APHID PARASITISM: A SUSTAINABLE BIOCONTROL OPTION AGAINST APHID PESTS OF PECAN IN THE SOUTHEASTERN US

by

EDDIE K. SLUSHER

(Under the Direction of Jason Schmidt)

ABSTRACT

Three species of pecan aphid are important pests of pecans in Georgia. Growers often react to pecan aphid outbreaks by applying insecticides, which can lead to resistance development and detrimental non-target effects on natural enemies such as ladybeetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), and *Aphelinus perpallidus* Gahan (Hymenoptera: Aphelinidae). *Aphelinus perpallidus* is a biocontrol agent of pecan aphids as it requires an aphid host in order to complete its life cycle. However, many portions of the ecology between pecan aphids and *A. perpallidus* are poorly understood. In the following studies we examined the effects of multiple abiotic and biotic factors on pecan aphids and *A. perpallidus* including seasonality, insecticide application, vertical stratification, and hyperparasitism. Through this research, it was found that pecan aphids are found in low numbers throughout the season in managed, commercial pecan orchards in Georgia but tend to follow the same seasonal trends as experimental orchards. *A. perpallidus* follows the same seasonal cycle as their hosts. Studies on the pecan aphid-parasitoid food-web, found five hyperparasitoid species associated with *A. perpallidus* in Georgia pecan orchards, one of the first times this had been analyzed genetically. It was also found that the

insecticides flonicamid, sulfoxaflor, and afidopyropen managed pecan aphids while not being harmful to adult *A. perpallidus* and mummy abundance. Finally, studies on vertical stratification effects on aphids and *A. perpallidus* found that aphids, mummies, and adult *A. perpallidus* were predominantly found in higher abundance in the lower canopy of pecan trees. The results of these studies contribute valuable information to growers and interested parties on pecan aphid-parasitoid interactions.

INDEX WORDS: aphicides, biological control, host parasitoid-interactions, hyperparasitoids

APHID PARASITISM: A SUSTAINABLE BIOCONTROL OPTION AGAINST APHID PESTS OF PECAN IN THE SOUTHEASTERN US

by

EDDIE K. SLUSHER

BS, Georgetown College, 2013

MS, Kentucky State University, 2017

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2021

APHID PARASITISM: A SUSTAINABLE BIOCONTROL OPTION AGAINST APHID PESTS OF PECAN IN THE SOUTHEASTERN US

by

EDDIE K. SLUSHER

Major Professor:	Jason Schmidt
Committee:	Angelita Acebes-Doria
	Ted Cottrell
	Will Hudson

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
December 2021

ACKNOWLEDGEMENTS

I would like to thank Dr. Angel Acebes and Dr. Jason Schmidt for their guidance as my major professors throughout my graduate career. I would especially like to thank Angel for taking a chance on me as a PhD student back in 2018 when she was just beginning her career as a professor. I would also like to thank my committee members Ted Cottrell and Will Hudson for their valuable knowledge that they passed onto me.

I would also like to thank all the technical assistance I received from my lab mates in the Tree Nut Entomology Lab including Pamela “Pam” Halliday (UGA-Tifton), Kameron “Kam” Walker (ABAC), Caitlyn Crawford (ABAC), Todd “T.J.” Walker (ABAC), Kate Phillips (UGA-Tifton), Kirsten Flinn (UGA), Jamal Hunter (UGA), Pedro Toledo (UGA-Tifton), and William “Hunter” Mclemore (UGA and ABAC). I would also like to thank the Biological Control Lab, especially Jason Schmidt and Melissa Thompson for their help with all the molecular work that was done for the second chapter of this dissertation. Furthermore, I would also like to thank Xuelin Luo for her help with all the statistics. I would also like to thank Tommy Bowling (Albany), Phillip Doles (Marshallville), Buck Paulk (Ray City), and Jeff Dorsey (Nashville) who allowed the use of their orchards for the following studies.

Finally, I would like to thank my parents, Eddie W. Slusher and Christine L. Slusher as well as my brothers, Cody and Brandon Slusher, for all their support throughout my graduate career. This would not have been possible without all of your support along the way.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

PECAN PRODUCTION AND PHYSIOLOGY

Pecans in Georgia

The pecan, *Carya illinoinensis* (Wangenh.) K. Koch, (Fagales: Juglandaceae) is a deciduous nut tree native to North America with a historical range stretching from northern Mexico to as far North as Indiana and Illinois (Harris 1983, Wells 2017). Natural populations of *C. illinoensis* can be found around the Mississippi River valley (Wells 2017). Domestically, pecan trees are primarily cultivated for nut production across most of the southern U.S. including Georgia, Texas, New Mexico, Arkansas, Oklahoma, and Kansas (Wells 2017). In addition, the country of Mexico contributes significantly to the world's pecan nut production (Wells 2017). Georgia has been a global leader in pecan production since the 1950's and by 1963 was producing ~453,592.37 kg of pecans annually (Well 2017). Today, pecan is still one of the top-ranking agricultural commodities in Georgia. In 2019, 52,204.4 bearing hectares of pecans were in production in Georgia yielding about 634.05 kg per hectare leading to a total production of roughly 33,100,199.82 kg of pecans (NASS 2020) . The farm gate value for pecan production in 2019 was $263,359,174 (Wolfe and Stubbs 2020).

Reproduction and Growth

Pecan budbreak in Georgia is usually around the beginning of April but can be influenced by winter and spring temperatures. For example, a warm winter and cool spring can lead to a delay in bud break (Sparks 2005, Wells 2007). Spring is also a time for shoot growth; however, younger

trees may go through additional shoot growth cycles in mid and late summer. The leaves, male flowers (catkins), and female flowers (pistillate) also develop during this time frame (Wells and Conner 2007, Wells 2017).

The pecan is monoecious with separate catkins and pistillates growing on the same tree (Casales et al. 2018). The catkins are long and slender often appearing in groups of two or three along new shoots (Wells and Conner 2007, Wells 2017, Casales et al. 2018). A single stalk of a catkin can contain as many as 110 flowers with each flower holding up to 2000 pollen grains (Wells 2007). The pistillates are found at the tips of new branches and consist of three to ten green flowers that turn bright yellow as they mature; these flowers were induced in June of the previous season (Wells 2007). Pecan anthesis and pistillate receptivity occur at different times on a tree; this is referred to as dichogamy. Dichogamy is a reproductive strategy that prevents self-pollination, which can have detrimental effects on fruit production (Sparks 2005, Wells 2007, Casales et al. 2018). However, this also presents an issue cultivating pecans as all the trees of one variety will release pollen at the same time when pistillates are unreceptive (Sparks 2005, Wells 2007, Casales et al. 2018). Therefore, two varieties are often needed in an orchard to have optimal pollination. Some pecans are protandrous, meaning they shed their pollen before the pistillates are receptive, while others are protogynous and have receptive pistillates before their pollen is shed. A combination of both types in an orchard is key to adequate pollination (Sparks 2005, Wells 2007, Casales et al. 2018). The environment during pollination can influence reproductive success. For example, low humidity can lead to a drying out of the pistillates while high temperatures and high humidity can prevent pollen shed (Wells 2007, Fronza et al. 2018).

The different stages of nut development occur throughout summer months and, while it differs by variety, most pecan cultivars follow similar stages of development (Byford 2005, Wells

2007). Early nut sizing occurs roughly 6 weeks after pollination (usually early June) as fertilization occurs. Rapid nut sizing occurs 9 weeks after pollination as well as the early water stage. Shell hardening and mid-water stage begin about 12 weeks post-pollination during late July. In early August, about 13 weeks post-pollination, shell hardening continues, and the kernel begins to fill. Fill stage continues in mid-August or 15 weeks post-pollination when shell hardening is completed, and the early dough and gel stages begin. Kernel development continues throughout late September and October usually reaching completion around late October when the green shuck separates from the mature nut (Wells 2007).

Pecan trees are naturally alternate bearing (i.e., exhibit masting), generally having a heavy crop one year followed by a lighter one the next year (Wood et al. 2003, Noperi-Mosqueda et al. 2020). This can create problems for commercial production as it leads to an inconsistent harvest year to year (Conner and Worley 2000). Alternate bearing is believed to be caused by the previous season's crop load as well as the amount of carbohydrate stores the tree has (Noperi-Mosqueda et al. 2020). Low carbohydrate stores during the winter months can have a negative impact on the following spring flowering. This can often be mitigated by nut thinning prior to induction of the pistillates in August as well as applying fertilizer late in the season (Conner and Worley 2000, Noperi-Mosqueda et al. 2020).

