects observed and yield collected during the summer of 2015 for treated (with floral resources) and control (without floral resources) plots at both locations for the first planting date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield (D) *Gryon sp.*, and *Geocoris spp.* Different letters indicate significant differences ($\alpha = 0.05$).

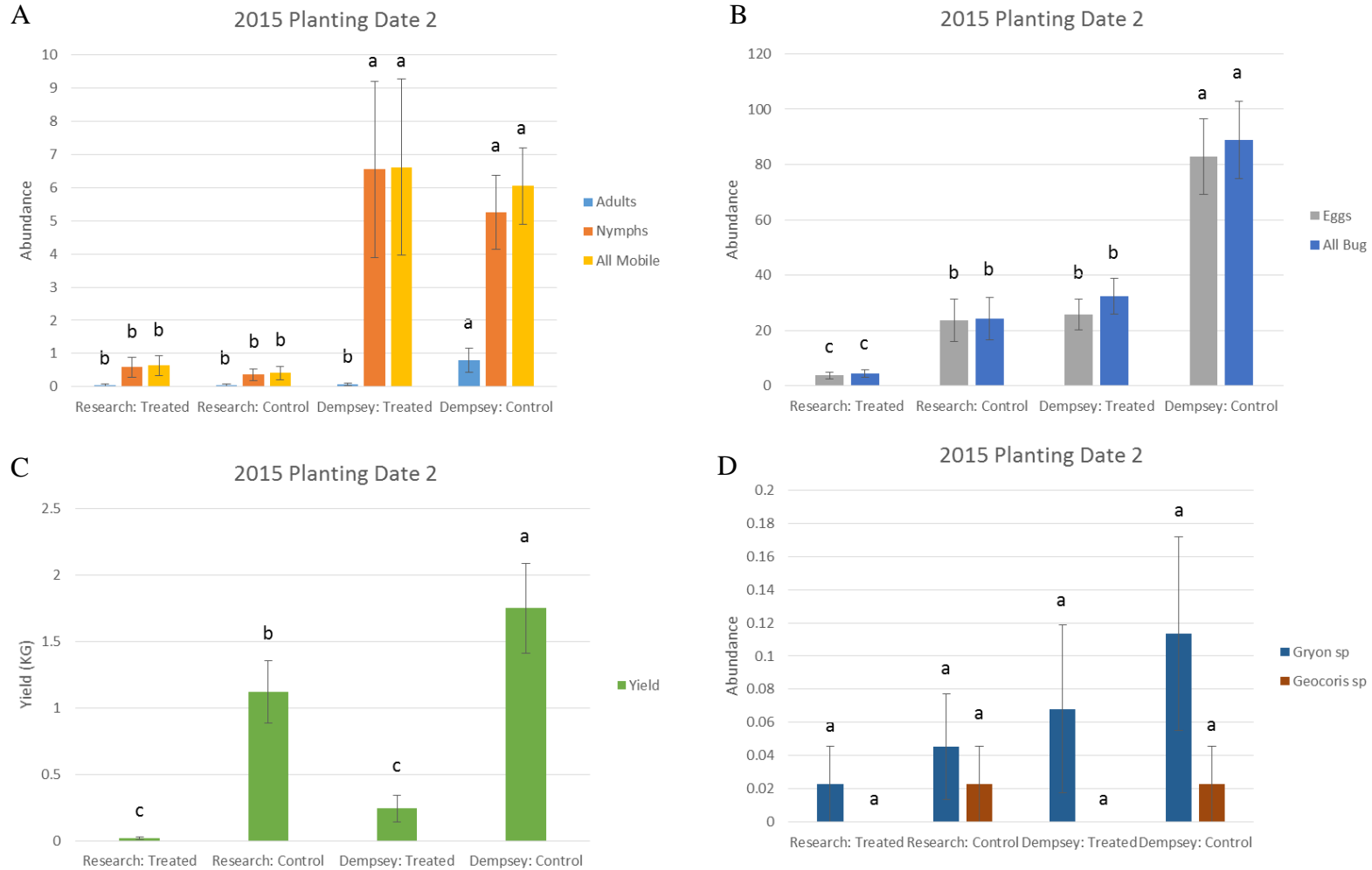


Figure 2.15. 2015 Data Second Planting Date Floral Resources. Graphs showing the mean (\pm SE) number of squash bugs, and beneficial insects observed and yield collected during the summer of 2015 for treated (with floral resources) and control (without floral resources) plots at both locations for the second planting date. (A) Adults, Nymphs, and All Mobile (B) Eggs, and All Bug (C) Yield (D) *Gryon* sp., and *Geocoris* spp. Different letters indicate significant differences ($\alpha = 0.05$).

CHAPTER 3
ASSESSMENT OF HABITAT MODIFICATION FOR BENEFICIAL INSECT ABUNDANCE
IN SQUASH²

² Fair, Conor, and Braman, Kris. To be submitted to Environmental Entomology.

Abstract

Georgia is the 5th largest producer of squash in the United States. The squash bug *Anasa tristis* (DeGeer) is an indigenous pest of squash and other cucurbits. Previous research has shown pesticides to be an effective form of control, but many growers seek alternative methods of management. Farmscaping is a method of cultural control which can be used to promote biological pest management using floral resources to attract beneficial insects to help control the pest populations. A common practice in farmscaping is to add floral resources with crops to attract more natural enemies and other beneficial insects like pollinators. In the summer of 2014 and 2015, field plots of squash were separated by a minimum of 150 meters and had floral resources planted along side with the squash. Pit fall traps and yellow pan traps were placed in both treated and control plots to test if floral resources increased abundance of beneficial insects. The abundance of Carabidae was higher in the treated than in the control plot for the Watkinsville, Griffin, and Dempsey, but not the Research location ($F_{1,11} = 2.65$, p value = 0.106), and ($F_{1,138} = 8.95$, p value = 0.0033) respectively. Sentinel egg masses placed in the field, then collected and reared in the lab resulted in no parasitism. Floral resources varied on location and year in their ability to attract beneficial insects. Furthermore, greater taxonomic resolution of the parasitic Hymenoptera might help further understand if there are *A. tristis* parasitoids attracted by these floral resources.

Introduction

In 2014, squash production in the Southeast accounted for 30.7% of the United States total acreage harvested (USDA 2015). Georgia (at 8.3%) was the 5th largest producer in the country, and 2nd largest in the Southeast. One major concern for squash producers is the squash bug, *Anasa tristis* (DeGeer). *A. tristis* is an indigenous pest of squash and other members of the Cucurbitaceae family including: pumpkin, watermelon, and zucchini. *A. tristis* causes plant damage by feeding, and through the transmission of the pathogen *Serratia marcescens* Bizio, which causes cucurbit yellow vine disease (Beard 1935, Bruton et al. 2003, and Pair et al. 2004). Research investigating the proper timing of pesticide applications showed that chemical control methods are successful in controlling squash bug populations (Palumbo et al. 1993). Furthermore, systemically treated squash (<1% of total hectareage) and semiochemical toxic baits can control early squash bug populations (Pair 1997). While these methods are shown to be successful in controlling squash bug populations, they were developed with large commercial growers in mind. Many of the small-scale growing operations do not have the materials or the ability to use such chemicals based on limited funds or regulations from organic certifications. The growing number of small-scale commercial vegetable growers who are producing for fresh markets in urban areas such as Atlanta, Athens, Savannah, Macon, and Columbus have different needs in terms of pest management.

Farmers have long been using beneficial insects to help aid them in controlling pest insects and increase pollination. There are many generalist predators and parasitoids that can be used to help farmers control squash bug populations. Spiders (Lycosidae and Linyphiidae) hemipteran predators *Geocoris punctipes* (Say), *G. uliginosis* (Say), beetles *Coccinella septempunctata* (Linnaeus), *Coleomegilla maculata* (DeGeer), *Hippodamia convergens* (Guérin-

Méneville), and species of Carabidae and Staphylinidae have been shown to be prominent predators of squash bugs (Decker and Yeargan 2008, Rondon et al. 2003, Schmidt et al. 2014). Rates of predation have ranged from 2-7% in one study to 11% in another (Decker and Yeargan 2008, Schmidt et al. 2014). Additionally, there are many parasitoids of the squash bug. One parasitoid of the nymph and adult stages is *Trichopoda pennipes*, a tachinid fly. There are three *A. tristis* egg parasitoid wasp species of the genus *Gryon* (Platygastridae): *Gryon pennsylvanicum* (Ashmead), *G. anasae* (Ashmead), and *G. carinatifrons* (Ashmead). In addition, there are three parasitoid species of *Ooencyrtus* (Encyrtidae): *Ooencyrtus anasae* (Ashmead) *O. sp.* ‘light form’ and *O. sp.* ‘dark form’ (Ashmead 1886 and Nechols et al. 1989). Conservative estimates have *T. pennipes* parasitizing up to 30% of adults, and *G. pennsylvanicum* parasitizing up to 31.4% of eggs (Decker and Yeargan 2008). However, egg parasitoid abundance was not high during the early season to provide the important early season control needed to suppress squash bug numbers. Farmers could purchase and release these parasitoids to potentially gain early season control, but costs are often very high. Additional measures can be taken to help these parasitoids control pest populations.

Conservation farming has gained a lot of interest in small-scale operations throughout the country. Methods of control include ecosystem engineering or farmscaping. Farmscaping refers to the addition of floral resources to support naturally occurring beneficial insects, which can aid in the management of pest species (Bugg and Pickett 1998). Adding floral resources and other plant species in strategic locations can also help reduce erosion, reduce runoff of agrochemicals, and they serve as an additional source of funds when sold as cut flowers. The floral resources attract and act as a supplemental food source for many beneficial insects, including many of the beneficial insects known to prey on the squash bug and other insect pests. However, there is also

the potential for additional pest insects to be attracted by the added sources of vegetation and nectar. Floral resource characteristics, such as floral attraction, nectar accessibility, and nutritional suitability, must be considered to meet the needs of the beneficial insects and reduce the risk of potential pest outbreaks (Wackers and Van Rijn 2005, Wackers et al. 2007, Winkler et al. 2010, Philips et al. 2014). There has been some research investigating potential candidates for floral resources to be used in farmscaping. Buckwheat (*Fagopyrum esculentum* Moench) has been shown to increase the lifespan of female parasitic wasps by approximately 6 days as compared to a water only diet (Nfziger and Fadamiro 2010). Dill (*Anethum graveolens* L.), Cosmos (*Cosmos bipinnatus* Cav.), Calendula (*Calendula officinalis* L.), and Marigolds (*Tagetes patula* L.) have been included in previous studies to attract beneficial insects (Grasswitz 2013, and Martinez-Uña et al. 2013). Other floral resources can be considered for their varying flower morphology, and the timing of their maturity so they coincide with other floral resources chosen. The objective of this study is to determine if a multi-species floral resource attracts beneficial insects and if the increase in beneficial insect abundance affects squash bug populations and yield.

Materials and Methods

Study Area and Experimental Design

The study was conducted over two summers (April-August) in 2014 and 2015. In 2014, the two locations were the University of Georgia's Horticulture farm in Watkinsville GA, and the Research and Education Garden in Griffin GA. In 2015, the two locations were the Dempsey Farm and University of Georgia's Research and Education Garden in Griffin GA. Plots (7.2 m x 15.84 m) were set up as unrandomized strips in a one way design consisting of five rows (four rows utilized for planting squash, and the middle row and surrounding buffer for the floral

resources) and a 0.6 meter buffer surrounding the plot with four contiguous blocks (Figure 2.1). There were two treatments 1) with floral resources (treated), and 2) without floral resources (control) at the whole-plot level. Treated and control plots at each location were separated by a minimum of 150 meters. The anticipated spillover effect of floral resources prevented our use of the more typical RCB design where blocks with and without flowers would be adjacent. Treatments were separated by a significant distance in order to better understand the value of beneficial insect habitat.

Experimental Plots

Plots were directly sowed with the straightneck squash *Curcubita pepo* L. variety ‘Zephyr’ (Johnny’s Selected Seeds, Waterville, ME). Three seeds were planted in the center of the beds every 0.6 m and were thinned to one plant immediately following seedling emergence. Plots were lightly mulched with wheat straw to maintain moisture during germination. The treated plots had the middle row and buffers equally hand-broadcast planted in sand with a floral mix including the following species intended to attract beneficial insects: **Buckwheat** (*Fagopyrum esculentum*), **White Dill** (*Anethum graveolens*), **Cosmos** (*Cosmos bipinnatus*), **Calendula** (*Calendula officinalis*), **Centaurea** (*Centaurea cyanus*), **Hybrid Dwarf Sunflowers** **Baby’s Breath** (*Helianthus annuus* var. Covent Market Garden), **Nasturtiums** (*Tropaeolum majus*), **Zinnias** (*Zinnia elegans*), and **n** (*Gypsophila elegans*) and **Johnny’s Select Seeds Beneficial Insect Attractant Mix**. The control plots were prepared in the same manner but did not have flowers planted in the middle or buffers. Planting dates were chosen based on soil temperature recommendations (above 17°C). In 2014, planting occurred on April 17th in Watkinsville, and April 10th in Griffin. In 2015, planting occurred on April 7th for both locations. A second planting of squash occurred one month later for both years. Results from

soil tests informed lime and fertilizer treatments. Irrigation amounts were adjusted based on natural rainfall to prevent overwatering.

Data Collecting

Pit fall traps and yellow pan traps (four of each at each location) filled with water and detergent were randomly placed in all plots each week for ten weeks (Figure 3.1, and 3.2). Samples were collected from the field after 72-96 hours had passed, and were taken back to the lab to be sorted and counted. Only specimens of the most abundant predator or omnivore or pollinator taxa are considered here. Data are combined for taxa in which immature and adult stages were present. Additionally, two sentinel egg masses per block were used during the second year to determine percent parasitism weekly

Data Analysis

A generalized linear mixed model was applied to determine the influence of sampling date, and the interaction between location and treatment on beneficial insect abundance. The data collected was subjected to ANOVA using a generalized linear mixed model (PROC GLIMMIX, SAS Software) for count data. The additional category of 'Pollinator' is comprised of Mordellidae, Halictidae, Apidae, Syrphidae, and Dolichopodidae families. Differences in least square means were determined by pairwise t-tests ($\alpha = 0.05$) as the multiple comparisons post hoc test to determine significant differences between levels of all factors. Least squared means was used as the multiple comparisons post hoc test to measure difference between levels of all factors. The negative binomial distribution was used to model the beneficial insect abundance data.

Results

Sentinel Egg Masses

Of all the 32 egg masses placed in the field during the summer of 2015, there were no parasitoids that emerged, nor were there any eggs that did not hatch.