Pecan Pest Assemblage

Pecan trees harbor numerous pest species that attack all stages of growth and can be found in all parts of the tree (Hudson 2007). Primary pests target the foliage making up a diverse assemblage of Lepidoptera, Coleoptera, Hemiptera and even Acari (Table 1.1). The nuts are commonly targeted by Hemiptera, Lepidoptera, and Coleoptera (Table 1.1). Finally, the bark and roots are attacked by various species of Coleoptera and one species of scale (Table 1.1). Most of

these pests may be minor or sporadic depending on the conditions present (Hudson 2007). However, a few of these pests mentioned can be problematic from year to year and require some form of management in order to prevent economic damage (Wood et al. 1987, Honaker et al. 2013, Shapiro–Ilan et al. 2013, Shapiro-Ilan et al. 2017, Knutson and Ree 2019). The pecan aphid complex is an example of such a pest that often requires management from growers (Wood et al. 1987, Cottrell et al. 2009, Paulsen et al. 2013, Shapiro–Ilan et al. 2013). Insecticides are often the first method of management implemented in response to an aphid outbreak in various systems (Kandil et al. 2017, Tang et al. 2017, Acebes-Doria and Hudson 2019, Mingeot et al. 2020, Ullah et al. 2020). However, the risk of resistance to insecticides across multiple agricultural commodities has led to consideration of alternative methods of control such as biological control (Kandil et al. 2017, Tang et al. 2017, Acebes-Doria and Hudson 2019, Mingeot et al. 2020, Ullah et al. 2020). The ecological interactions between pecan aphids, *A. perpallidus*, and the various biotic and abiotic factors of their environment are mostly unknown and poorly researched. Thus, the focus of this research was to examine how some of these environmental factors pecan aphids and *A. perpallidus* face affect their abundance and distribution.

PECAN APHIDS

Pecan Aphid Morphology and Life History

Three species of aphid (Hemiptera: Aphididae) are common pests of pecans in the Southeast: yellow pecan aphid *Monelliopsis pecani*s Bissell, blackmargined aphid *Monellia caryella* (Fitch), and black pecan aphid *Melanocallis caryaefoliae* (Davis) (Tedders 1978, Wood et al. 1987, Cottrell et al. 2009, Paulsen et al. 2013). Yellow pecan aphid and blackmargined aphids make up what is known as the ‘yellow aphid’ complex and are difficult to distinguish as

nymphs under field sampling conditions (Tedders 1978, Wood et al. 1987, Cottrell et al. 2009, Paulsen et al. 2013). Yellow pecan aphids and blackmargined aphid nymphs are apterous, bullet-shaped, and pale to bright yellow with red eyes (Tedders 1978, Hudson 2007). They often have black markings on the body and antennae that vary throughout the year. Alate adults are easier to distinguish as yellow pecan aphid adults have wings that they hold gable-like over the body while blackmargined aphid adults have wings that are held flat across the back (Tedders 1978, Hudson 2007). Blackmargined adults also have distinct black markings that run across the front edge of the wing (Tedders 1978, Hudson 2007). Adults of both species can be alate or apterous depending on the time of the year (Tedders 1978, Hudson 2007). The characteristic cornicles of many aphids are reduced in both yellow aphid species (Tedders 1978, Hudson 2007).

Black pecan aphid nymphs are olive green and get darker with successive instars. Early season populations consisting of the fundatrices and the next few generations of alate females are yellow (Tedders 1978, Hudson 2007). They are typically oval shaped and have red eyes. Alate adults are solid black, but apterous females may retain their nymphal colors as adults. The adult's wings have a brown and black tinge and are held gable-like. Small, white spots are often present on the dorsal part of the body (Tedders 1978, Hudson 2007). Like yellow aphids, the black pecan aphid has reduced cornicles (Tedders 1978, Hudson 2007) . In contrast to yellow pecan aphid and blackmargined aphid, which are frequently found on the lower surface of pecan leaves, black pecan aphid nymphs have been found to be evenly distributed on both upper and lower surfaces of the leaf (Paulsen et al. 2013). This is believed to be a strategy to avoid enemies as black aphid nymphs must remain in a feeding area long enough to cause lesions. The failure to do so may result in increased development time and decreased adult size (Cottrell et al. 2009).

The life history of all three aphid species is similar with a few exceptions. Their life cycle is both holocyclic (both asexual and sexual morphs are present during certain points in the season) and monoecious (pecan aphids complete their entire life cycle on pecan) (Tedders 1978, Paulsen 2011). All three species emerge in late March – late April as apterous fundatrices which hatch from eggs that were laid on the bark the previous winter. The fundatrices will spawn anywhere from 15-20 generations of females that reproduce asexually (Tedders 1978, Hudson 2007, Cottrell et al. 2009, Paulsen et al. 2013). The progeny produced during this time can range from as few as five to over 200 (Tedders 1978, Hudson 2007, Cottrell et al. 2009, Paulsen et al. 2013). Yellow pecan aphids and blackmargined populations typically have bimodal peaks with the first peak between May and June and a second peak occurring between August and October (Tedders 1978, Hudson 2007, Mizell 2007). Black pecan aphid are present throughout the season, but are often only present in damaging numbers until August to October (Tedders 1978, Hudson 2007). Between mid-October and early December, apterous females and alate males will appear. The males and females will mate and the females will lay eggs on or in the crevices of the bark of small limbs. These eggs overwinter and produce the fundatrices of the next generation (Tedders 1978, Hudson 2007, Paulsen et al. 2013).

Pecan Aphid Feeding and Damage

All three aphid species feed on the leaf veins, extracting nutrients from the phloem (Tedders 1978, Kaakeh and Dutcher 1994, Cottrell et al. 2009, Paulsen et al. 2013). Black aphids feed mainly on quaternary veins, yellow pecan aphid feeds mainly on the tertiary veins, and blackmargined aphid feeds mostly on the primary and secondary veins (Kaakeh and Dutcher 1994, Paulsen et al. 2013). Feeding can deplete carbohydrates in the leaf, reduce leaf chlorophyll and area, decrease net photosynthesis, and, in severe infestations, cause defoliation (Tedders 1978,

Cottrell et al. 2009, Paulsen et al. 2013). In addition, pecan aphids excrete honeydew that can coat the leaf causing it to have a glossy appearance. Over time, honeydew deposition allows the growth of sooty mold which covers the leaf surface and hinders photosynthesis (Tedders 1978, Cottrell et al. 2009, Paulsen et al. 2013). Black pecan aphid is of special concern because, in addition to the damage caused above, black pecan aphid causes a chlorotic lesion around the feeding site (Tedders 1978, Wood and Reilly 1998, Cottrell et al. 2009). Symptoms are typically restricted by the leaf veins but may become larger over time even if the aphid is removed. The severity of this symptom can be affected by aphid age/instar, length of feeding time, and the age/health of the leaf (Tedders 1978, Cottrell et al. 2009). These chlorotic lesions appear to be important to black pecan aphid nymph development as they rarely move from their initial feeding site until they become adults. Laboratory studies that forced black aphid nymphs onto a new leaf everyday found that they took 5 days longer to develop, were smaller in size, and had a higher mortality rate than aphids that were not moved (Cottrell et al. 2009). Black pecan aphid feeding damages the foliage and can lead to defoliation as the tissue in the chlorotic area turns brown and dies (Cottrell et al. 2009).

Despite a thorough understanding of the life history of these aphids, most of these studies have been done in a laboratory setting or in an experimental orchard. This leaves out potential factors that vary between commercial orchards such as differences in spray regimes in addition to seasonal changes in pecan aphid numbers. Analysis of aphid populations in commercial orchards could potentially provide valuable information on pecan aphid populations in the southeast.

Pecan Aphid Management

In Georgia, the yellow aphid complex is present throughout the growing season. It is recommended in the University of Georgia pecan spray guide (Acebes-Doria and Hudson 2019) that the yellow aphid complex not be treated in the early season. Early season populations are often

controlled by the presence of beneficial aphid predators and parasitoid such as ladybeetles, lacewings, and *Aphelinus perpallidus* Gahan (Hymenoptera: Aphelinidae) (Bueno Jr and Stone 1983, Bueno Jr and Stone 1985, Acebes-Doria and Hudson 2019). However, in the event that yellow aphids may require insecticidal management there are several options available. These include acetamiprid, clothianidin, flonicamid, flupyradifurone, imidacloprid, pymetrozine, pyridaben, pyrifluquinazon, sulfoxaflor, thiamethoxam, and tolfenpyrad (Acebes-Doria and Hudson 2019).