Seasonal Abundance

The data collected using the pit fall traps shows various peaks and valleys of beneficial insect and natural enemy abundance throughout the duration of sampling dates. Important predators such as Araneae and Carabidae peaked mid-season at the Watkinsville, Griffin, and Research locations, but had high abundance throughout the middle into the end of the season at the Dempsey location (Figure 3.3A, 3.3B, 3.4B, 3.5B, and 3.6B.). Parasitic Hymenoptera and predaceous Hemiptera peaked at various sampling dates depending on the year and location (Figure 3.3A, 3.3B, 3.4A, 3.5A, and 3.6A). *Geocoris* spp. never peaked in great abundance for any year or location except at the very end of the season at the Watkinsville location (Figure 3.3B, 3.4B, 3.5B, and 3.6B).

The data collected using the yellow pan traps show many inconsistent peaks and valleys for each beneficial insect and natural enemy between location and year sampled. The abundance of pollinators, predaceous Hemiptera, and parasitic Hymenoptera peaked early in the season for both the Watkinsville and Griffin locations in the summer of 2014, but the abundance had a mid-season peak for the Research and Dempsey locations in the summer of 2015 (Figure 3.9A, 3.10A, 3.11A, and 3.12A). The abundance of Araneae, *Geocoris* sp, *Linepithema humile*, and *Solenopsis invicta* had various peaks with a few increases of abundance in the ants towards the end of the season (Figure 3.9B 3.10B, 3.11B, and 3.12B).

Floral Resources

The data collected using the pit fall traps in 2014 and 2015 showed varying results in attracting beneficial insects and natural enemies to the treated plots. The abundance of parasitic Hymenoptera was significantly greater in the control plots at the Watkinsville location, and there was no significant difference between the treated and control plots at the Griffin location ($F_{1,131} = 2.19$, p value = 0.1412) (Figure 3.7A). The abundance of parasitic Hymenoptera was significantly greater in the control plots at the Research location, and there was significantly higher abundance found in the treated plot than found in the control plot at the Dempsey location ($F_{1,138} = 216.28$, p value = $<.0001$) (Figure 3.8A). The abundance of Hemiptera was not significantly different between the control plots at the Watkinsville location, and there was significantly greater abundance found in the treated plot than found in the control plot at the Griffin location ($F_{1,131} = 4.62$, p value = 0.0335) (Figure 3.7A). The abundance of Hemiptera was significantly greater in the treated plot than the control plot at the Research location, and there was no significant difference in abundance between the treated and control plots at the Dempsey location ($F_{1,138} = 12.77$, p value = 0.0005) (Figure 3.8A). The abundance of Araneae was not significantly different between the treated and control plots at the Watkinsville location, and there was significantly greater abundance found in the treated plot than found in the control plot at the Griffin location ($F_{1,131} = 6.29$, p value = 0.0134) (Figure 3.7B). The abundance of Araneae was not significantly different between the treated and control plots at the Research location, and there was significantly greater abundance found in the treated plot than found in the control plot at the Dempsey location ($F_{1,138} = 4.31$, p value = 0.0397) (Figure 3.8B). The abundance of Carabidae was significantly higher in the treated plot than in the control plot for both the Watkinsville and Griffin locations ($F_{1,11} = 2.65$, p value = 0.106) (Figure 3.7B). The

abundance of Carabidae did not significantly differ between the treated and control plots at the Research location, but the Carabidae abundance was significantly higher in the treated plot than in the control plot for the Dempsey location ($F_{1,138} = 8.95$, p value = 0.0033) (Figure 3.8B). The abundance of *Geocoris* spp. did not significantly differ between the treated and control plots for all locations ($F_{1,131} = 0.11$, and $F_{1,138} = 0.74$, p value = 0.7376, and 0.3922 respectively) (Figure 3.7B, and 3.8B). The abundance of *Linepithema humile* was significantly higher in the treated plot than in the control plot at the Watkinsville location, and there was no significant difference between the treated and control plots at the Griffin location ($F_{1,131} = 7.9$, p value = 0.0057) (Figure 3.7C). The abundance of *Linepithema humile* was significantly higher in the treated plot than in the control plot at both the Research and Dempsey locations ($F_{1,138} = 0.92$, p value = 0.338) (Figure 3.8C). The abundance of *Solenopsis invicta* was significantly greater in the control plot than in the treated plot for both the Watkinsville and Griffin locations ($F_{1,131} = 0.59$, p value = 0.4427) (Figure 3.7C). The abundance of *Solenopsis invicta* was not significantly different between the treated and control plots at the Research locations, and was significantly greater in the treated plot than in the control plot at the Dempsey location ($F_{1,138} = 14.73$, p value = 0.0002) (Figure 3.8C).

The data collected using the yellow pan traps in 2014 and 2015 showed few instances where the floral resources attracted beneficial insects and natural enemies to the treated plots. The abundance of parasitic Hymenoptera was significantly greater in the control plots at both the Watkinsville and Griffin locations ($F_{1,110} = 1.57$, p value = 0.2126) (Figure 3.13A). The abundance of parasitic Hymenoptera was significantly greater in the control plot than the treated plot at the Research location, and there was no significant difference between the treated and control plot at the Dempsey location ($F_{1,124} = 5.86$, p value = 0.0169) (Figure 3.14A). The

abundance of Hemiptera was not significantly different between the control plots at the Watkinsville location, and there was significantly greater abundance found in the treated plot than found in the control plot at the Griffin location ($F_{1,110} = 4.73$, p value = 0.0317) (Figure 3.13A). The abundance of Hemiptera was significantly greater in the treated plot than the control plot at the Research location, and there was no significant difference in abundance between the treated and control plots at the Dempsey location ($F_{1,124} = 1.9$, p value = 0.17) (Figure 3.14A). The abundance of Araneae was significantly greater in the control plot than the treated plot at the Watkinsville location, and there was no significant difference between the treated and control plots at the Griffin location, ($F_{1,110} = 10.63$, p value = 0.0015) (Figure 3.13B). The abundance of Araneae was not significantly different between the treated and control plots at both the Research and Dempsey locations ($F_{1,124} = 0.28$, p value = 0.5976) (Figure 3.14B). The abundance of pollinators was not significantly different between the treated and control plots at both the Watkinsville and Griffin locations ($F_{1,110} = 2.75$, p value = 0.1003) (Figure 3.13A). The abundance of pollinators was significantly higher in the treated plot than the control plot at the Research location, but the abundance of pollinators did not significantly differ between the treated and control plots at the Dempsey location ($F_{1,124} = 0.2$, p value = 0.6578) (Figure 3.14A). The abundance of *Geocoris spp.* did not significantly differ between the treated and control plots for all locations ($F_{1,110} = 0.17$, and $F_{1,124} = 0.13$, p value = 0.6777, and 0.7234 respectively) (Figure 3.13B, and 3.14B). The abundance of *Linepithema humile* did not significantly differ between the treated and control plots at both the Watkinsville and Griffin locations ($F_{1,110} = 4.51$, p value = 0.0359) (Figure 3.13C). The abundance of *Linepithema humile* was significantly higher in the treated plot than in the control plot at the Research location, and the abundance of *Linepithema humile* was significantly higher in the control plot than the treated plot at the

Dempsey location ($F_{1,124} = 14.39$, p value = 0.0002) (Figure 3.14C). The abundance of *Solenopsis invicta* was significantly greater in the control plot than in the treated plot for both the Watkinsville and Griffin locations ($F_{1,110} = 1.29$, p value = 0.2587) (Figure 3.13C). The abundance of *Solenopsis invicta* was significantly greater in the control plot than in the treated plot for the Research locations, but the abundance of *Solenopsis invicta* did not significantly differ between the treated and control plots at the Dempsey location ($F_{1,124} = 1.81$, p value = 0.1814) (Figure 3.14C).

Discussion

Season long monitoring of the seasonal abundance of the beneficial insects and natural enemies demonstrated different trends based on location and sampling year. Monitoring the seasonal abundance of the target beneficial insects and/or natural enemies can help inform future choices of the floral resources. For example, an early blooming flower might help to attract pollinators to increase the pollination of the cash crop. Other flowers with small corolla planted earlier could help draw in more parasitoids during the crucial time of control needed in the early season for squash.

There are many instances where the added floral resources failed to attract the beneficial insects and/or natural enemies to the treated plots. Attraction of *Solenopsis invicta* to the control plots over the treated plots, or attraction of *Linepithema humile* to the treated plots over the control plots could be explained by the biology of the organisms. As they live in large colonies with either one or multiple queens, the occurrence of a neighboring nest near the location of a trap could cause the abundance to be higher than if there were no colonies directly adjacent to the trap. There were also many times that parasitic Hymenoptera were attracted to the control plots over the treated plots. As a factor of our experimental design, the control plots had a lack

of floral resources as compared to the treated plots. The distinct yellow pan traps could be viewed more easily in the control plots than in the treated plots, thus explaining why there would be more parasitic Hymenoptera found in the control yellow pan traps versus the treated yellow pan traps. This is further supported by the fact that there was one result in which the treated pit fall traps collected more parasitic Hymenoptera than the control pit fall traps, while the yellow pan traps had no such results. This might also be resolved if the parasitic Hymenoptera were identified beyond ordinal level. This would allow for better taxonomic resolution and the species of interest could be isolated for analysis, rather than including all parasitic Hymenoptera.

Floral resources often attracted more beneficial insects and natural enemies (Table 3.1, and 3.2), but not in all plots. Floral resources could be manipulated to address the issue of attracting beneficial insects and natural enemies for the entire growing season. Selecting plant species to lengthen the time in which flowers are at full bloom could help to encourage more beneficial insects and natural enemies during the crucial early season (Fargo et al. 1988, Palumbo et al. 1991). Furthermore, individual flower species can be chosen to encourage the attraction of specific beneficial insects and natural enemies based on flower and insect morphology (Nfzinger and Fadamiro 2010, and Campbell et al. 2012). Short-corolla flowers can be added to increase the attraction of parasitoids with small mouth parts. Buckwheat is a great option as it grows quickly, and you can re-seed so that you have blooms throughout the entire growing season.

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Tables

Table 3.1. Pit Fall Traps, Treatment Outcome for each Response Variable for each Year, and Location. Shows how the treatment of floral resources impacted each response variable: positive (+) negative (-), and non-significant (0) effect for each year, and all locations for pit fall trap data.

2014	Parasitic Hymenoptera	Araneae	Carabidae	Hemiptera	<i>Geocoris</i> sp.	<i>Linepithema humile</i>	<i>Solenopsis invicta</i>
Location 1	-	0	+	0	0	+	-
Location 2	0	+	+	+	0	0	-
2015							
Location 1	-	0	0	+	0	+	0
Location 2	+	+	+	0	0	+	+

Table 3.2. Yellow Pan Traps, Treatment Outcome for each Response Variable for each Year and Location. Shows how the treatment of floral resources impacted each response variable: positive (+) negative (-), and non-significant (0) effect for each year, and all locations for yellow pan trap data.

2014	Parasitic Hymenoptera	Araneae	Pollinators	Hemiptera	<i>Geocoris</i> sp.	<i>Linepithema humile</i>	<i>Solenopsis invicta</i>
Location 1	-	-	0	0	0	0	-
Location 2	-	0	0	+	0	0	-
2015							
Location 1	-	0	+	+	0	+	-
Location 2	0	0	0	0	0	-	0

Figures

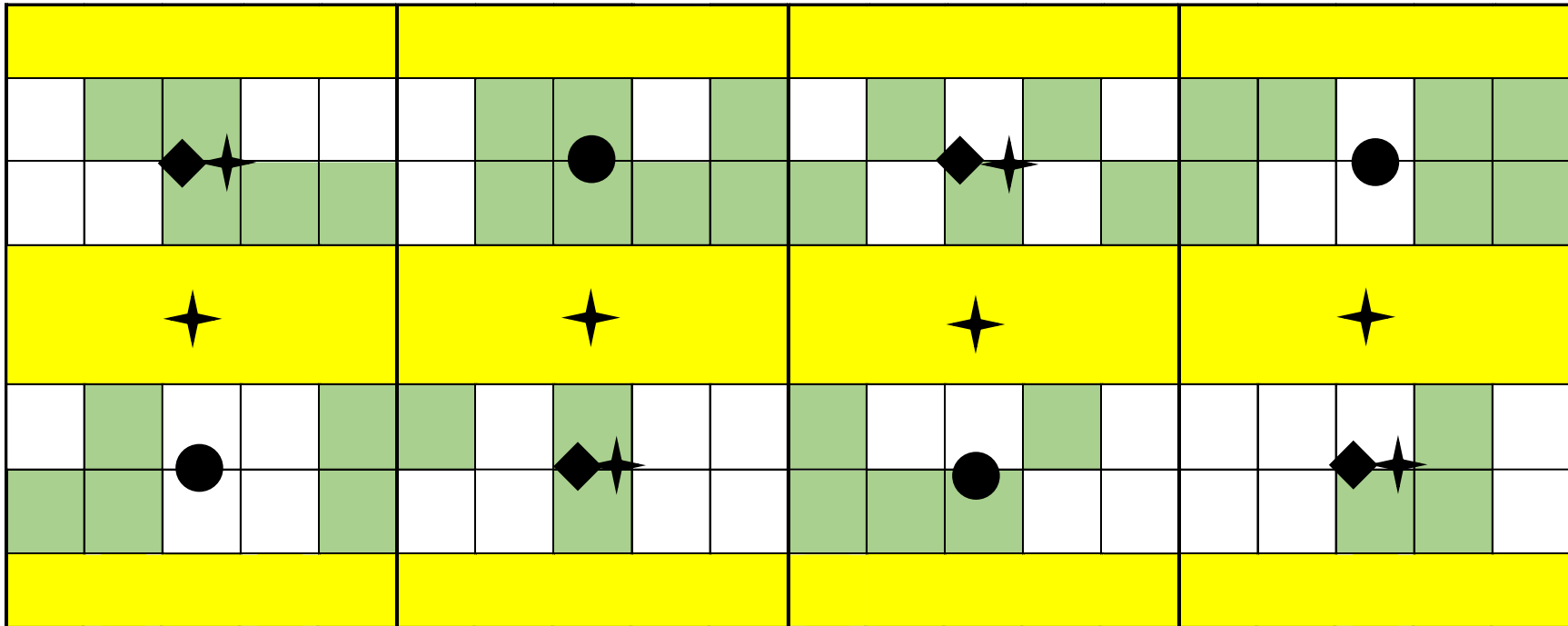


Figure 3.1. Placement of Data Collection Methods. Diamonds indicate the location of the pit fall traps. Circles indicate the location of yellow pan traps. Stars indicate the location of yellow sticky traps (data not included).



Figure 3.2. Images Showing Collection Methods. Image on the left shows the yellow pan traps. Image on the right shows the pit fall traps.

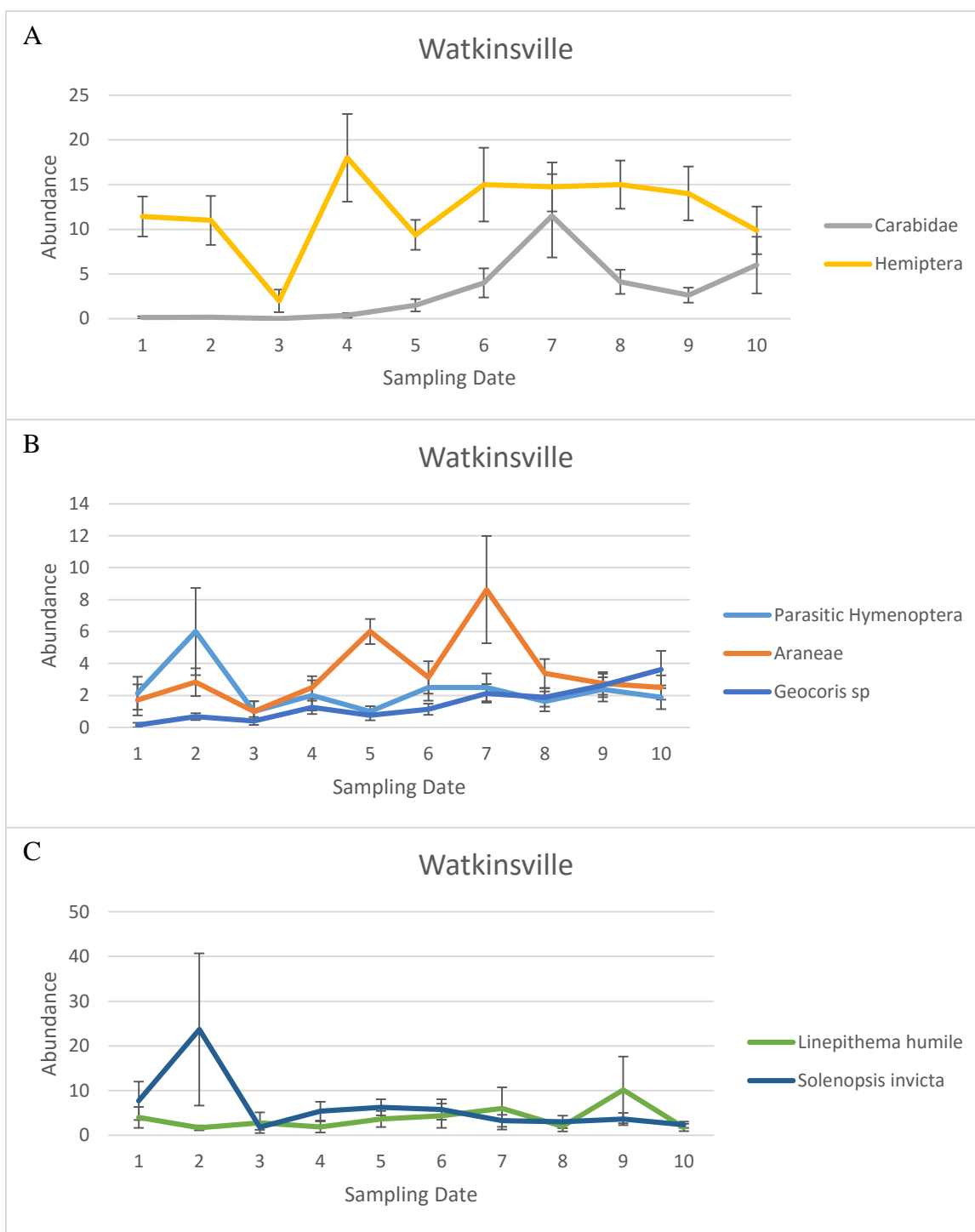


Figure 3.3. Watkinsville Pit Fall Traps Seasonal Abundance. Graphs showing mean (\pm SE) of beneficial insects collected using the pit fall traps during the summer of 2014 at the Watkinsville location. (A) Carabidae, Hemiptera, (B) Parasitic Hymenoptera, Araneae, and *Geocoris* spp. (C) *Linepithema humile*, and *Solenopsis invicta*.

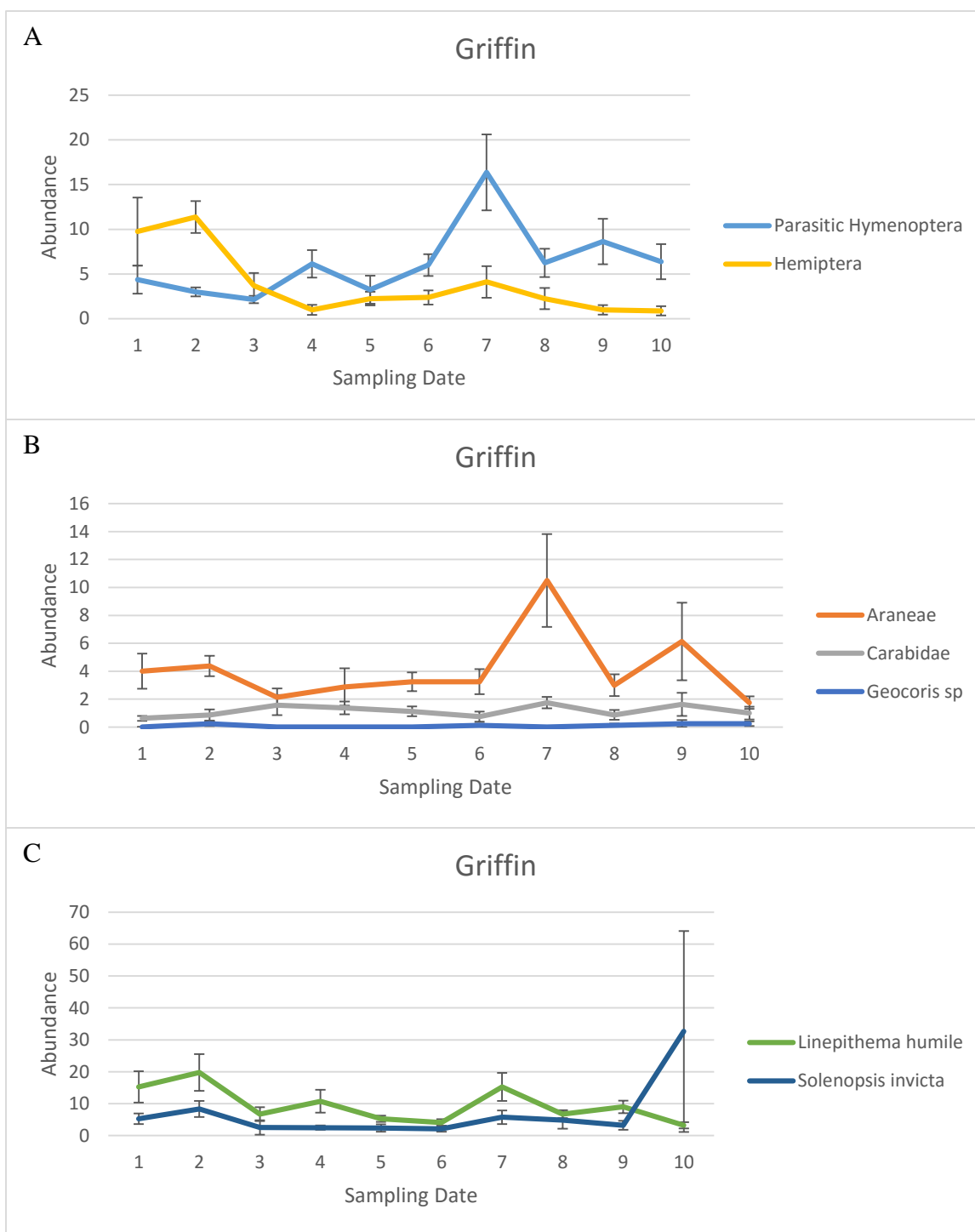


Figure 3.4. Griffin Pit Fall Traps Seasonal Abundance. Graphs showing mean (\pm SE) of beneficial insects collected using the pit fall traps during the summer of 2014 at the Griffin location. (A) Parasitic Hymenoptera, Hemiptera, (B) Araneae, Carabidae, and *Geocoris* spp. (C) *Linepithema humile*, and *Solenopsis invicta*

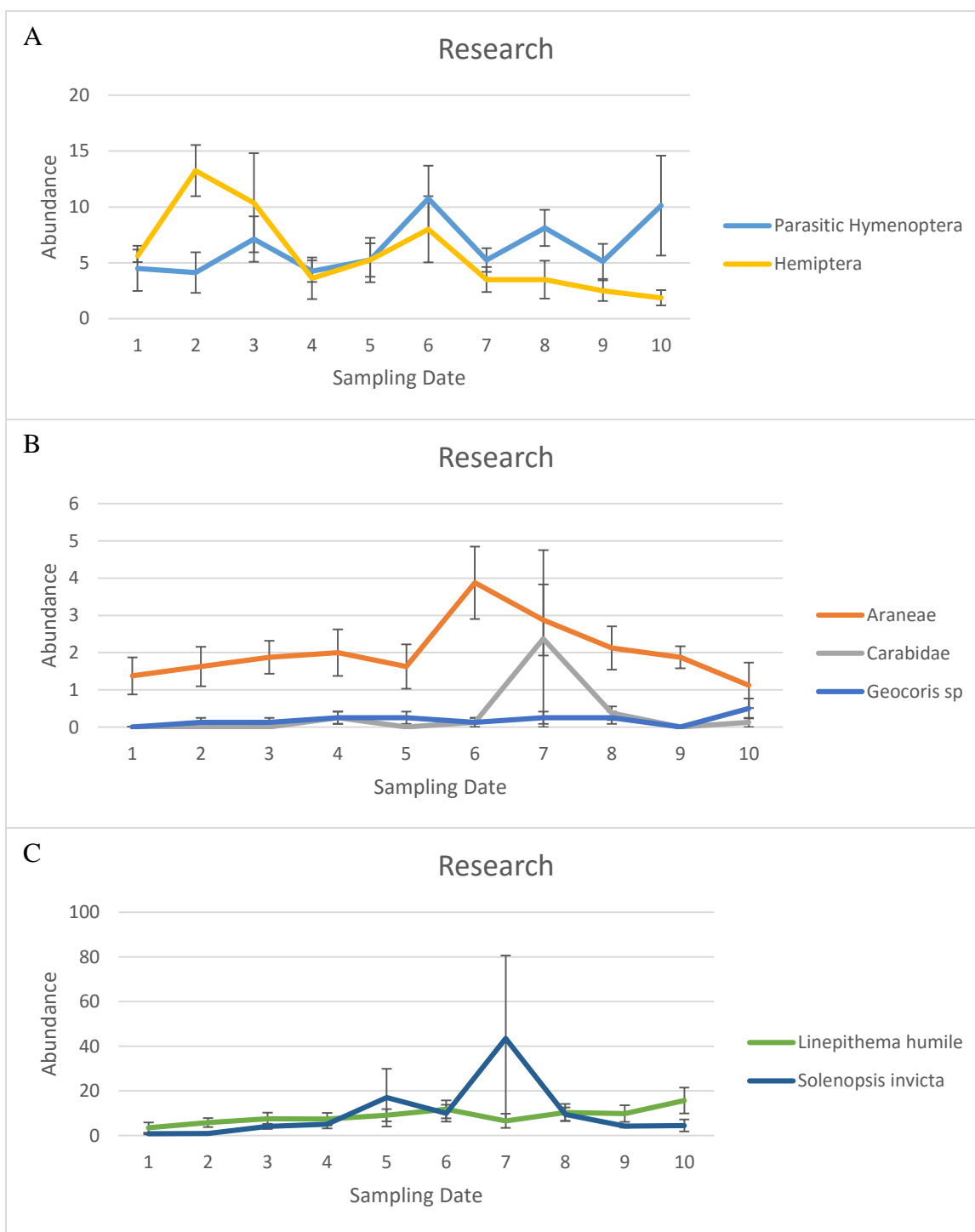


Figure 3.5. Research Pit Fall Traps Seasonal Abundance. Graphs showing mean (\pm SE) of beneficial insects collected using the pit fall traps during the summer of 2015 at the Research location. (A) Parasitic Hymenoptera, Hemiptera, (B) Araneae, Carabidae, and *Geocoris* spp. (C) *Linepithema humile*, and *Solenopsis invicta*.

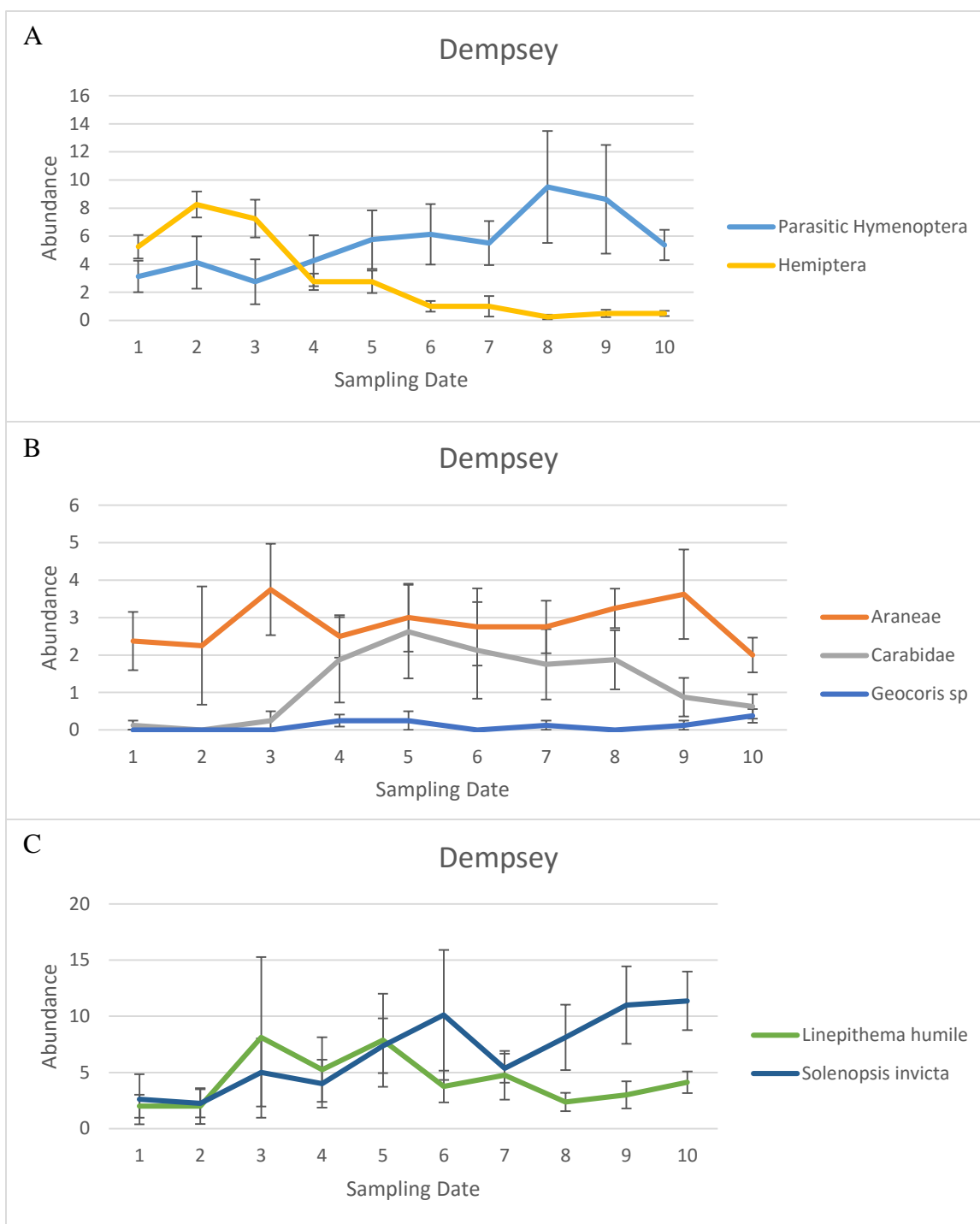


Figure 3.6. Dempsey Pit Fall Traps Seasonal Abundance. Graphs showing mean (\pm SE) of beneficial insects collected using the pit fall traps during the summer of 2015 at the Dempsey location. (A) Parasitic Hymenoptera, Hemiptera, (B) Araneae, Carabidae, and *Geocoris* spp. (C) *Linepithema humile*, and *Solenopsis invicta*.