Black pecan aphids can be managed using the same insecticides plus chlorpyrifos (Acebes-Doria and Hudson 2019). Black pecan aphids should be scouted for by checking the compound leaves on 10 terminals on 10 trees. If it is prior to the 1st of July, one should treat if 25% of the terminals have 2 black pecan aphids or more. After the 1st of July, treatment should be applied if 15% of terminals have more than one black pecan aphid (Acebes-Doria and Hudson 2019). Gibberellic acid is also an option for black pecan aphid management. Application of gibberellic acid does not directly harm black pecan aphids but has been shown to mitigate the ability of the black pecan aphid to elicit leaf chlorosis and lessens black pecan aphid establishment (Cottrell et al. 2010, Acebes-Doria and Hudson 2019).

Pyrethroid materials such as cypermethrin or bifenthrin should not be used, especially in the early to mid-season (Acebes-Doria and Hudson 2019). These insecticides often inhibit natural enemies that feed on aphids, potentially causing an outbreak as aphid populations rebound with fewer predators and parasitoids to control them. Populations of the yellow aphid complex have also been found to exhibit resistance to neonicotinyl insecticides such as imidacloprid, thiamethoxam, acetamiprid, and clothianidin (Acebes-Doria and Hudson 2019).

Insecticide resistance inhibits the ability to manage many different species of aphid. In addition, lack of understanding of some of these resistance mechanisms has made addressing this problem more difficult. The first case of resistance in *Myzus persicae* Sulzer (Hemiptera: Aphididae) was reported in 1955. However, it was only discovered in the 1990's that carboxylesterases in *M. persicae* were key to its resistance to organophosphates, pyrethroids, and carbamates (Tang et al. 2017). Since this time, *M. persicae* has been shown to possess as many as seven independent resistance mechanisms with several being novel methods of resistance (Bass et al. 2014). In addition to *M. persicae,* resistance to pyrethroids in the upper midwestern United States has been documented in *Aphis glycines* Matsumura (Hemiptera: Aphididae) which have shown up to a 39-fold decrease in mortality compared to laboratory populations when exposed to pyrethroids (Hanson et al. 2017). Furthermore, a strain of *Aphis craccivora* Koch (Hemiptera: Aphididae) that had been exposed to pirimicarb for 12 generations exhibited 47-fold resistance to the insecticide compared to laboratory populations. In addition, the resistant strain was also cross-resistant for carbosulfan, malathion, chlorpyrifos-methyl, and thiamethoxam (Kandil et al. 2017).

In recent years, there has been a move from using broad spectrum insecticides for aphid management to insecticides formulated to target insects with piercing-sucking mouthparts (Ball 1977, Harris and Cutler 1978, Acebes-Doria and Halliday 2020, Mulder Jr et al. 2020). This allows for management of the target insect while mitigating non-target effects to natural enemy populations. By reducing non-target effects, natural enemy populations can be sustained and assist in the control of pest populations. Non-target effects have been documented in the pecan orchard system. For example, carbaryl used for pecan weevil management led to an outbreak of aphid populations due to reduction in natural enemy populations (Dutcher et al. 1985). In addition, the

use of tebufenozide and chlorpyrifos for hickory shuckworm control had non-target effects on lacewings and ladybeetles (Quiñones-Pando et al. 2009).

Resistance development can be managed by rotating insecticides of different chemical classes based on guidelines published by the Insecticide Resistance Action Committee (IRAC) (Sparks and Nauen 2015). For example, aphicides such as sulfoxaflor, flonicamid, and afidopyropen can be used as part of a rotation for aphid management, as they have different modes of action (Sparks and Nauen 2015). However, continued evaluation and research on insecticide effects both in the lab and in the field is critical in order to spot potential signs of resistance in aphid populations. To date, few studies have been done to evaluate the effects of insecticides on pecan aphids and their parasitoids in a field setting. Such information can be useful in order to alter pest management tactics including rotation of chemical classes and elimination of ineffective chemistries. In addition, evaluation of non-target effects is also important in order to identify discrepancies in biological and chemical control.

PARASITOIDS AS BIOLOGICAL CONTROL AGENTS

Aphid Parasitoid Ecology

Aphids are primarily parasitized by Hymenoptera parasitoids that specialize on aphids. Aphidiinae, a monophyletic subfamily of Braconidae (Hymenoptera) (Kambhampati et al. 2000, Boivin et al. 2012) are primarily solitary koinobiont (i.e. a single parasitoid develops as the host continues to live) (Boivin et al. 2012). Aphelinidae (Hymenoptera) mainly parasitize the Hemipteran suborder Sternorryncha which contains whiteflies, aphids, and scales. Four Aphelinid genera are known to attack aphids: *Aphelinus, Marietta, Protaphelinus*, and *Mesidiopsis* (Viggiani 1984, Boivin et al. 2012). The genus *Aphelinus* consists entirely of solitary, koinobiont aphid

parasitoids (Van Lenteren et al. 1997, Boivin et al. 2012). One Dipteran genus *Endaphis* (Diptera: Cecidomyiidae) contains six species that are known aphid parasitoids (Muratori et al. 2009, Boivin et al. 2012). The life cycle of *Endaphis* parasitoids is unique from Hypmenoptera because the eggs are deposited near aphid colonies instead of within an individual aphid. The eggs hatch and the first instar larvae seek out a host to parasitize. The parasitoid larva only lives within the host until its last instar, when it emerges and drops to the group to pupate (Muratori et al. 2009, Boivin et al. 2012).

The aphid parasitoid lifecycle begins when a female deposits a single egg into or near aphid. After the eggs hatch, the larva feeds on an aphid as the aphid continues to feed and grow (Boivin et al. 2012). It is only during the last larval instar of the parasitoid that the larva kills the host. The larval parasitoid then spins a silken cocoon in which it pupates (Boivin et al. 2012). The remaining aphid cuticle hardens and dries, becoming what is known as a mummy (Boivin et al. 2012, Hall et al. 2017). The larva develops inside the pupa until it becomes an adult. The adult parasitoid then chews a hole in the mummy and escapes (Boivin et al. 2012). Female parasitoids often mate near the emergence site and then begin to search for a host. Most females parasitize hosts that are either on the same plant or a few meters away (Boivin et al. 2012). Only in the event of host scarcity will female parasitoids travel long distances. For example, an individual *Aphidius eadyi* Stary, Gonzalez, and Hall (Hymenoptera: Braconidae) was found to be able to disperse up to 120 km within 1 year when pursuing *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) (Cameron et al. 1981).

Parasitoids in Aphid Biological Control

Parasitoids are a common method employed for aphid management. The first attempt to introduce a parasitoid for aphid management was the use of *Aphelinus mali* (Haldeman)

(Hymenoptera: Aphelinidae) to control *Eriosoma lanigerum* Hausm (Hemiptera: Aphididae) in apples (Howard 1929). *Aphelinus mali* was introduced to North America in 1921 and has since been used worldwide as an effective biological control agent of woolly apple aphid achieving field parasitism rates of 50-90% (Howard 1929, Shaw and Walker 1996, Su et al. 2017).

Aphelinus abdominalis Dalman (Hymenoptera: Aphelinidae) is a commercially available polyphagous solitary aphid parasitoid. In addition, it is deuterotokous, producing diploid females and haploid males parthenogenetically (Wahab 1985). Native to Europe, *A. abdominalis* has since been introduced to Asia, Africa, and North America (Barrette et al. 2009). *A. abdominalis* has been used for management of *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae), *Nasonovia ribisnigri* (Mosely) (Hemiptera: Aphididae), *Macrosiphum rosae* (L.) (Hemiptera: Aphididae), *M. persicae* and *Rhodobium porosum* (Sanderson) (Hemiptera: Aphididae) (Shrestha et al. 2017).

Members of the *Aphidius* genus have also been used successfully in aphid biological control. *Aphidius colemani* Viereck (Hymenoptera: Braconidae) was introduced from Argentina to North America in the 1990's as a biological control agent for *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) (Lin and Ives 2003). *A. colemani* has since been introduced to Europe, Africa, Asia, Australia, and New Zealand and has been used for management of several economically important aphids such as *Aphis gossypii* Glover (Hemiptera: Aphididae) in glasshouse crops and *M. persicae* on eggplants (Perdikis et al. 2004). In addition, *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) provides biological control for many aphid species including: *A. pisum*, *M. euphorbiae*, *M. rosae*, *A. solani*, *M. persicae*, and *R. porosum* (Pennacchio et al. 1995, Boivin et al. 2012).

Perennial aphid-parasitoid systems

Aphid-parasitoid interactions have been well documented across several systems, especially perennial systems. As mentioned previously, *A. mali* has been used in apple orchards to manage *E. lanigerum* and several other parasitoids have been used or assessed for aphid management in other perennial cropping systems. *Ephedrus sp.* (Hymenoptera: Braconidae) parasitizes *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae), a major pest in organic apple orchards (Dib et al. 2010) at rates of 0.66% to 6.93%. *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) is used to manage *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) (Weathersbee et al. 2004). *T. citricida* is a vector of the citrus tristeza virus is found in Southeast Asia, Australia, New Zealand, South Africa, South America, and Hawaii. While not a native host species, *L. testaceipes* has adapted to *T. citricida* as a host and has become the most common primary parasitoid collected in Florida *L. testaceipes* populations (Weathersbee et al. 2004). In addition, *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae), *A. colemani*, *Ephedrus persicae* Froggatt (Hymenoptera: Braconidae), *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae), *Praon volucre* (Haliday) (Hymenoptera; Braconidae), *Trioxys angelicae* (Haliday) (Hymenoptera: Braconidae), and *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae) have been found to be associated with *T. citricida* in Tunisian citrus orchards (Boukhris-Bouhachem 2011). *Praon unicum* Smith (Hymenoptera: Braconidae), *A. ervi*, and two *Aphidius* spp. have been identified as parasitoids of blueberry aphid, *Ericaphis fimbriata* (Richards) (Hemiptera: Aphididae), a vector of the blueberry scorch virus. The strawberry system is plagued by numerous aphid pests including *Acyrtosiphon malvae* (Theobald) (Hemiptera: Aphididae), *A. gossypii, A. solani, Chaetosiphon fragaefolii* (Cockerell) (Hemiptera: Aphididae), *M. euphorbiae, M. rosae, M. persicae*, and *R. porosum* (de Menten 2011, Cingolani and Greco

2018). Mummy samples have revealed various parasitoids associated with each species including several commercially species released into strawberry fields (de Menten 2011, Cingolani and Greco 2018) (Table 1.2).

Pecan Aphid Parasitoid

Aphelinus perpallidus is a small yellow wasp in the family Aphelinidae (Tedders 1978). The biology of *A. perpallidus* is largely unknown with only a few manuscripts being published that mainly focus on the relationships between it and its primary host, pecan aphids (Bueno Jr and Stone 1983, Bueno Jr and Stone 1985, Bueno Jr and Stone 1987, Bueno Jr and Van Cleve 1997a, b). *A. perpallidus* has been documented to parasitize both yellow pecan aphid and blackmargined aphid. Parasitism of black pecan aphid in lab studies was reported by Tedder (1978), but recent studies suggest that black pecan aphids have no known parasitoid (Paulsen et al. 2013). Like most *Aphelinus*, female *A. perpallidus* lay a single egg inside of a pecan aphid. The hatched larva will begin to eat the pecan aphid from the inside eventually killing it. Mummies of pecan aphids are shiny black and often stay attached to the leaf surface. *A. perpallidus* exhibits arrhenotokous parthenogenesis, in that mated females produce both male and female offspring, whereas unmated females produce only males (Bueno Jr and Van Cleve 1997b).

The seasonal phenology of *A. perpallidus* is mostly unknown. Tedders (1978) reported low numbers of adults in early April and low numbers of mummies from May until late July, and the highest number of mummies were recorded in October. All three aphid species were usually parasitized as first instars and occasionally as second instars. Black pecan aphid mummies consisted of only third and fourth instars with no adults collected suggesting that most are killed between the third and fourth instar. Assessment of yellow pecan aphid and blackmargined aphid mummies suggest these aphids are typically killed as adults or during their fourth and third instars

(Tedders 1978). Tedders (1978) estimated a 7-10 day period for *A. perpallidus* to complete its the life cycle, and Tedders also reported parasitism appeared to be proportional to pecan aphid abundance. Bueno and Stone (1987) found a significant relationship between *A. perpallidus* fecundity and aphid density, suggesting that aphid densities of 0.50-0.75 aphids per cm^2 were needed in order to have enough parasitism to develop a high proportion of female progeny.

A few studies have attempted to document the impact of parasitism on pecan aphids with most of these studies being done in the Southwest. Watterson and Stone (1982) collected samples of 300 blackmargined aphids across multiple orchards and documented percentage parasitism. During most of the growing season, less than 6% of blackmargined aphids were parasitized. However, in one orchard sampled this number increased to 52%. Bueno Jr. and Stone (1985) found peak percent parasitism from 6% - 17% in 1981 and 25% to 30% in 1982. Mansour et al. (1988) found a peak of only 6.4% parasitism in a laboratory study and no parasitism when *A. perpallidus* were released into the field in Israel. Therefore, it appears that *A. perpallidus* may have some impact on pecan aphid populations but this impact varies according to time of year and location.

The effects of hyperparasitism on *A. perpallidus* were documented in a couple of studies. Bueno and Stone (1983) reported low hyperparasitism rates of 1.2%, 1.7%, and 3.3% at sites in Texas. Bueno and Stone (1985) identified several of these hyperparasitoids and they include: *Alloxysta schlingeri* (Andrews) (Hymenoptera: Charipidae), *Signophora* spp. (Hymenoptera: Signophoridae), and *Aphidencyrtus* spp. (Hymenoptera: Encyrtidae).

Despite this substantial body of work, the seasonal phenology and effects of environmental factors such as canopy height, insecticide application, and hyperparasitoid pressure have not been thoroughly studied in *A. perpallidus*. There are many questions to be answered about *A.*

perpallidus' habits and its relationship and role within the pecan aphid food web. This could help provide support for the efficacy of *A. perpallidus* as a biological control organism.

VERTICAL STRATIFICATION OF ARTHROPODS IN AGRICULTURE

Vertical Stratification in Ecology

Vertical stratification defines the distribution of organisms along a vertical plane (Basset et al. 2003). The distribution of organisms across a vertical plane has become a subject of interest in several different systems including forest, water bodies, and soil (Basset et al. 2003, Shapiro-Ilan et al. 2017, McGregor et al. 2018, Procházka et al. 2018, Seo et al. 2018, Knutson and Ree 2019, Oliveira and Scheffers 2019, Rissanen et al. 2019, Chmel et al. 2021, Littlefair et al. 2021). The presence of vertical strata has been linked to greater species richness, more niche space, and habitat partitioning (Oliveira and Scheffers 2019).

While many groups of organisms have had their vertical stratification preferences assessed, insects have been among the groups most heavily studied, especially those that are pests of forests or disease vectors (McGregor et al. 2018, Procházka et al. 2018, Šigut et al. 2018). The effects of vertical stratification on agricultural pests and natural enemies has been underrepresented, especially in the orchards system as most studies are usually done on row crops. Even in studies conducted on orchards systems, most studies have focused primarily on parts accessible from the ground. Assessment and understanding of the effects of vertical stratification can provide valuable information on the distribution of pests and natural enemies, helping to influence many different aspects of agricultural management including insecticide application and monitoring. For example, a study by Teulon and Penman (1987) found that Froggatt's apple leafhopper, *Typhlocyba froggatti* Baker (Hemiptera: Cicadellidae), populations were affected by increasing height. Male

leafhoppers were captured in higher numbers as the trap height increased from 0.9-2.7 m. Thus, it was recommended that monitoring efforts should focus on upper portions of the tree where apple leafhopper were congregating (Teulon and Penman 1987).

Furthermore, understanding canopy location preference can be important for understanding pest-predator/parasitoid dynamics and to assess impact of biological control programs. Examination of *Halyomorpha halys* (Stal) (Hemiptera: Pentatomidae) and its parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) at different canopy locations in tree of heaven (*Ailanthus altissima* (Mill.) Swingle) and hackberry (*Celtis occidentalis* L.) revealed differences in habitat preference. Both adult and nymph *H. halys* were captured in significantly higher numbers in the upper canopy than in the middle or lower canopy. However, egg masses were collected in higher numbers in the mid-canopy where *T. japonicus* also emerged in higher numbers (Quinn et al. 2019). This yielded important information about the effects of vertical stratification on the ecology and distribution of both pest and parasitoid.

Effects of Vertical Stratification on Aphids

Aphids are believed to distribute themselves throughout the canopy for a number of reasons. Several previous studies have shown that aphids often prefer the lower canopy in many different tree systems. This is likely due to avoidance of the upper portions of tree which have leaves with lower nutritional value (Dixon 2005, Platková et al. 2020). In addition, the upper canopy is exposed to more solar radiation and temperatures can differ by as much as 10 ºC (Dixon 2005). Aphids may also use vertical strata as a space use component to avoid predation and parasitism (Costamagna and Landis 2011, Platková et al. 2020). Aphids are considered easy prey for many different predators and parasitoids due to their lack of physical defenses, however, they have been shown to display numerous other strategies to survive such as changes in vertical space

(Costamagna and Landis 2011). However, this varies according to the predator with some studies finding that aphids congregate in the lower canopy as predation pressure is higher in the upper and middle parts of the canopy, while other studies have found predation pressure in the lower canopy from predators such as earwigs (Dixon 1969, Clements and Yeargan 1997, Costamagna and Landis 2011, Kirstová et al. 2017, Platková et al. 2020).