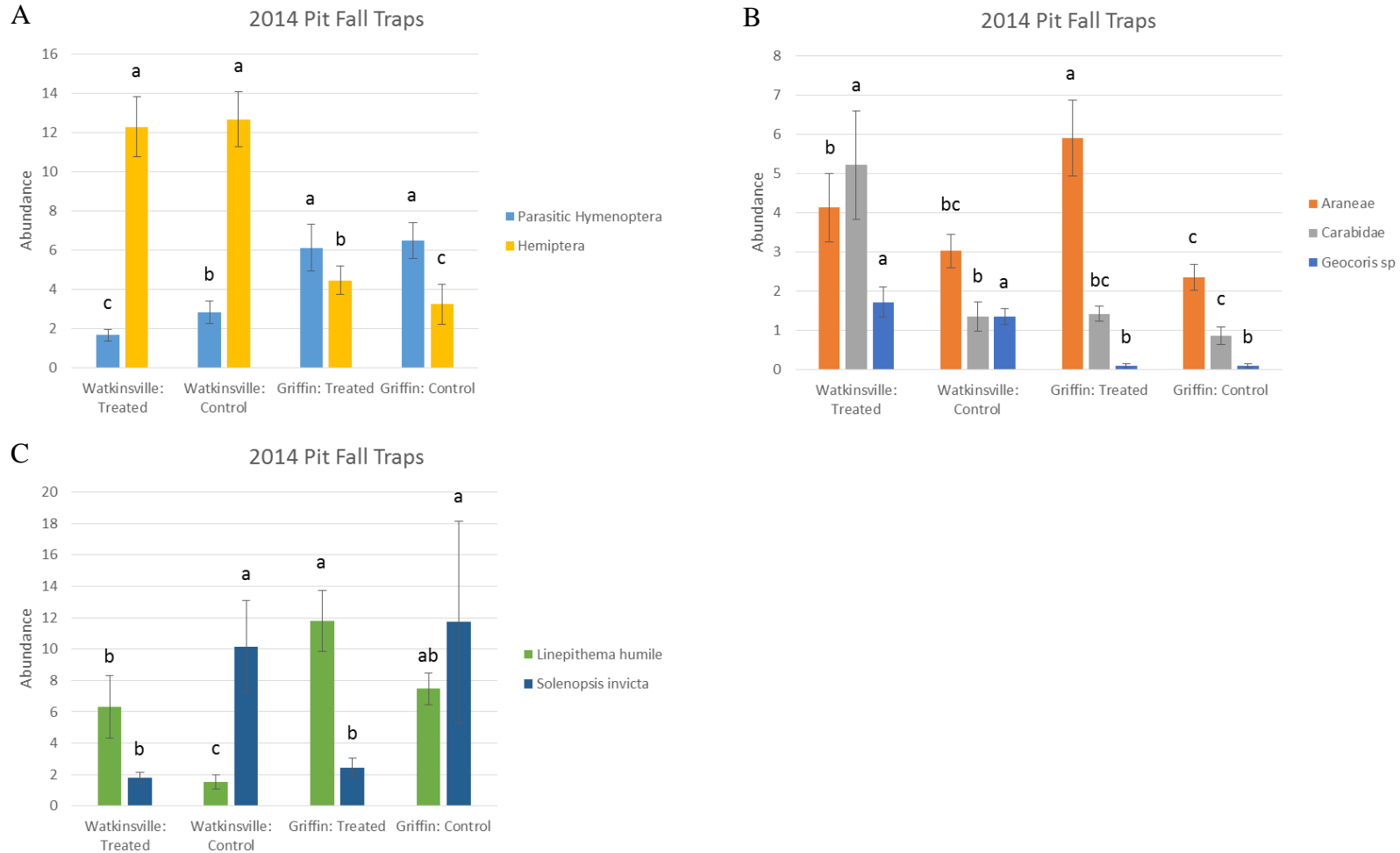


Figure 3.7. 2014 Data Pit Fall Traps Floral Resources. Graphs showing the mean (\pm SE) number of beneficial insects collected using pit fall traps during the summer of 2014 for treated (with floral resources) and control (without floral resources) plots at both locations. (A) Parasitic Hymenoptera, and Hemiptera (B) Araneae, Carabidae, and *Geocoris* sp. (C) *Linepithema humile*, and *Solenopsis invicta*. Different letters indicate significant differences ($\alpha=0.05$).

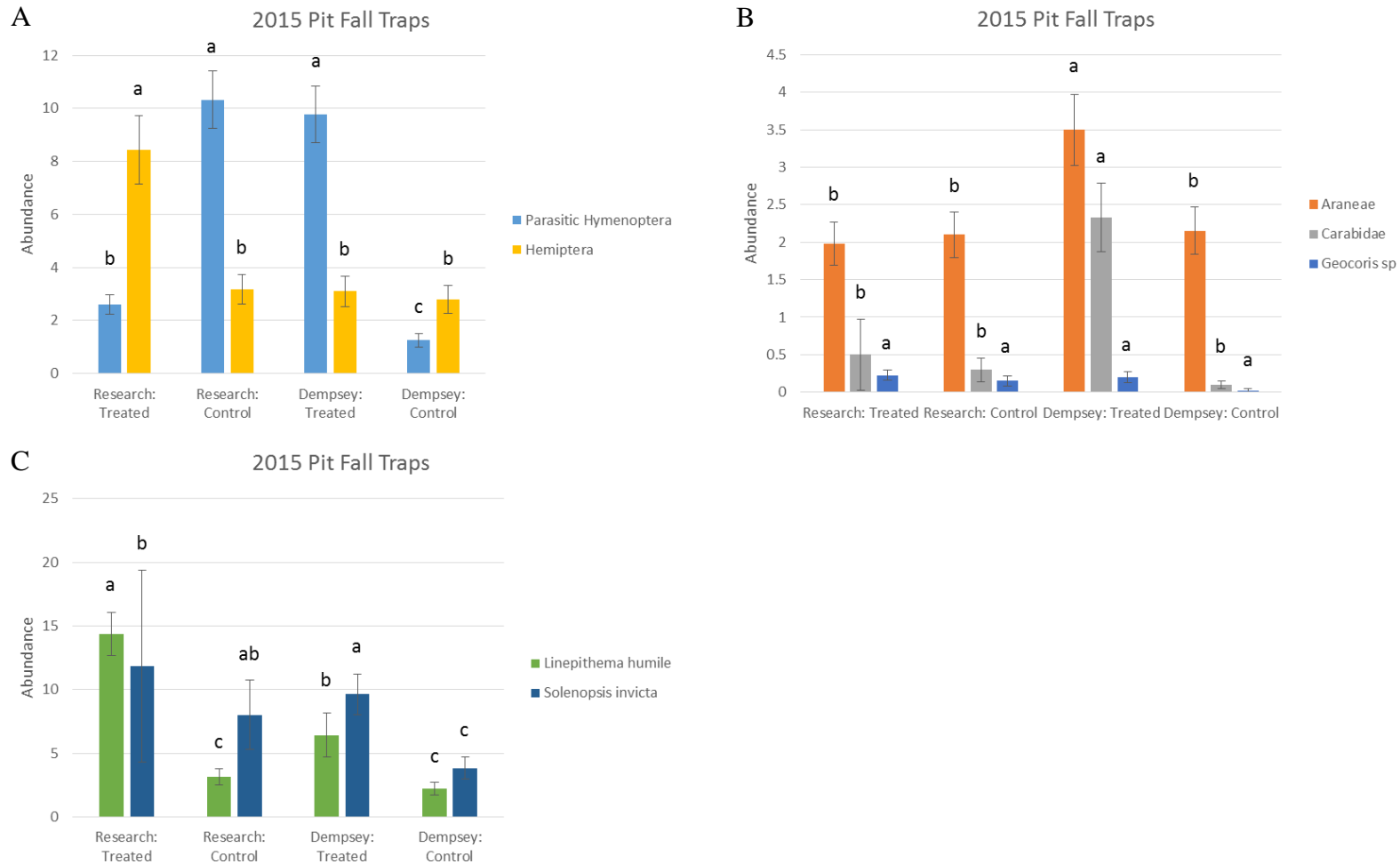


Figure 3.8. 2015 Data Pit Fall Traps Floral Resources. Graphs showing the mean (\pm SE) number of beneficial insects collected using pit fall traps during the summer of 2014 for treated (with floral resources) and control plots (without floral resources) at both locations. (A) Parasitic Hymenoptera, and Hemiptera (B) Araneae, Carabidae, and *Geocoris* spp. (C) *Linepithema humile*, and *Solenopsis invicta*. Different letters indicate significant differences ($\alpha=0.05$).

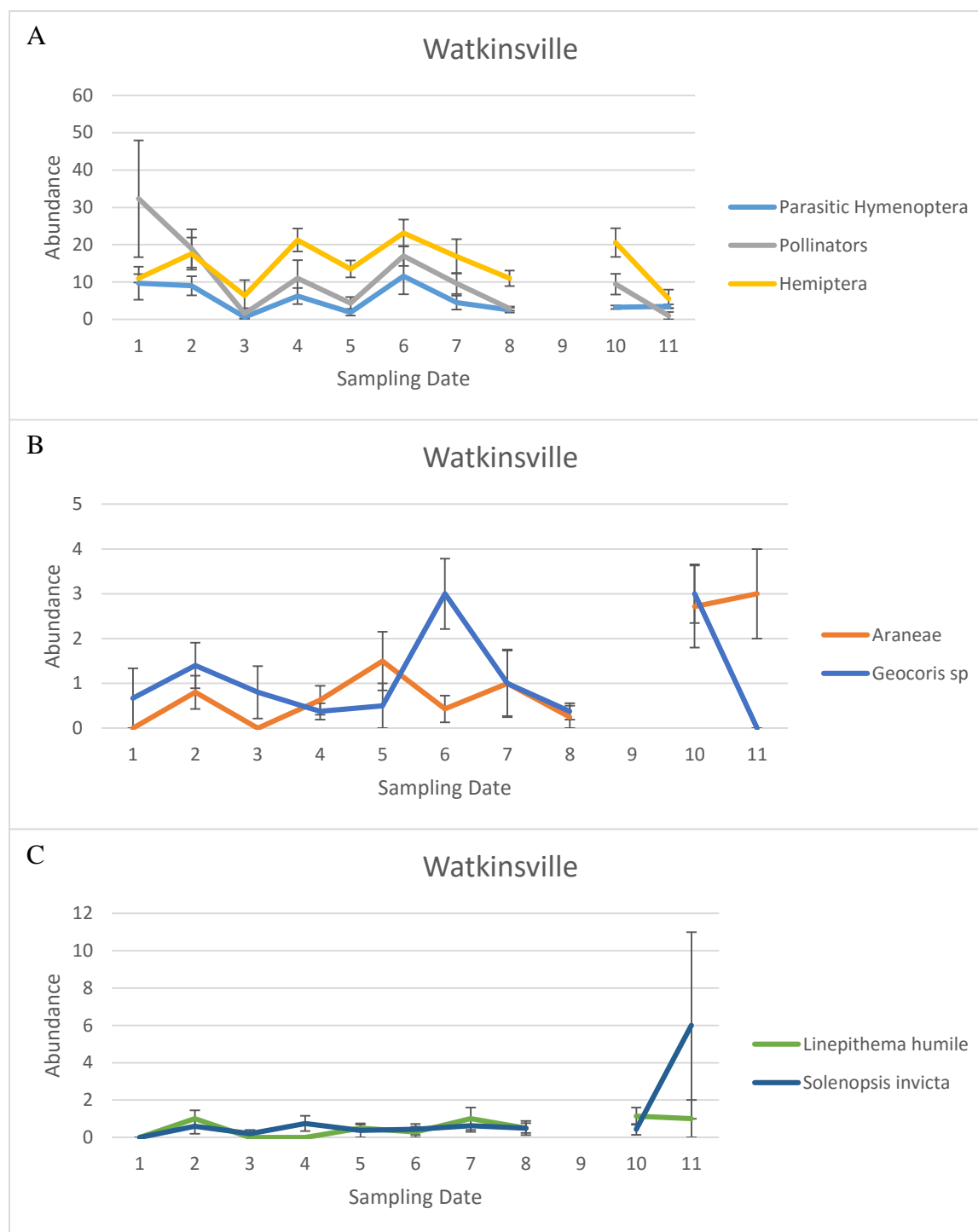


Figure 3.9. Watkinsville Yellow Pan Traps Seasonal Abundance. Graphs showing mean (\pm SE) of beneficial insects collected using the yellow pan traps during the summer of 2014 at the Watkinsville location. (A) Parasitic Hymenoptera, Hemiptera, (B) Araneae, Carabidae, and *Geocoris spp.* (C) *Linepithema humile*, and *Solenopsis invicta*. Sample date 9 is removed due to lack of data.