Another reason aphids may disperse is in response to density of the population in parts of the canopy (Costamagna and Landis 2011, Fernandes et al. 2012, Platková et al. 2020). For example, *M. pecanis* were found to prefer the lower portion of 13-meter pecan trees until the population density reached a certain point, after which, the aphid showed no canopy preference (Polles and Mullinix 1977). Thus, it appears that the vertical distribution of pecan aphids may be population driven. Further analysis of the relationships between pecans aphids and the factors mentioned above is critical in order to understand what drives pecan aphids to prefer certain portions of the canopy over others.

Effects of Vertical Stratification on Natural Enemies

Vertical stratification may determine the location of aphid populations and other pests, and likely has implications for interactions with their natural enemies. For example, lady beetles (Hymenoptera: Coccinellidae) can respond positively (*Olla v-nigrum* (Mulsant)), negatively (*Coccinella septempunctata* (Linnaeus), *Coleomegilla maculate* De G), and neutrally (*Harmonia axyridis* (Pallas)) to increases in canopy height depending on the individual lady beetle species (Cottrell 2017). Parasitoids have also been found to be affected by vertical strata. For example, parasitism rates were found to decrease from the first to the third level of various trees in Czech Republic deciduous forest. In addition, less host-specific taxa such as Ichneumonidae and

Braconidae were found more frequently in the upper canopy than in the lower canopy while more host-specific taxa such as Tachinids mirrored their host distribution (Šigut et al. 2018).

The effects of vertical stratification on *A. perpallidus* abundance have yet to be studied. In addition, the relationships between pecan aphid and *A. perpallidus* populations in pecan trees has also not been thoroughly studied. Such information is useful for implementing pest management plans by providing information on where in pecan trees growers should scout for aphids, where to best target pesticide coverage, and where biological control is most effective.

CLARIFYING HOST-PARASITOID INTERACTIONS THROUGH MOLECULAR BARCODING

Molecular Techniques versus Traditional Rearing

A major obstacle in developing a successful biological control program is the poor understanding of the food-web interactions between the pest of interest and its parasitoids (Kitson et al. 2019). Understanding host-parasitoid interactions is often constrained by traditional insect rearing, which is often slow and time-consuming (Lefort et al. 2017, Šigut et al. 2017, Kitson et al. 2019, Sow et al. 2019). Furthermore, in the case of understanding complex host-parasitoid interactions where multiple parasitoids target the same host, traditional rearing only reveals the 'winner' of these interactions (Lefort et al. 2017). The use of molecular techniques can help document both the winner and the "losers(s)" by recovering DNA from host cadavers and eggs (Bon et al. 2008, Traugott et al. 2008, Desneux et al. 2009, Gariepy and Messing 2012, Zhou et al. 2014, Gómez-Marco et al. 2015, Hall et al. 2017, Lefort et al. 2017, Šigut et al. 2017, Ye et al. 2017b, Alhmedi et al. 2018, Kanturski et al. 2018, Gariepy et al. 2019, Kitson et al. 2019, Sow et al. 2019, Zhu et al. 2019) . For example, use of DNA barcoding on millet head miner, *Heliocheilus*

albipuntella (de Joannis) (Lepidoptera: Noctuidae), provided a higher indication of parasitoid diversity as well as rates of parasitism compared to rearing. In addition, DNA barcoding was also able to detect multi-parasitism and cryptic species, something that could not be done with insect rearing (Sow et al. 2019).

Use of molecular techniques to uncover aphid-parasitoid food webs

Aphid mummies can provide valuable information on previous parasitoid occupants. Studies are just beginning to harvest the power of DNA to uncover aphid-parasitoid food webs. A study done on *A. gossypii* in China used a combination of multiplex polymerase chain reactions (PCR) and several singleplex PCRs to reveal the interactions of the *A. gossypii* food-web (Zhu et al. 2019). They uncovered three primary parasitoids and seven hyperparasitoids associated with *A. gossypii*. Further work using DNA barcoding on *A. glycines* in China identified fifteen hymenopteran parasitoids representing 10 genera and five families (Zhou et al. 2014). There is even the possibility of uncovering novel interactions. For example, barcoding of mummies found in high-tunnel strawberry cultivation unveiled the first occurrence of the aphid *Aphis ruborum* (Borner & Schilder) (Hemiptera: Aphididae) in Mississippi. It also revealed two cryptic parasitoids associated with the aphid, *Aphelinus varipes* (Foerster) (Hymenoptera: Aphelinidae) and *A. albipodus* (Riddick et al. 2019).

Uncovering the complexities of aphid-parasitoid food webs can provide many benefits for biological control programs, including reasons many programs fail. For example, the low success rate of biological control in a cabbage aphid system was revealed using sequencing of aphid mummies (Lefort et al. 2017). The authors used next generation sequencing (i.e. NGS) on mummies of the *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) in both its native range (United Kingdom) and its invasive range (New Zealand) to uncover unique trophic structure at

each site. The UK site consisted of three primary parasitoids and two hyperparasitoids associated with *B. brassicae* while New Zealand consisted of a single primary parasitoid and eight hyperparasitoids (Lefort et al. 2017). A similar DNA-based approach revealed that *Binodoxys angelicae* Haliday (Hymenoptera: Braconidae), a primary parasitoid of *Aphis spiraecola* Patch (Hemiptera: Aphididae), also experiences heavy top-down pressure from hyperparasitoids with six hyperparasitoids that use *B. angelicae* as a host (Gómez-Marco et al. 2015).

To date, no studies have tried to genetically characterize the pecan aphid-parasitoid system, and only a few have begun to unravel different aphid-parasitoid systems with most focusing on cereal crops. Performing an initial genetic characterization of these communities is critical in order to identify the key-players. In addition, many specimens in the pecan aphid-parasitoid system require DNA-barcoding and voucher specimens for use in online DNA-barcoding libraries. Characterization of these interactions can be useful for evaluating the effectiveness of *A. perpallidus* as a biological control agent by identifying potential issues such as top-down pressure from hyperparasitoids as presented in Lefort et al. (2017) and Gomez-Marco et al. (2015).

SUMMARY

In this dissertation, I will focus on the pecan aphid-parasitoid food web in the Southeast. While there has been some previous work on pecan aphid – *A. perpallidus* interactions, most of this work has been done in the southwest with only a few studies documenting pecan aphid-parasitoid interactions in the southeast (Tedders 1977, Tedders 1978, Bueno Jr and Stone 1983, Bueno Jr and Stone 1985, Bueno Jr and Stone 1987, Bueno Jr and Van Cleve 1997a, b). This research will use an integration of field work and lab-based genetic analysis in order to characterize

and detail some of the environmental interactions affecting the three pecan aphid species and their primary parasitoid *A. perpallidus* in Georgia pecan orchards.

For my first objective, I will analyze the seasonal phenology of the three pecan aphid species and both the mummies and adults of *A. perpallidus* in multiple southeast Georgia commercial pecan orchards with different management regimes throughout the pecan growing season. While previous work has been done documenting the seasonal presence of the yellow aphid complex and black pecan aphids in experimental orchards in the southeast, there has been no effort to characterize the seasonal phenology of the pecan aphids in commercial orchards, where they may be subjected to different amounts and types of insecticide pressure (Tedders 1977, Tedders 1978, Dutcher et al. 2010, Dutcher et al. 2012). In addition, there has been little effort to characterize the seasonal phenology of both adult *A. perpallidus* and their mummies in commercial pecan orchards (Tedders 1978). I hypothesize that pecan aphids and their parastioids will be present in lower numbers due to insecticidal pressure, but will still present a similar phenology that has been observed in experimental pecan orchards (Dutcher et al. 2010, Dutcher et al. 2012, Dutcher 2016).

Second, I will analyze the effects of three aphicides on the abundance of both pecan aphids, mummies, and *A. perpallidus* in an experimental orchard. As previously mentioned in the *pecan aphid management* section, there is always the risk of resistance and non-target effects when using insecticides for aphid management (Purcell et al. 1994, Koo et al. 2014, Sparks and Nauen 2015, Hanson et al. 2017, Tang et al. 2017, Koch et al. 2018, Jiang et al. 2019, Joseph 2020, Koch et al. 2020). While some lab-based studies have been done with grower standard products for pecan aphid management such as sulfoxaflor and flonicamid as well as new products such as afidopyropen, there has been minimal work done to assess the efficacy of these products in a field-

based setting (Sparks and Nauen 2015, Jiang et al. 2019, Joseph 2020). In addition, there has been no work done to test the effects of these products on *A. perpallidus* or its mummies in a field setting. Thus, I will evaluate the effects of commonly used pecan aphid management products sulfoxaflor, flonicamid, and afidopyropen on pecan aphids and *A. perpallidus* in an experimental pecan orchard. I hypothesize that due to the specific chemistries of these products, that pecan aphid populations will be managed while not causing non-target effects on *A. perpallidus*.