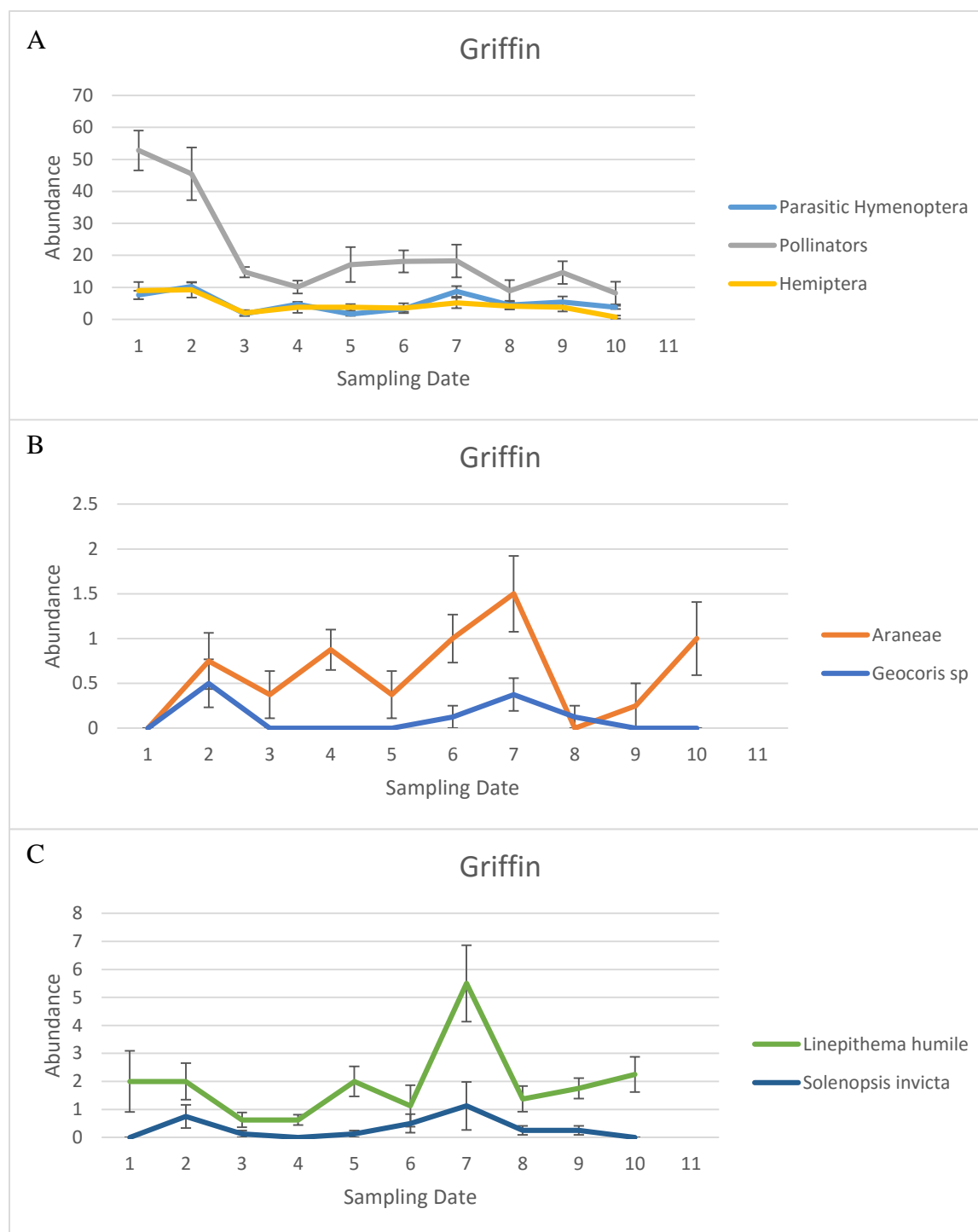


Figure 3.10. Griffin Yellow Pan Traps Seasonal Abundance. Graphs showing mean (\pm SE) of beneficial insects collected using the yellow pan traps during the summer of 2014 at the Griffin location. (A) Parasitic Hymenoptera, Hemiptera, (B) Araneae, Carabidae, and *Geocoris spp.* (C) *Linepithema humile*, and *Solenopsis invicta*. Sample Date 11 is removed due to lack of data.

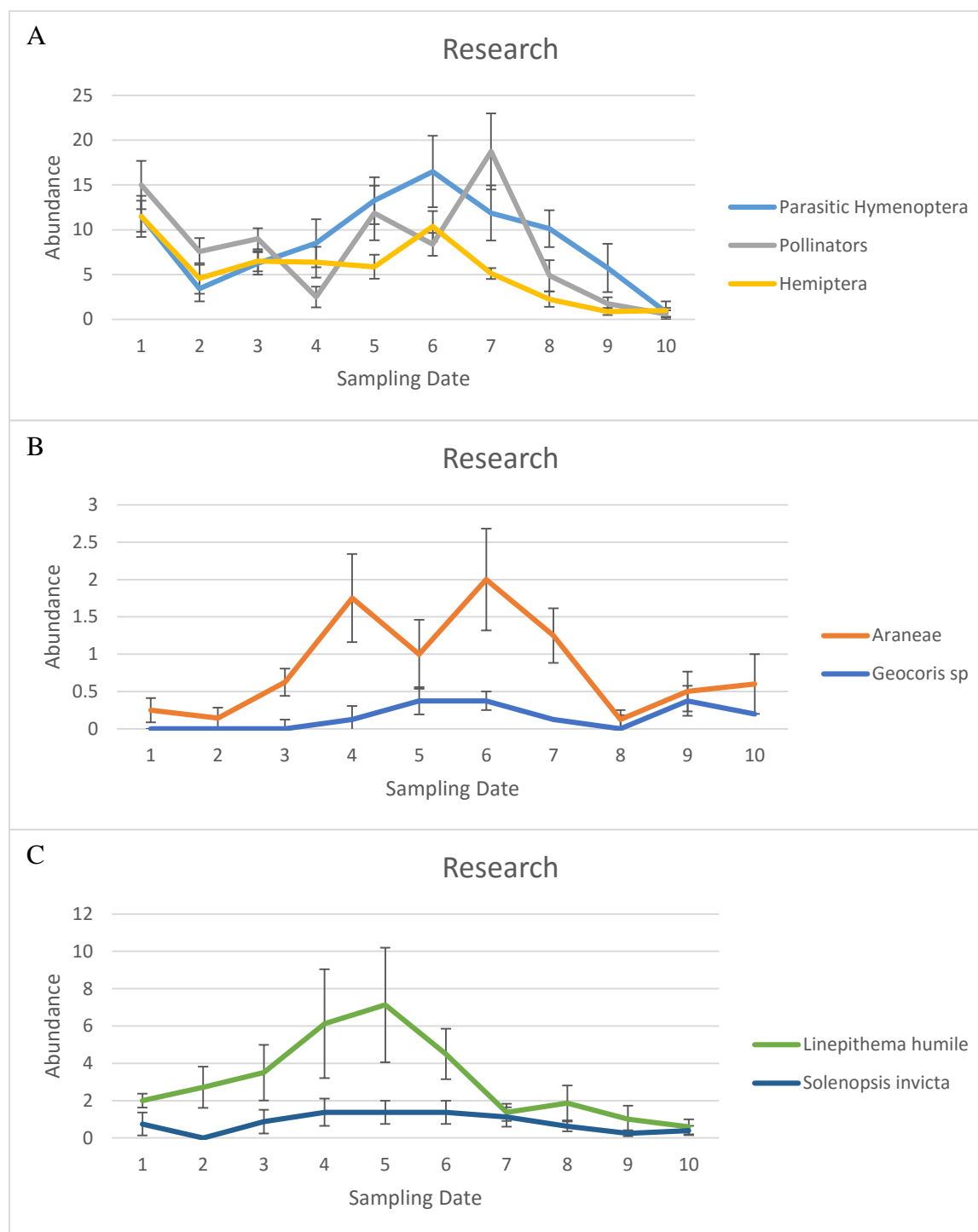


Figure 3.11. Research Yellow Pan Traps Seasonal Abundance. Graphs showing mean (\pm SE) of beneficial insects collected using the yellow pan traps during the summer of 2015 at the Research location. (A) Parasitic Hymenoptera, Hemiptera, (B) Araneae, Carabidae, and *Geocoris* spp. (C) *Linepithema humile*, and *Solenopsis invicta*.

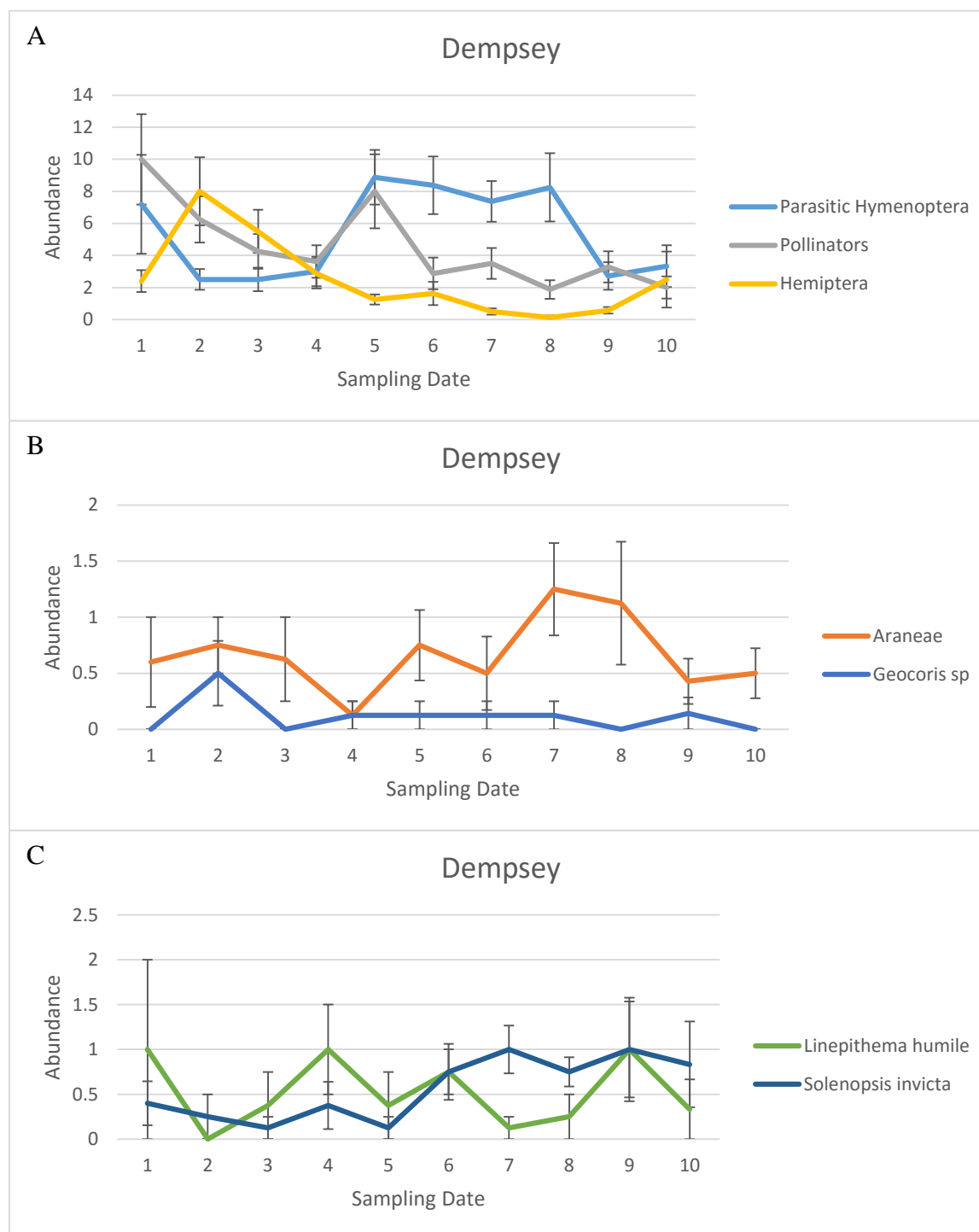


Figure 3.12. Dempsey Yellow Pan traps Seasonal Abundance. Graphs showing mean (\pm SE) of beneficial insects collected using the yellow pan traps during the summer of 2015 at the Dempsey location. (A) Parasitic Hymenoptera, Hemiptera, (B) Araneae, Carabidae, and *Geocoris spp.* (C) *Linepithema humile*, and *Solenopsis invicta*.

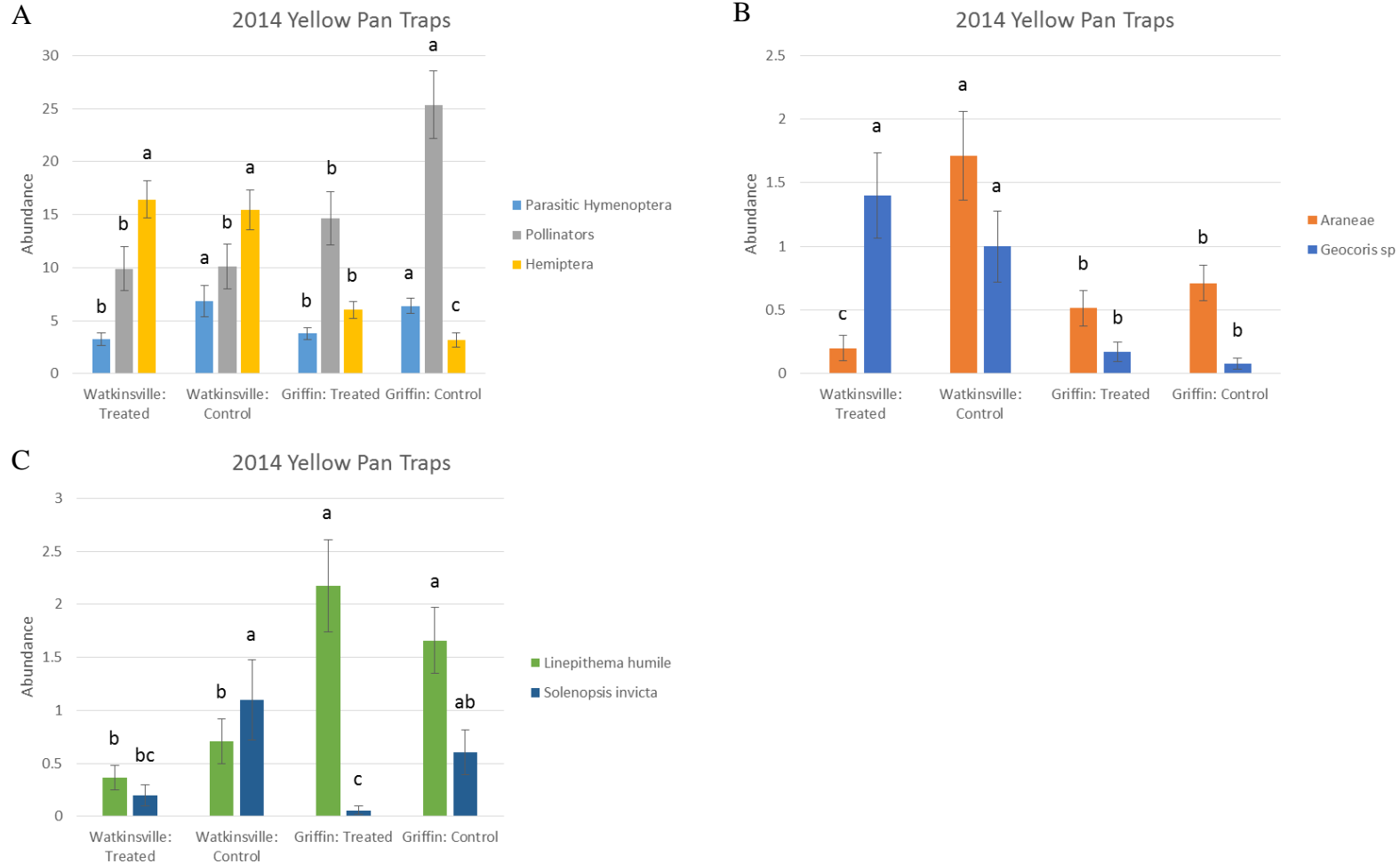


Figure 3.13. 2014 Data Yellow Pan Traps Floral Resources. Graphs showing the mean (\pm SE) number of beneficial insects collected using yellow pan traps during the summer of 2014 for treated (with floral resources) and control (without floral resources) plots at both locations. (A) Parasitic Hymenoptera, Pollinators, and Hemiptera (B) Araneae, and *Geocoris* sp (C) *Linepithema humile*, and *Solenopsis invicta*. Different letters indicate significant differences ($\alpha=0.05$).

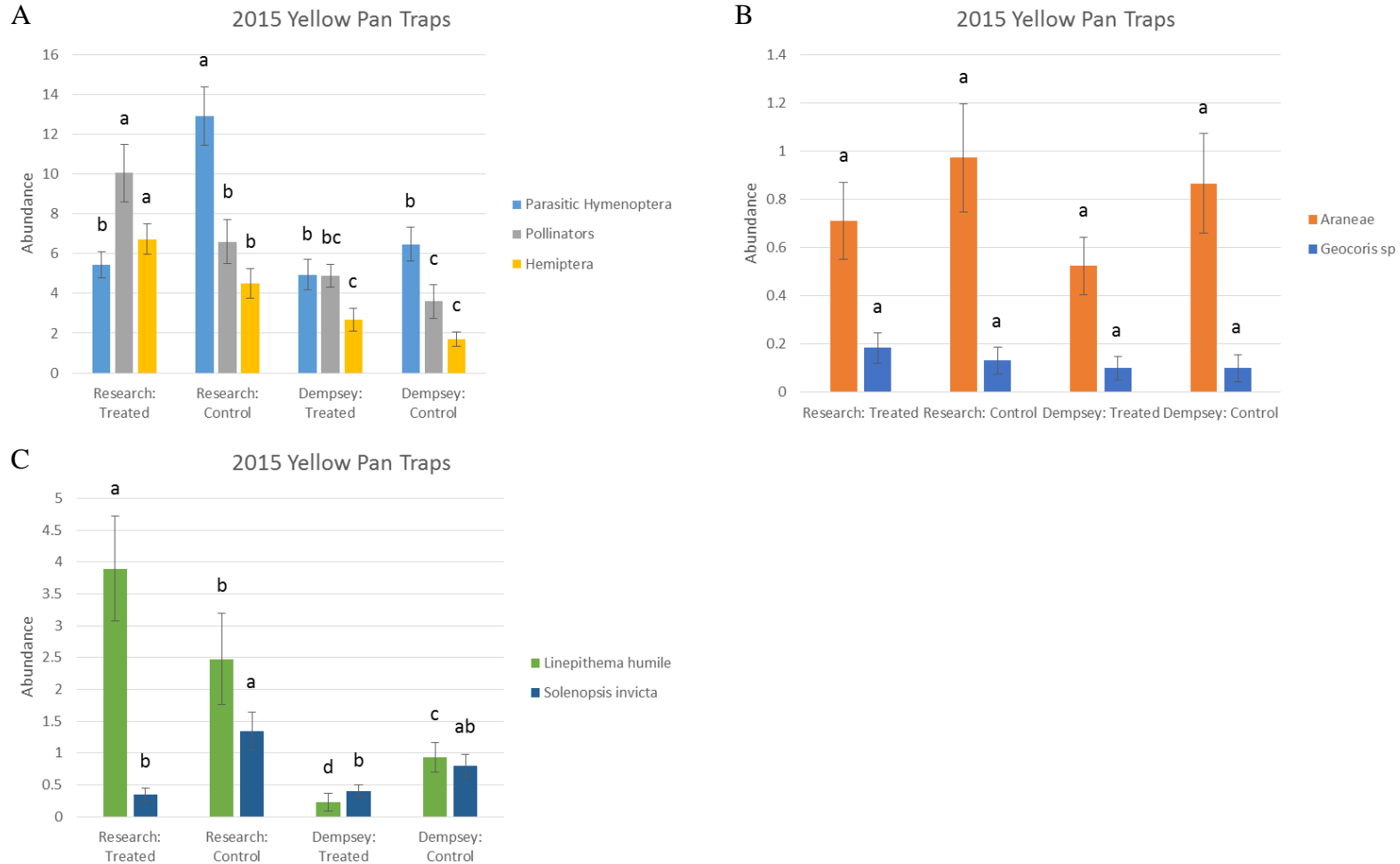


Figure 3.44. 2015 Data Yellow Pan Traps Floral Resources. Graphs showing the mean (\pm SE) number of beneficial insects collected using yellow pan traps during the summer of 2015 for treated (with floral resources) and control (without floral resources) plots at both locations. (A) Parasitic Hymenoptera, Pollinators, and Hemiptera (B) Araneae, and *Geocoris* sp (C) *Linepithema humile*, and *Solenopsis invicta*. Different letters indicate significant differences ($\alpha=0.05$).

CHAPTER 4

QUANTIFICATION OF PREDATION RATES FOR *Geocoris punctipes* ON *Anasa tristis*³

³ Fair, Conor, and Braman, Kris. To be submitted to Journal of Entomological Science.

Abstract

Georgia is the 5th largest producer of squash in the United States. The squash bug *Anasa tristis* (DeGeer) is an indigenous pest of squash and other cucurbits. Small growers seek alternative methods of control to insecticides to manage squash bug populations. The introduction of natural enemies to control pest populations has been commonly used in integrated pest management. The big eyed bug, *Geocoris punctipes*, is a well-established predator of *A. tristis*. Wild *G. punctipes* adult male and females were collected from stands of mixed grasses in Spalding Co., GA and allowed to prey on *A. tristis* eggs, first and second instars in no choice tests. Consumption of first and second instar squash bugs was significantly greater by females than males ($F_{1,48} = 53.11$, and 38.16 , p value $<.0001$ respectively). Predation of eggs could be underestimated as probed eggs were reared in the lab, and protected from potentially harmful environmental conditions. The control group experienced significantly more non-predation mortality than the male and female groups for the first and second instars ($F_{2,72} = 5.61$, and 3.57 , p value = 0.0054 , and 0.0331 respectively). These data further expand on established knowledge of this predator. With this information, farmers will be able to better inform their integrated pest management methods. The big eyed bug, *G. punctipes*, is a significant predator of the squash bug *A. tristis*, and should be considered for release as a biological control agent and/or target natural enemy to attract via added floral resources used in farmscaping.

Introduction

In 2014, the United States harvested 38,530 acres, or \$191,532,000 of squash. (USDA 2015). Additionally, there is a growing market for small-scale commercial vegetable production, with greatest number of Georgia growers found in urban regions such as Atlanta, Athens, Savannah, Macon, and Columbus. As the nation shifts its preference towards locally grown food, a substantial number of consumers are willing to pay premiums, especially for certain types of produce (Wolf et al. 2005). In 2011, Georgia had 23 organic certified vegetable farms that produced \$2,761,182 (2012 Certified Organic Production Survey). As of 2014, there were 124 farms with Certified Naturally Grown certification and 154 farmers' markets in Georgia. There has been a large increase in the number of farmers' markets since 2003 when Georgia had one nine (2012 Certified Organic Production Survey, and 2014 Good Food Guide, Georgia Organics). Furthermore, these farmers can reach more consumers with programs like Community Supported Agriculture (CSA), which provide a diverse share of products to their customers on a weekly basis. According to the latest Census of Agriculture, direct sales of food products from farmers to individual consumers rose by nearly 50% between 2002 and 2007 (2013 Farm Futures Aug). This organic production market is growing, although production and harvesting expenses remain limiting (e.g., Biermacher et al. 2007). One of the major pests for squash (especially non-conventional) production is the squash bug.

The squash bug, *Anasa tristis* (DeGeer) has long been considered a significant indigenous pest of squash and other members of the Cucurbitaceae family including: pumpkin, watermelon, and zucchini. *A. tristis* feed on the leaves, stems, and vines of the squash plant by sticking their stylets into the phloem tissue. When the squash bug feeds, it causes damage to plants by consuming their nutrients and by reducing their photosynthetic capacity due to leaf chlorosis and

necrosis (Beard 1935). Furthermore, squash farmers in the eastern United States have experienced significant loss of yield due to cucurbit yellow vine disease caused by the bacterial pathogen, *Serratia marcescens* Bizio. The disease was first observed in 1988 when farms in Texas and Oklahoma experienced significant yield loss due to the yellowing and wilting of their squash and pumpkin plants (Bruton et al. 2003), but it was not until the study by Pair et al. (2004), when it was conclusively shown that *A. tristis* was a competent vector of *Serratia marcescens* Bizio.

Chemical control methods have had varying success in controlling squash bug populations. Farmers encountered difficulties in reducing yield loss caused by squash bug damage with early insecticide application recommendations (Walton 1946, Roberts & Saluta 1985, and Criswell 1987). Treatments were ineffective when farmers applied insecticides after the nymphs had become numerous or plant damage had become excessive. Palumbo et al. (1993) found that early spraying is more effective than spraying right before harvest. Scientists have also used systemically treated squash (<1% of total hectareage) and semiochemical toxic baits to successfully control early squash bug populations (Pair 1997). While chemical control is a tool that conventional farmers can use, small-scale growers and organic farmers may not be able to use them to control their pest populations. Other pest management methods such as cultural control have been studied to examine their efficacy.

Cultivars with resistance to squash bug feeding have been developed in an attempt to limit damage done to the crops. Squash bugs reared in the lab on resistant and susceptible varieties of squash are able to overcome resistant cultivars, but the researchers believe that ecological and agricultural factors would prevent the breakdown of resistance in the field (Margolies et al. 1998). Other approaches include various types of cloth or plastic row covers

and different mulches including aluminum and different plastics have had moderate success (Kring 1964 and Chalfant et al. 1977). Natwick and Durazo (1985) reported data that showed fabric row covers reduce the incidence of virus diseases transmitted by the whitefly in summer squash. However, Cartwright et al. (1990) observed a strong preference towards the soils with mulch for the squash bugs, and no added benefit to row covers. They cite the protection they receive from the mulch from ground dwelling predators as a potential reason for their preference over bare ground. Mulch systems, while increasing soil moisture and reducing irrigation, may increase squash bug control costs, and therefore the benefits are likely nullified.

Another method of control includes the use of beneficial insects to biological control agents to reduce pest insect populations. Many beneficial insects act as predators or parasitoids of pest insects. There have been many studies to assess the natural predators of the squash bug. Studies have found the most prevalent predators to be spiders (Lycosidae and Linyphiidae), Hemiptera (*Geocoris punctipes* (Say), *G. uliginosu* (Say)), and Coleoptera, especially ladybird beetles (*Coccinella septempunctata* (Linnaeus), *Coleomegilla maculata* (DeGeer), and *Hippodamia convergens* (Guérin-Méneville)), and species of Carabidae and Staphylinidae (Decker and Yeargan 2008 Rondon et al. 2003, Schmidt et al. 2014). Derek and Yeargan (2008) found lower estimates of squash bug predation (2-7%) than Schmidt et al. (2014) (11%). Furthermore, squash bug nymphs and adults spend much of their time on the ground beneath the plants, and would therefore be subject to many ground dwelling predators (Britton 1919, Palumbo et al. 1991). *G. punctipes* has been studied before in regards to its predation potential on *A. tristis* and other economically important insect pests. (Joseph and Braman 2009, and Rondon et al. 2003). However, further information is needed to better inform farmers on the potential for biological control.

Big eyed bugs, *G. punctipes* are common generalist omnivores found throughout the southern United States (Tamki and Weeks 1972). Previously research on big eyed bugs has shown that they significantly reduce fall armyworm numbers in turf grass (Braman et al. 2003), and prey on spider mites, plant bugs, leafhoppers, aphids, chinch bugs, and various lepidopteran larvae (Dunbar 1971). *G. punctipes* is also known to feed on plant material, but Hunter (2009) assessed the tritrophic interaction, and determined that the net effect is usually in favor of the plant. The study by Rondon et al. (2003) determined that *G. punctipes* third instars and adults did consume *A. tristis* first instar nymphs, but at low levels. The objective of this study seeks to expand on this finding by determining the difference in predation rates for both sexes of *G. punctipes*, and if the prey range of *G. punctipes* includes later instars of *A. tristis*. If evidence can be brought forth that shows *G. punctipes* has greater potential as a biological control agent against *A. tristis* farmers can add another option for integrated pest management (Figure 4.2).

Materials and Methods

Experimental Design

Big eyed bugs *Geocoris punctipes* were collected using a sweep net from the fields in Spalding Co. GA. during August to October of 2015 and brought into the lab. They were placed in a petri dish with moist filter paper and kept in a growth chamber at 25° C and 14:10 (L:D) photoperiod and starved for 24 hours (Figure 4.3). After the 24 hour starving period was complete, a food source was introduced into the petri dish with the individual *G. punctipes*. The food source collected from the greenhouse colony was either 10 first instar squash bugs *Anasa tristis*, 10 second instar *A. tristis* or 10 *A. tristis* eggs (Figures 4.4, and 4.5). *G. punctipes* had access to their potential food item for 72 hours, after which the number of consumed *A. tristis* individuals (either 1st instar, 2nd instar, or eggs) was recorded. Controls for first instars, second

instars, and eggs were completed without the presence of *G. punctipes*. Each challenge was replicated 25 times (n=25) for each group (male, female, and control) for a total of 75 challenges. Consumption of a nymphal instar and eggs can be seen in Figures 4.2, 4.3, 4.4, and 4.5. Figure 4.5 shows an actively feeding *G. punctipes*, and Figure 4.3 shows an *A. tristis* nymph that was previously consumed. Figure 4.2 shows *G. punctipes* probing an egg mass in the field. Figure 4.4 shows *G. punctipes* probing an egg mass in the lab. Furthermore, the egg in Figure 4.7 was empty of any developing nymph, and was determined to be consumed by *G. punctipes*. While probing indicates the potential for feeding, only vacant eggs were considered to be consumed. Background mortality (unconsumed squash bug eggs and nymphs) was also determined.