Third, I will assess the effects of vertical strata on pecan aphids and *A. perpallidus* in both a commercial orchard with shorter trees (~6-9 m) and in an experimental orchard with taller trees (~15-18 m). There has been little effort to date to characterize the relationships between pecan aphids, *A. perpallidus*, and canopy height. As mentioned previously, vertical stratification can have a variety of effects on pest and predator interactions, with some species preferring a particular canopy location based on factors ranging from predator avoidance to food quality (Edelson and Estes 1987, Dixon 2005, Costamagna and Landis 2011, Fernandes et al. 2012, Platková et al. 2020). For this chapter, I will attempt to characterize the effects of vertical stratification on pecan aphids and *A. perpallidus* at various pecan tree canopy locations. I hypothesize that pecan aphids will be found lower in the canopy where there is better food quality and more protection from abiotic factors such as weather. I also theorize that *A. perpallidus* will have the same preference for canopy location as its host.

Finally, I will provide a proof-of-concept by genetically characterizing the pecan aphid-parasitoid food web using DNA barcoding. As mentioned previously there has been no effort to characterize the key-players in the pecan aphid-parasitoid food web with many species lacking genetic sequences in DNA sequencing libraries. Furthermore, little has been done to characterize the hyperparasitoid assemblage in the pecan aphid-parasitoid food web. As a first step to

characterize the pecan aphid food web I will used DNA extraction techniques and PCR analysis to define the trophic interactions of pecan aphids and their primary and hyperparasitoids. This information will be used to characterize the pecan aphid-parasitoid food web for the first time while also revealing valuable information such as potential top-down pressure from hyperparasitoids.

LITERATURE CITED

Acebes-Doria, A. L., and W. G. Hudson. 2019. University of Georgia commercial pecan spray guide. University of Georgia

Acebes-Doria, A. L., and P. L. Halliday. 2020. Insecticide efficacy against pecan aphids and pecan leaf scorch mites, 2018. Arthropod Manag. Tests 45: tsaa087.

Alhmedi, A., S. Raymaekers, Ž. Tomanović, D. Bylemans, and T. Beliën. 2018. Food web structure of aphids and their parasitoids in Belgian fruit agroecosystems. Entomol. Sci. 21: 279-291.

Ball, J. C. 1977. Aphid control on pecans, 1976. Insecticide and Acaricide Tests 2: 44-44.

Barrette, M., G.-M. Wu, J. Brodeur, L.-A. Giraldeau, and G. Boivin. 2009. Testing competing measures of profitability for mobile resources. Oecologia 158: 757-764.

Bass, C., A. M. Puinean, C. T. Zimmer, I. Denholm, L. M. Field, S. P. Foster, O. Gutbrod, R. Nauen, R. Slater, and M. S. Williamson. 2014. The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. Insect Biochem. Mol. Biol. 51: 41-51.

Basset, Y., P. M. Hammond, H. Barrios, J. D. Holloway, and S. E. Miller. 2003. Vertical stratification of arthropod assemblages. *in Arthropods of Tropical Forests*: 17-27.

Boivin, G., T. Hance, and J. Brodeur. 2012. Aphid parasitoids in biological control. Can. J. Plant Sci. 92: 1-12.

Bon, M., K. Hoelmer, D. Coutinot, and N. Ramualde. 2008. PCR-Based, rapid diagnosis of parasitism of lygus spp.(Hemiptera: Miridae) by *Peristenus relictus* (Hymenoptera: Braconidae). Biocontrol Sci. Technol. 18: 505-516.

Boukhris-Bouhachem, S. 2011. Aphid enemies reported from Tunisian citrus orchards. Tunis. J. Plant Prot. 6: 21-28.

Bueno Jr, R., and J. Stone. 1983. Phenology of a parasite of the blackmargined aphid in west Texas [*Aphelinus perpallidus, Monellia caryella*]. Southwest. Entomol.

Bueno Jr, R., and J. D. Stone. 1985. *Aphelinus perpallidus* Parasitism of *Monellia caryella* populations in far west Texas. J. Entomol. Sci. 20: 325-330.

Bueno Jr, R., and J. Stone. 1987. Reproductive response of *Aphelinus perpallidus* (Hymenoptera: Aphelinidae) to age of its parent and density of its host, *Monellia caryella* (Homoptera: Aphidae). Environ. Entomol. 16: 877-880.

Bueno Jr, R., and H. Van Cleve. 1997a. The effect of cold storage on the emergence of *Aphelinus perpallidus*, a parasitoid of *Monellia caryella*. Southwest. Entomol.

Bueno Jr, R., and H. Van Cleve. 1997b. The effect of temperature and host density on the reproduction of *Aphelinus perpallidus*. Southwest. Entomol.

Byford, R. 2005. Growth and development of pecan nuts. New Mexico State University.

Cameron, P., G. Walker, and D. Allan. 1981. Establishment and dispersal of the introduced parasite *Aphidius Eadyi* (Hymenoptera: Aphidiidae) in the North Island of New Zealand, and its initial effect on pea aphid. N. Z. J. Zoology 8: 105-112.

Casales, F. G., E. Van der Watt, and G. M. Coetzer. 2018. Propagation of pecan (*Carya illinoensis*): a review. Afr. J. Biotechnol. 17: 586-605.

Chmel, K., S. M. Kamga, T. Awa, F. L. Ewome, G. Uceda-Gómez, D. Hořák, J. Mlíkovský, L. L. Molua, J. Riegert, and Š. Janeček. 2021. Vertical stratification and seasonal changes of the avian community in Mount Cameroon lowland rainforest. Afr. J. Ecol.

Cingolani, M. F., and N. Greco. 2018. Spatio-Temporal variation of strawberry aphid populations and their parasitoids. Appl. Entomol. Zool. 53: 205-214.

Conner, P. J., and R. E. Worley. 2000. Alternate bearing intensity of pecan cultivars. hortscience 35: 1067-1069.

Costamagna, A. C., and D. A. Landis. 2011. Lack of strong refuges allows top-down control of soybean aphid by generalist natural enemies. Biol. Control 57: 184-192.

Cottrell, T. 2017. Trap Height Affects Capture of lady beetles (Coleoptera: Coccinellidae) in pecan orchards. Environ. Entomol. 46: 343-352.

Cottrell, T. E., B. W. Wood, and X. Ni. 2009. Chlorotic feeding injury by the black pecan aphid (Hemiptera: Aphididae) to pecan foliage promotes aphid settling and nymphal development. Environ. Entomol. 38: 411-416.

Cottrell, T. E., B. W. Wood, and X. Ni. 2010. Application of plant growth regulators mitigates chlorotic foliar injury by the black pecan aphid (Hemiptera: Aphididae). Pest Manag. Sci. 66: 1236-1242.

de Menten, N. 2011. Fresaprotect: The use of a cocktail of parasitoids against aphids in strawberries-a case study. IOBC/WPRS Bulletins 70: 217-223.

Desneux, N., P. Starý, C. J. Delebecque, T. D. Gariepy, R. J. Barta, K. A. Hoelmer, and G. E. Heimpel. 2009. Cryptic species of parasitoids attacking the soybean aphid (Hemiptera: Aphididae) in Asia: *Binodoxys communis* and *Binodoxys koreanus* (Hymenoptera: Braconidae: Aphidiinae). Ann. Entomol. Soc. Am. 102: 925-936.

Dib, H., S. Simon, B. Sauphanor, and Y. Capowiez. 2010. The role of natural enemies on the population dynamics of the rosy apple aphid, *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) in organic apple orchards in South-Eastern France. Biol. Control 55: 97-109.

Dixon, A. 1969. Population dynamics of the sycamore aphid *Drepanosiphum platanoides* (Schr.)(Hemiptera: Aphididae): migratory and trivial flight activity. J. Anim. Ecol. 585-606.

Dixon, A. F. G. 2005. Insect herbivore-host dynamics: tree-dwelling aphids. Cambridge University Press.

Dutcher, J., J. Payne, and W. Neel. 1985. The impact of pecan weevil control strategies on non-target arthropods. *in Pecan Weevil: Research Perspective*: 39-50 Quail Ridge Press, Brandon, MS.

Dutcher, J. D. 2016. A suction trap for sampling aphids and aphidophagous insects in pecan trees. Int. J. Insect Sci. 2: 1-11.

Dutcher, J. D., H. Karar, and M. G. Abbas. 2010. Effectiveness of two insecticides for control of foliage feeding pecan aphids. Arthropod Manag. Tests 35.