Data Analysis

A generalized linear mixed model was applied to determine the influence of *G. punctipes* sex, and the food source (*A. tristis* egg, 1st or 2nd instar) on *G. punctipes* predation rates of *A. tristis* as well as the *A. tristis* background mortality data. The data collected were subjected to ANOVA using a generalized linear mixed model (PROC GLIMMIX, SAS Software). Predation data were modeled as the outcome of ten Bernoulli trials using a binomial distributions and logit transformation. Differences in least square means were determined by pairwise t-tests (alpha = 0.05) as the multiple comparisons post hoc test to determine significant differences between levels of all factors.

Results

Male *Geocoris punctipes* consumed on average 1.08 ± 0.24404 first instar squash bugs, 1.68 ± 0.4111772 second instar squash bugs, and 0 eggs during the 72 hour exposure. Female *G. punctipes* consumed on average 4.12 ± 0.5607138 first instar squash bugs, 4.28 ± 0.5642694 second instar squash bugs, and 0.12 ± 0.0663325 eggs. Consumption of first and second instars

was significantly greater in females than in males ($F_{1,48} = 53.11$, and 38.16 , p value $<.0001$ respectively). The control group experienced significantly more non-predation mortality than the male and female groups for the first and second instars (Table 4.1) ($F_{2,72} = 5.61$, and 3.57 , p value $= 0.0054$, and 0.0331 respectively). Images taken of *A. tristis* eggs did show signs of probing done by *G. punctipes*. Figure 4.6 shows an egg that had been probed on the side of the egg, but the squash bug still hatched. Figure 4.7 shows an egg that had been probed along the operculum, but the squash bug did not hatch.

Discussion

Previous literature has shown that the big eyed bug *Geocoris punctipes* is a generalist predator that consumes a wide variety of insects, including the squash bug *Anasa tristis* (Braman et al. 2003, and Rondon et al. 2003). Rondon et al. demonstrated the ability of *G. punctipes* to consume first instar squash bugs (*Anasa tristis*), and this research widens the prey range to include both eggs and second instars. One experimental design artifact that was not taken into account prior to the experiment was the age of the eggs and nymphs presented to *G. punctipes*. There could be preference towards more recently laid eggs, or nymphs who have recently molted. If the age of the food source could be kept constant throughout the study, this question could be answered, and a more accurate account of *G. punctipes* predation on *A. tristis* would be elucidated. The likelihood of *G. punctipes* consuming third or later squash bug instars is unlikely as the size begins to favor the squash bugs. We were able to provide the difference in predation rates between sexes of *G. punctipes*. Female *G. punctipes* consumed first and second instar nymphs of *A. tristis* almost four times more than males. This discrepancy does not come as a surprise because the nutritional requirement of females would be larger than that of males due to requirements for egg nutrient production (vitellogenin). Additionally, there is the

potential that predation rates of *A. tristis* eggs could be underestimated. There were many instances where eggs were probed and punctured by a *G. punctipes*, and the egg had hatched while kept in the petri dish in the growth chamber. We believe that had those probed or punctured eggs experienced the variable temperature and potential pathogens found in the wild, they might not have hatched. This could be investigated with a field study, which might expand the egg predation rate of *G. punctipes* on *A. tristis*. The more important conclusion is that the big eyed bug *G. punctipes* should be considered a significant predator of the squash bug *A. tristis*. The prey range of *G. punctipes* now includes the eggs as well as the early instars of *A. tristis*. This information improves the potential *G. punctipes* has as a biological control agent in controlling the important early season squash bug populations. Human releases of *G. punctipes*, or modifying floral resources to attract *G. punctipes* early in the season would be recommended for best results in controlling squash bug populations.

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Tables

Table 4.1. Background *Anasa tristis* Mortality. Table showing mean and SE numbers of natural non-predation mortality of squash bugs that occurred for each group (n=25). Different letters indicate significant differences ($\alpha = 0.05$).

Male	Mean	Standard Error
Egg	0.2a	0.0816497
First	0.68b	0.3039737
Second	0.88b	0.3479464
Female		
Egg	0.2a	0.1
First	0.72b	0.3342654
Second	0.8b	0.2581989
Control		
Egg	0.12a	0.0879394
First	1.48a	0.3791218
Second	1.48a	0.4168133

Figures

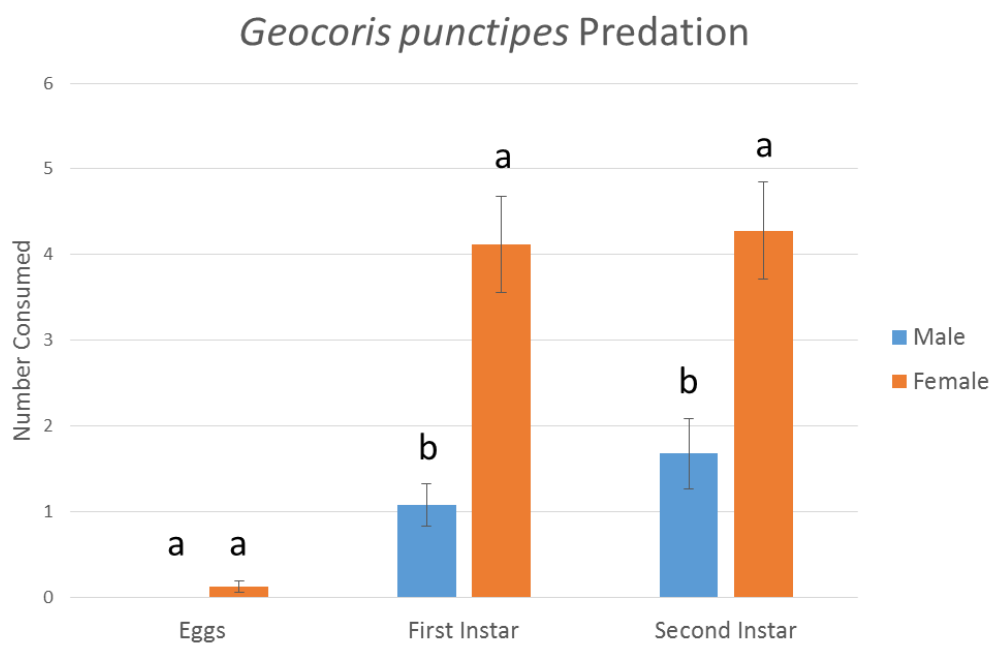


Figure 4.1. *Geocoris punctipes* Predation Rates. Graph showing the mean and SE± of *Geocoris punctipes* predation rates on *Anasa tristis* from 72 hours of exposure. Different letters indicate significant differences (alpha = 0.05).



Figure 4.2. *Geocoris punctipes* Feeding on *Anasa tristis* Eggs in the Wild. Image showing big eyed bug *Geocoris punctipes* probing squash bug *Anasa tristis* eggs in the field.

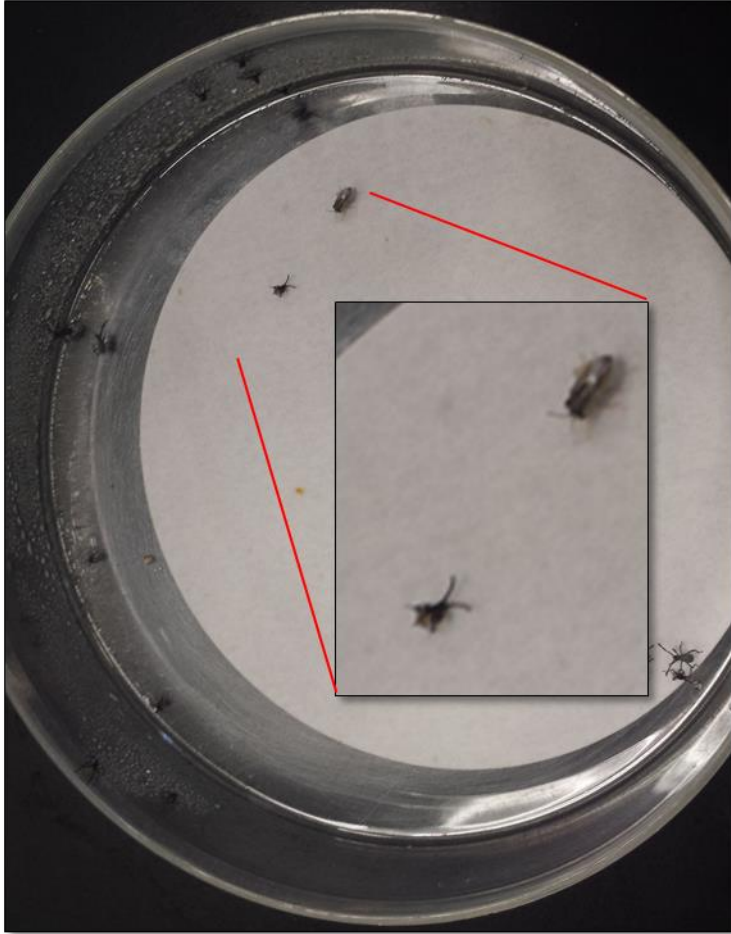


Figure 4.35 Experimental Set Up. Image showing the petri dish used in the experimental design. Enlarged image also shows evidence of *Geocoris punctipes* consuming *Anasa tristis* nymph.

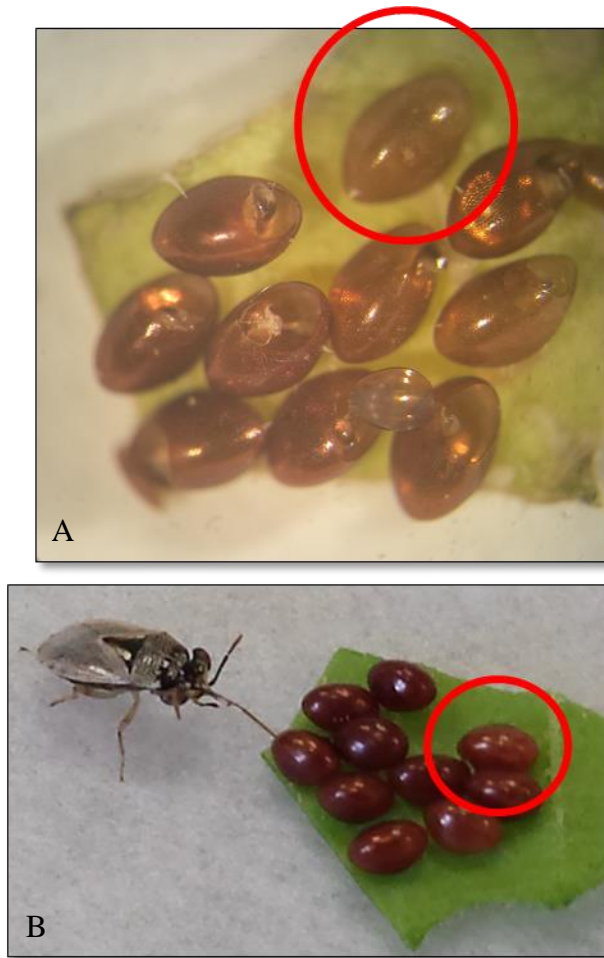


Figure 4.4. *Geocoris punctipes* Feeding on *Anasa tristis* Eggs in the Lab. (B) Image showing *Geocoris punctipes* feeding on *Anasa tristis*. (A) Egg in red circle did not hatch, while the other nine eggs did, including the egg seen being probed by *Geocoris punctipes* (B).

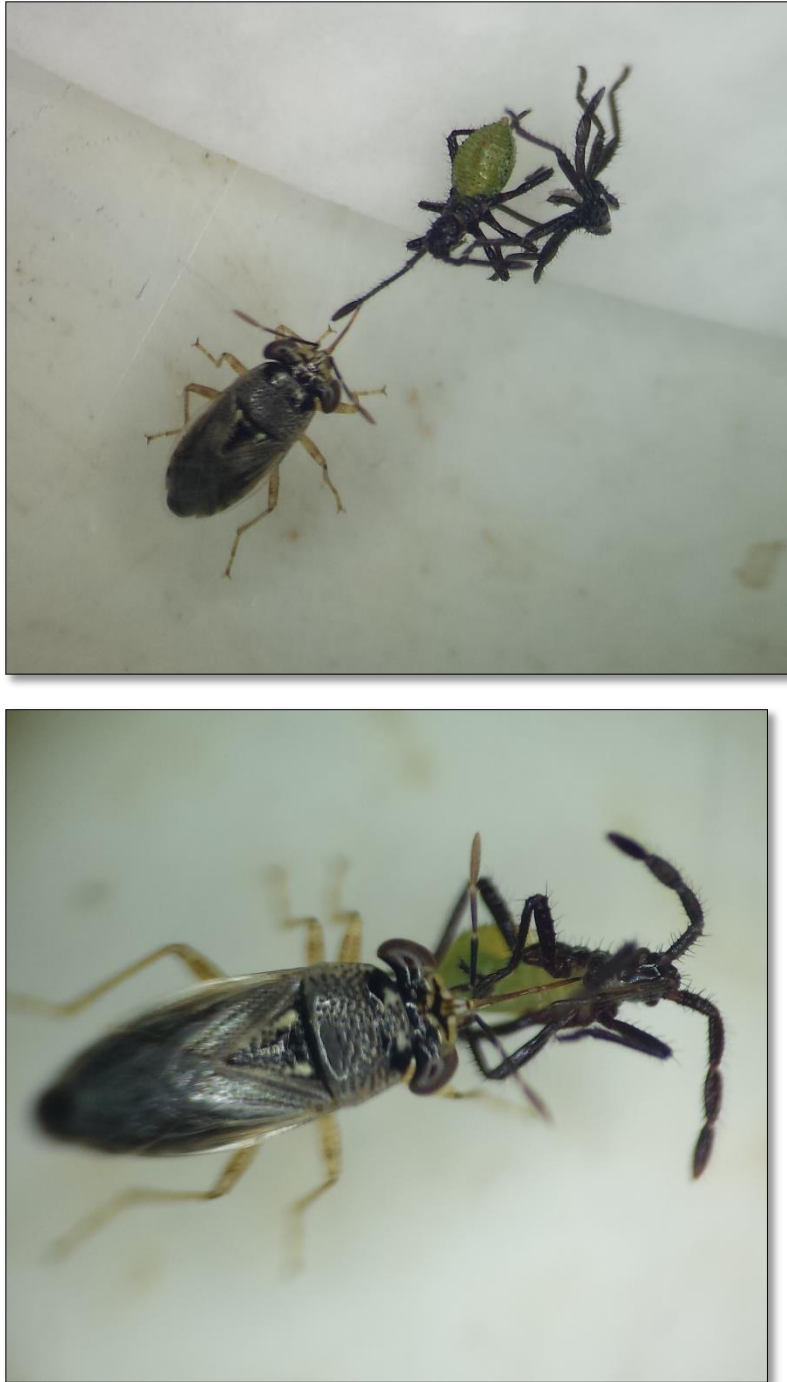


Figure 4.5.6 *Geocoris punctipes* Feeding on *Anasa tristis* Nymphs in the Lab. Top image shows early attempt of *G. punctipes* feeding on *A. tristis* nymphs. Target feeding location is vulnerable space in between antennal segments. Bottom image shows later stage of *G. punctipes* feeding on *A. tristis* nymphs. Once *A. tristis* nymph ceases escaping behavior, *G. punctipes* moves to another vulnerable location for further feeding.