Dutcher, J. D., H. Karar, and G. Abbas. 2012. Seasonal abundance of aphids and aphidophagous insects in pecan. Insects 3: 1257-1270.

Edelson, J., and P. Estes. 1987. Seasonal abundance and distribution of predators and parasites associated with *Monelliopsis pecanis* Bissell and *Monellia caryella* (Fitch)(Homoptera: Aphidae). J. Entomol. Sci. 22: 336-347.

Fernandes, F. S., F. S. Ramalho, J. B. Malaquias, J. L. Nascimento Junior, E. T. Correia, and J. C. Zanuncio. 2012. Within-Plant distribution of cotton aphid (Hemiptera: Aphididae) in cotton cultivars with colored fibers. An. Acad. Bras. Ciênc. 84: 707-721.

Fronza, D., J. J. Hamann, V. Both, R. d. O. Anese, and E. A. Meyer. 2018. Pecan cultivation: general aspects. Ciênc. Rural 48.

Gariepy, T., and R. Messing. 2012. Development and use of molecular diagnostic tools to determine trophic links and interspecific interactions in aphid–parasitoid communities in Hawaii. Biol. Control 60: 26-38.

Gariepy, T. D., A. Bruin, J. Konopka, C. Scott-Dupree, H. Fraser, M. C. Bon, and E. Talamas. 2019. A modified dna barcode approach to define trophic interactions between native and exotic pentatomids and their parasitoids. Mol. Ecol. 28: 456-470.

Gómez-Marco, F., A. Urbaneja, J. A. Jaques, P. Rugman-Jones, R. Stouthamer, and A. Tena. 2015. Untangling the aphid-parasitoid food web in citrus: can hyperparasitoids disrupt biological control? Biol. Control 81: 111-121.

Hall, A. A., M. J. Steinbauer, G. S. Taylor, S. N. Johnson, J. M. Cook, and M. Riegler. 2017. Unravelling mummies: cryptic diversity, host specificity, trophic and coevolutionary interactions in psyllid–parasitoid food webs. BMC Evol. Biol. 17: 127.

Hanson, A. A., J. Menger-Anderson, C. Silverstein, B. D. Potter, I. V. MacRae, E. W. Hodgson, and R. L. Koch. 2017. Evidence for soybean aphid (Hemiptera: Aphididae) resistance to pyrethroid insecticides in the upper midwestern United States. J. Econ. Entomol. 110: 2235-2246.

Harris, M. K. 1983. Integrated pest management of pecans. Annu. Rev. Entomol. 28: 291-318.

Harris, M. K., and B. L. Cutler. 1978. Pecan, pecan weevil and hickory shuckworm management, 1977. Insecticide and Acaricide Tests 3: 59-60.

Honaker, J., S. Skrivanek, J. Lopez, D. Martin, L. Lombardini, L. Grauke, and M. Harris. 2013. Blackmargined aphid (*Monellia caryella* (Fitch); Hemiptera: Aphididae) honeydew production in pecan and implications for managing the pecan aphid complex in Texas. Southwest. Entomol. 38: 19-32.

Howard, L. 1929. *Aphelinus mali* and its travels. Ann. Entomol. Soc. Am. 22: 341-368.

Hudson, W. 2007. Insects and mites associated with pecans. 142-170. In L. Wells (ed.), *in Southeastern Pecan Growers' Handbook*. University of Georgia Athens, United States.

Jiang, J., X. Liu, Z. Zhang, F. Liu, and W. Mu. 2019. Lethal and sublethal impact of sulfoxaflor on three species of *Trichogramma* parasitoid wasps (Hymenoptera: Trichogrammatidae). Biol. Control 134: 32-37.

Joseph, S. V. 2020. Effect of afidopyropen against *Myzus persicae* (Hemiptera: Aphididae) and its predator, *Adalia bipunctata* (L.) in a greenhouse. J. Entomol. Sci. 55: 584-587.

Kaakeh, W., and J. D. Dutcher. 1994. Probing behavior and density of *Monelliopsis pecanis*, *Monellia caryella*, and *Melanocallis caryaefoliae* (Homoptera: Aphididae) on pecan cultivars. J Econ Entomol 87: 951-956.

Kambhampati, S., W. Völkl, and M. Mackauer. 2000. Phylogenetic relationships among genera of Aphidiinae (Hymenoptera: Braconidae) based on dna sequence of the mitochondrial 16S rRNA gene. Systematic Entomology 25: 437-445.

Kandil, M. A., I. S. Abdallah, H. M. Abou-Yousef, N. A. Abdallah, and E. A. Fouad. 2017. Mechanism of resistance to pirimicarb in the cowpea aphid *Aphis craccivora*. Crop Prot. 94: 173-177.

Kanturski, M., Y. Lee, J. Choi, and S. Lee. 2018. DNA barcoding and a precise morphological comparison revealed a cryptic species in the *Nippolachnus piri* Complex (Hemiptera: Aphididae: Lachninae). Sci. Rep. 8: 1-16.

Kirstová, M., P. Pyszko, J. Šipoš, P. Drozd, and P. Kočárek. 2017. Vertical distribution of earwigs (Dermaptera: Forficulidae) in a temperate lowland forest, based on sampling with a mobile aerial lift platform. Ento. Sci. 20: 57-64.

Kitson, J. J., C. Hahn, R. J. Sands, N. A. Straw, D. M. Evans, and D. H. Lunt. 2019. Detecting host–parasitoid interactions in an invasive lepidopteran using nested tagging dna metabarcoding. Mol. Ecol. 28: 471-483.

Knutson, A. E., and B. Ree. 2019. Biology and management of the pecan nut casebearer (Lepidoptera: Pyralidae). J. Integr. Pest Manag. 10: 29.

Koch, R. L., E. W. Hodgson, J. J. Knodel, A. J. Varenhorst, and B. D. Potter. 2018. management of insecticide-resistant soybean aphids in the upper midwest of the United States. J. Integr. Pest Manag. 9: 23.

Koch, R. L., O. da Silva Queiroz, R. C. Aita, E. W. Hodgson, B. D. Potter, T. Nyoike, and C. D. Ellers-Kirk. 2020. Efficacy of afidopyropen against soybean aphid (Hemiptera: Aphididae) and toxicity to natural enemies. Pest Manag. Sci. 76: 375-383.

Koo, H.-N., J.-J. An, S.-E. Park, J.-I. Kim, and G.-H. Kim. 2014. Regional susceptibilities to 12 insecticides of melon and cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae) and a point mutation associated with imidacloprid resistance. Crop Prot. 55: 91-97.

Kunkel, B. A., and T. E. Cottrell. 2007. Oviposition response of green lacewings (Neuroptera: Chrysopidae) to aphids (Hemiptera: Aphididae) and potential attractants on pecan. environ. entomol. 36: 577-583.

Lefort, M.-C., S. Wratten, A. Cusumano, Y.-D. Varennes, and S. Boyer. 2017. Disentangling higher trophic level interactions in the cabbage aphid food web using high-throughput dna sequencing. Metabarcoding & Metagenomics: e13709.

Lin, L. A., and A. R. Ives. 2003. The effect of parasitoid host-size preference on host population growth rates: an example of *Aphidius colemani* and *Aphis glycines*. Ecol. Entomol. 28: 542-550.

Littlefair, J. E., L. E. Hrenchuk, P. J. Blanchfield, M. D. Rennie, and M. E. Cristescu. 2021. Thermal stratification and fish thermal preference explain vertical eDNA distributions in lakes. Mol. Ecol. 30: 3083-3096.

McGregor, B. L., A. E. Runkel, S. M. Wisely, and N. D. Burkett-Cadena. 2018. Vertical stratification of culicoides biting midges at a florida big game preserve. Parasites & Vectors 11: 1-11.

Mingeot, D., L. Hautier, and J. P. Jansen. 2020. Structuration of multilocus genotypes associated with insecticide resistance of the peach potato aphid, *Myzus persicae* (Sulzer), in potato fields in Southern Belgium. Pest Manag. Sci.

Mizell, R. F. 2007. Impact of *Harmonia Axyridis* (Coleoptera: Coccinellidae) on native arthropod predators in pecan and crape myrtle. Fla. Entomol. 90: 524-536.

Mulder Jr, P., S. Seuhs, and M. Payton. 2020. Insecticide efficacy for controlling pecan aphids, 2018. Arthropod Manag. Tests 45: tsaa008.

Muratori, F. B., R. J. Gagne, and R. H. Messing. 2009. Ecological traits of a new aphid parasitoid, *Endaphis fugitiva* (Diptera: Cecidomyiidae), and its potential for biological control of the banana aphid, *Pentalonia nigronervosa* (Hemiptera: Aphididae). Biol. Control 50: 185-193.