Figure 4.6. *Geocoris punctipes* Evidence of Feeding. Image showing hatched *Anasa tristis* egg and puncture hole. Same egg as seen in Figure 4.3B being probed by *Geocoris punctipes*.



Figure 4.7. *Geocoris punctipes* Evidence of Egg Consumption. Image showing unhatched and consumed *Anasa tristis* egg by *Geocoris punctipes*. Presumed puncture hole seen in red circle. This is the same egg seen in Figure 4.3A and 4.3B

CHAPTER 5
CONCLUSIONS

Mitigating Squash Bug Populations and Damage

Visual observations of squash bug abundance indicated that there were likely two generations during both the 2014 and 2015 field seasons (May-August). It is possible that extending the duration of the observations could result in data indicating a third generation. The visual observations also showed how the two plantings of squash experienced squash bug infestation differently. This information is important for growers to inform their decisions regarding controlling this pest. Recommendations for planting dates have the potential to help in controlling squash bug infestations, but the additional IPM tactic of added floral resources may further help. A later season harvest would benefit from the added floral resources as the *Gryon* sp. parasitoids will by then have had enough time to increase in abundance to provide the beneficial control.

Data from the 2014 and 2015 field experiments do not offer consistent recommendations for floral resource additions to agricultural systems in the Southeast. Much of the floral resource data is inconsistent from year to year, location to location, and for the two planting dates. There were few instances in which plots with the added floral resources had a lower abundance of squash bugs, and the yield was subsequently higher in the treated plots. The majority of the data indicates either a non-significant or negative result. Some data show a control in squash bug abundance, but a reduction in the yield in the treated plots. This continues the narrative published by previous researchers investigating this method of control (Forehand et al. 2006a, and Forehand et al. 2006b). The inconsistency between the successful reduction in squash bug abundance and the reduction in yield in our study is likely related to the unanticipated attraction of other pest insects. The Squash Vine Borer, *Melittia cucurbitae*, is a well-known pest of squash, and can be very damaging to plant health and yield. *M. cucurbitae* was seen during both

the 2014 and 2015 field seasons. The attraction of additional pest insects is important to consider when making choices regarding the composition of the floral resources to be used for controlling pest insects. Farmscaping may require a very specific mixture and dosage of floral resources unique for each location and cropping system. The goal is to attract just enough beneficial insects and natural enemies but not to attract additional pest insects. The proportion of floral resources to cash crop area may have been too high in this study. Further investigations regarding how much space is required to attract beneficial insects would be useful for farmers so that they do not limit the production of their cash crop. The types of floral resources used in this study could also have caused the attraction of additional pests. The addition of single species floral resources might provide sufficient attraction of beneficial insects (Phillips et al. 2014). Regardless of flower species or area set aside for floral resources, the characteristics of the flower species and the beneficial insects desired to attract should be taken into consideration (Nfzinger and Fadamiro 2010, Grasswitz 2013, and Campbell et al. 2012). While a diversity of floral resources is thought to attract a wide range of beneficial insects and natural enemies (Smukler et al. 2010), there seems to be a threshold at which the attraction of additional pest insects overcomes the benefits of the beneficial insects and natural enemies.

Attracting Beneficial Insects and Natural Enemies

Data collected on the seasonal abundance of the beneficial insects and natural enemies help to inform future manipulations of the floral resources added. For example, an early blooming flower might help to attract pollinators to increase the pollination of the cash crop. Other small corolla flowers planted earlier could help draw in more parasitoids during the crucial time of control needed in the early season for squash.

The comparison of beneficial insect and natural enemy abundance between treated (with floral resources) and control (without floral resources) plots helps to inform growers how to attract target taxa. The many times that more *Solenopsis invicta* was attracted to the control plots over the treated plots, or the times that more *Linepithema humile* was attracted to the treated plots over the control plots, could be explained by the biology of the organism. As they live in large colonies with either one or multiple queens, the occurrence of a neighboring nest to the location of a trap could cause the abundance to be higher than if there were no colonies directly adjacent to the trap. There was also many times that parasitic Hymenoptera were attracted to the control plots over the treated plots. As a factor of our experimental design, the control plots had a lack of floral resources as compared to the treated plots. The distinct yellow pan traps could be viewed more easily in the control plots than in the treated plots, thus explaining why there would be more parasitic Hymenoptera found in the control yellow pan traps versus the treated yellow pan traps. This is further supported by the fact that there was one result in which the treated pit fall traps collected more parasitic Hymenoptera than the control pit fall traps, while the yellow pan traps had no such results. This might also be resolved if the parasitic Hymenoptera were identified beyond the ordinal level. This would allow for better taxonomic resolution and the species or morphospecies of interest could be isolated for analysis, rather than including all parasitic Hymenoptera.

Overall, there were many times that the floral resources attracted more beneficial insects and natural enemies (Table 2, and 3). The instances that failed to do so have potential explanations as to why. There is the potential for some manipulation of the floral resources to address the issue of attracting beneficial insects and natural enemies for the entire growing season. Selecting plant species to lengthen the time in which flowers are at full bloom could

help to encourage more beneficial insects and natural enemies during the crucial early season (Fargo et al. 1988, Palumbo et al. 1991). Furthermore, individual flowers can be chosen to encourage the attraction of specific beneficial insects and natural enemies based on flower and insect morphology (Nfzinger and Fadamiro 2010, and Campbell et al. 2012). Flowers with a short corolla could be added to increase the attraction of parasitoids with small mouth parts. Buckwheat is a good option as it grows very rapidly, and reseeding could ensure that there are blooms throughout the entire growing season.

Predation of *Geocoris punctipes* on *Aanasa tristis*

Data collected on *G. punctipes* predation on *A. tristis* help to expand on previously published articles. The prey range now includes two additional developmental stages, eggs and second instar nymphs. The likelihood of *G. punctipes* consuming third instar nymphs or later stages was not studied but is unlikely as the size difference begins to favor the squash bugs. This study provided evidence of the difference in predation rates between sexes of *G. punctipes*. Additionally, there is the potential that predation rates of *A. tristis* eggs could be underestimated. There were many observations of eggs being probed and punctured by *G. punctipes*, however, they still hatched while kept in the petri dish in the growth chamber. Probed or punctured eggs experiencing the variable temperature and potential pathogens found in the wild may not have survived. This issue could be investigated with a field study, which might expand the predation rates of *G. punctipes* on *A. tristis* eggs. The more important conclusion is that the big eyed bug, *G. punctipes*, should be considered a significant predator of the squash bug *A. tristis*. The prey range of *G. punctipes* includes the eggs as well as the early instars of *A. tristis*, and is therefore an excellent candidate for biological control agent in controlling the important early season squash bug populations. Human releases of *G. punctipes* or modifying floral resources to attract

G. punctipes early in the season would be recommended for best results in controlling squash bug populations.

While the data collected do not provide a fully detailed recommendation to implement this method of additional floral resources, important lessons can be learned so that future research may be more fine-tuned to better achieve successful control of pest populations. Growers can use information about the number of squash bug generations occurring in the Southeast to inform planting dates. We provided information to aid growers in choosing floral resources to attract particular beneficial insects and natural enemies, as well as when to best attract them. Finally, this research demonstrated the potential *G. punctipes* has as a biological control agent. Growers can either manipulate floral resources or implement releases of *G. punctipes* to aid in the control of *A. tristis*.

Appendix

Tables

Table A.1. Chapter 2 Location*Treatment ANOVA Table. GLIMIX Model for Location*Treatment Effect. Table showing the ANOVA results for both years, and planting dates.

			Num DF	Den DF	F Value	P Value
2014	Planting Date 1	Adults	1	166	27.74	<.0001
		Nymphs	1	166	30	<.0001
		All Mobile	1	166	30.53	<.0001
		Eggs	1	166	18.18	<.0001
		All Bugs	1	166	19.69	<.0001
		Yield	1	117	1.77	0.1862
	Planting Date 2	Adults	1	166	32.24	<.0001
		Nymphs	1	166	12.82	0.0004
		All Mobile	1	166	18.12	<.0001
		Eggs	1	166	2.98	0.0863
2015	Planting Date 1	Adults	1	152	14.64	0.0002
		Nymphs	1	152	11.94	0.0007
		All Mobile	1	152	16.42	<.0001
		Eggs	1	152	2.74	0.0999
		All Bugs	1	152	7.84	0.0058
		Yield	1	152	0.09	0.7611
	Planting Date 2	Gryon sp	1	152	2.36	0.1265
		Geocoris spp.	1	152	0.02	0.8921
		Adults	1	152	3.35	0.069
		Nymphs	1	152	2.29	0.1327
Planting Date 1	All Mobile	1	152	3.45	0.0651	
	Eggs	1	152	0.65	0.4213	
	All Bugs	1	152	0.16	0.6918	
	Yield	1	152	1.77	0.1856	
	Gryon sp	1	152	0.01	0.9338	
	Geocoris spp.	1	152	0	0.993	

Table A.2. Chapter 2 Location*date ANOVA Table. GLIMIX Model for Location*Date Effect. Table showing the ANOVA results for both years, and planting dates.

			Num DF	Den DF	F Value	P Value	
2014	Planting Date 1	Adults	11	166	1.5	0.1345	
		Nymphs	11	166	0.9	0.4542	
		All Mobile	11	166	1.51	0.1319	
		Eggs	11	166	1.17	0.3132	
		All Bugs	11	166	1.04	0.4096	
		Yield	7	117	7.24	<.0001	
		Planting Date 2	Adults	Adults	11	166	0.1
Nymphs	11			166	1.35	0.2034	
All Mobile	11			166	1.61	0.1006	
Eggs	11			166	2.3	0.0119	
All Bugs	11			166	2.15	0.0192	
Yield	7			117	10.4	<.0001	
2015	Planting Date 1			Adults	10	152	1.87
		Nymphs	10	152	1.88	0.0526	
		All Mobile	10	152	3.49	0.0004	
		Eggs	10	152	3.14	0.0011	
		All Bugs	10	152	3.09	0.0013	
		Yield	10	152	2.1	0.0277	
		Gryon sp	10	152	0.62	0.7981	
	Geocoris spp.	10	152	0.19	0.997		
	Planting Date 2	Adults	Adults	10	152	0.49	0.8935
			Nymphs	10	152	1.77	0.0715
			All Mobile	10	152	1.61	0.1083
			Eggs	10	152	1.08	0.3818
			All Bugs	10	152	1.06	0.3955
			Yield	10	152	1.99	0.0378
Gryon sp			10	152	0.29	0.9834	
Geocoris spp.	10	152	0	1			

Table A.3. Chapter 3 Location*Treatment ANOVA Table. GLIMIX Model for Location*Treatment Effect. Table showing the ANOVA results for both years and sampling techniques.

Pit Fall Traps			Num DF	Den DF	F Value	P Value
	2014	Parasitic Hymenoptera	1	131	2.19	0.1412
		Araneae	1	131	6.29	0.0134
		Carabidae	1	131	2.65	0.106
		Hemiptera	1	131	4.62	0.0335
		Geocoris spp.	1	131	0.11	0.7376
		Linepithema humile	1	131	7.9	0.0057
		Solenopsis invicta	1	131	0.59	0.4427
	2015	Parasitic Hymenoptera	1	138	216.28	<.0001
		Araneae	1	138	4.31	0.0397
		Carabidae	1	138	8.95	0.0033
		Hemiptera	1	138	12.77	0.0005
		Geocoris spp.	1	138	0.74	0.3922
		Linepithema humile	1	138	0.92	0.338
		Solenopsis invicta	1	138	14.73	0.0002
Yellow Pan Traps			Num DF	Den DF	F Value	P Value
	2014	Parasitic Hymenoptera	1	110	1.57	0.2126
		Araneae	1	110	10.63	0.0015
		Pollinators	1	110	2.75	0.1003
		Hemiptera	1	110	4.73	0.0317
		Geocoris spp.	1	110	0.17	0.6777
		Linepithema humile	1	110	4.51	0.0359
		Solenopsis invicta	1	110	1.29	0.2587
	2015	Parasitic Hymenoptera	1	124	5.86	0.0169
		Araneae	1	124	0.28	0.5976
		Pollinators	1	124	0.2	0.6578
		Hemiptera	1	124	1.9	0.17
		Geocoris spp.	1	124	0.13	0.7234
		Linepithema humile	1	124	14.39	0.0002
		Solenopsis invicta	1	124	1.81	0.1814

Table A.4. Chapter 3 Location*Date ANOVA Table. GLIMIX Model for Location*Date Effect. Table showing the ANOVA results for both years and sampling techniques.

Pit Fall Traps			Num DF	Den DF	F Value	P Value
	2014	Parasitic Hymenoptera	9	131	2.25	0.0226
		Araneae	9	131	1.26	0.2674
		Carabidae	9	131	2.56	0.0097
		Hemiptera	9	131	7.55	<.0001
		Geocoris spp.	9	131	0.28	0.979
		Linepithema humile	9	131	1.92	0.0544
		Solenopsis invicta	9	131	2.38	0.0157
	2015	Parasitic Hymenoptera	9	138	1.38	0.2037
		Araneae	9	138	0.64	0.7652
		Carabidae	9	138	0.42	0.9203
		Hemiptera	9	138	2.63	0.0077
		Geocoris spp.	9	138	0.24	0.9881
		Linepithema humile	9	138	0.84	0.5834
		Solenopsis invicta	9	138	3.07	0.0022
Yellow Pan Traps			Num DF	Den DF	F Value	P Value
	2014	Parasitic Hymenoptera	8	110	3.41	0.0015
		Araneae	8	110	1.42	0.1944
		Pollinators	8	110	3.46	0.0014
		Hemiptera	8	110	2.16	0.0359
		Geocoris spp.	8	110	0.4	0.9175
		Linepithema humile	8	110	0.38	0.9284
		Solenopsis invicta	8	110	0.25	0.9804
	2015	Parasitic Hymenoptera	9	124	1.77	0.081
		Araneae	9	124	2.2	0.0266
		Pollinators	9	124	3.59	0.0005
		Hemiptera	9	124	5.27	<.0001
		Geocoris spp.	9	124	0.23	0.9898
		Linepithema humile	9	124	1.83	0.0693
		Solenopsis invicta	9	124	1.52	0.149

Table A.5. Chapter 4 Predation and Mortality ANOVA Table. GLIMIX Model for Group Effect. Table showing the ANOVA results for both Predation and Mortality data.

		Num DF	Den DF	F Value	P Value
Predation	Egg	1	48	0	0.9694
	First	1	48	53.11	<.0001
	Second	1	48	38.16	<.0001
Mortality	Egg	2	72	0.31	0.737
	First	2	72	5.61	0.0054
	Second	2	72	3.57	0.0331