NASS, U. 2020. United States Department of Agriculture National Agricultural Statistics Service. 2017. Quick stats database.

Noperi-Mosqueda, L. C., J. M. Soto-Parra, E. Sanchez, E. Navarro-LeóN, R. PéRez-Leal, M. A. Flores-Cordova, N. A. Salas-Salazar, and R. M. YáñEz-MuñOz. 2020. Yield, quality, alternate bearing and long-term yield index in pecan, as a response to mineral and organic nutrition. Not. Bot. Horti Agrobot. Cluj-Napoca. 48: 342-353.

Oliveira, B. F., and B. R. Scheffers. 2019. Vertical stratification influences global patterns of biodiversity. Ecogeg 42: 249-249.

Paulsen, C., T. Cottrell, and J. Ruberson. 2013. Distribution of the black pecan aphid, *Melanocallis caryaefoliae,* on the upper and lower surface of pecan foliage. Entomol. Exp. Appl. 146: 252-260.

Paulsen, C. M. 2011. The distribution of the black pecan aphid *Melanocallis Caryaefoliae* (Homoptera: Aphididae) between the upper and lower surfaces of pecan foliage. University of Georgia.

Pennacchio, F., M. Digilio, and E. Tremblay. 1995. Biochemical and metabolic alterations in *Acyrthosiphon pisum* parasitized by *Aphidius ervi*. Arch. Insect Biochem. Physiol. 30: 351-367.

Perdikis, D. C., D. P. Lykouressis, N. G. Garantonakis, and S. A. Iatrou. 2004. Instar preference and parasitization of *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) by the parasitoid *Aphidius colemani* (Hymenoptera: Aphidiidae). Eur. J. Entomol. 101: 333-336.

Platková, H., P. Pyszko, A. Coeur d´ Acier, E. Jousselin, and P. Drozd. 2020. Spatial distribution of aphids in the canopy of a temperate forest: where can they be found? Agric. For. Entomol. 22: 379-389.

Polles, S., and B. Mullinix. 1977. Distribution of yellow pecan aphids within pecan trees. Environ. Entomol. 6: 429-434.

Procházka, J., L. Cizek, and J. Schlaghamerský. 2018. Vertical stratification of scolytine beetles in temperate forests. Insect Conserv. Divers. 11: 534-544.

Purcell, M. F., J. D. Stark, and R. H. Messing. 1994. Insecticide effect on three tephritid fruit flies and associated braconid parasitoids in Hawaii. J. Econ. Entomol. 87: 1455-1462.

Quinn, N. F., E. J. Talamas, A. L. Acebes-Doria, T. C. Leskey, and J. C. Bergh. 2019. Vertical sampling in tree canopies for *Halyomorpha halys* (Hemiptera: Pentatomidae) life stages and its egg parasitoid, *Trissolcus japonicus* (Hymenoptera: Scelionidae). Environ. Entomol. 48: 173-180.

Quiñones-Pando, F. J., S. H. Tarango-Rivero, and C. A. Blanco. 2009. Effect of two insecticides on hickory shuckworm (Lepidoptera: Tortricidae) and predators of pecan pests. Southwest. Entomol. 34: 227-238.

Riddick, E. W., G. L. Miller, C. L. Owen, G. R. Bauchan, J. M. Schmidt, T. Gariepy, R. L. Brown, and M. J. Grodowitz. 2019. Discovery of *Aphis ruborum* (Hemiptera: Aphididae) and *Aphelinus varipes* (Hymenoptera: Aphelinidae) on cultivated strawberry in Mississippi, USA. J. Insect Sci. 19: 17.

Rissanen, A. J., S. Peura, P. A. Mpamah, S. Taipale, M. Tiirola, C. Biasi, A. Mäki, and H. Nykänen. 2019. Vertical stratification of bacteria and archaea in sediments of a small boreal humic lake. FEMS Microbiology Letters 366: fnz044.

Shapiro-Ilan, D. I., T. E. Cottrell, C. Bock, K. Mai, D. Boykin, L. Wells, W. G. Hudson, and R. F. Mizell III. 2017. Control of pecan weevil with microbial biopesticides. Environ. Entomol. 46: 1299-1304.

Shapiro–Ilan, D. I., T. E. Cottrell, M. A. Jackson, and B. W. Wood. 2013. Control of key pecan insect pests using biorational pesticides. J Econ Entomol 106: 257-266.

Shaw, P., and J. Walker. 1996. Biological control of woolly apple aphid by *Aphelinus mali* in an integrated fruit production programme in Nelson. 59-63. *In Proceedings of the New Zealand Plant Protection Conference, 1996*.

Shrestha, G., A. Enkegaard, G. V. Reddy, H. Skovgård, and T. Steenberg. 2017. Susceptibility of larvae and pupae of the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae) to the entomopathogenic fungus *Beauveria bassiana*. Ann. Entomol. Soc. Am. 110: 121-127.

Šigut, M., M. Kostovčík, H. Šigutová, J. Hulcr, P. Drozd, and J. Hrček. 2017. Performance of DNA metabarcoding, standard barcoding, and morphological approach in the identification of host–parasitoid interactions. PLOS One 12: e0187803.

Šigut, M., H. Šigutová, J. Šipoš, P. Pyszko, N. Kotásková, and P. Drozd. 2018. Vertical canopy gradient shaping the stratification of leaf-chewer–parasitoid interactions in a temperate forest. Ecol. Evol. 8: 7297-7311.

Sow, A., T. Brévault, L. Benoit, M.-P. Chapuis, M. Galan, A. Coeur d'acier, G. Delvare, M. Sembène, and J. Haran. 2019. Deciphering host-parasitoid interactions and parasitism rates of crop pests using DNA metabarcoding. Sci. Rep. 9: 1-12.

Sparks, D. 2005. Adaptability of pecan as a species. Hortscience 40: 1175-1189.

Sparks, T. C., and R. Nauen. 2015. IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Phys. 121: 122-128.

Su, M., X. Tan, Q. Yang, H. Zhou, and F. Wan. 2017. Relative efficacy of two clades of *Aphelinus mali* (Hymenoptera: Aphelinidae) for control of woolly apple aphid (Hemiptera: Aphididae) in China. J. Econ. Entomol. 110: 35-40.

Tang, Q.-L., K.-S. Ma, Y.-M. Hou, and X.-W. Gao. 2017. Monitoring insecticide resistance and diagnostics of resistance mechanisms in the green peach aphid, *Myzus persicae* (Sulzer)(Hemiptera: Aphididae) in China. Pestic. Biochem. Phys. 143: 39-47.

Tedders, W. L. 1977. *Trioxys pallidus* and *Trioxys complanatus* as parasites of *Monellia costalis*, *Monelliopsis nigropunctata* and *Tinocallis caryaefoliae*. Ann. Entomol. Soc. Am. 70: 687-690.

Tedders, W. L. 1978. Important biological and morphological characteristics of the foliar-feeding aphids of pecan, vol. 1578-1587, Department of Agriculture, Science and Education Administration, 1978.

Traugott, M., J. Bell, G. Broad, W. Powell, F. Van Veen, I. Vollhardt, and W. O. C. Symondson. 2008. Endoparasitism in cereal aphids: molecular analysis of a whole parasitoid community. Mol. Ecol. 17: 3928-3938.

Ullah, F., H. Gul, K. Tariq, N. Desneux, X. Gao, and D. Song. 2020. Functional analysis of cytochrome P450 genes linked with acetamiprid resistance in melon aphid, *Aphis gossypii*. Pestic. Biochem. Phys. 170: 104687.

Van Lenteren, J., Y. Drost, H. Van Roermund, and C. Posthuma-Doodeman. 1997. Aphelinid parasitoids as sustainable biological control agents in greenhouses. J. Appl. Entomol. 121: 473-485.

Viggiani, G. 1984. Bionomics of the Aphelinidae. Annu. Rev. Entomol. 29: 257-276.

Wahab, W. 1985. Observations on the biology and behaviour of *Aphelinus abdominalis* Dalm.(Hym., Aphelinidae), a parasite of aphids. Z. Angew. Entomol. 100: 290-296.

Weathersbee, A., C. Mckenzie, and Y. Tang. 2004. Host plant and temperature effects on *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae), a native parasitoid of the exotic brown citrus aphid (Homoptera: Aphididae). Ann. Entomol. Soc. Am. 97: 476-480.

Wells, L. 2007. Pecan Physiology pp. 1-7. *In L. Wells (ed.), Southeastern Pecan Growers' Handbook*. University of Georgia Extension, Athens, United States.

Wells, L. 2017. Pecan: America's Native Nut Tree, University of Alabama Press.

Wells, L., and P. Conner. 2007. Southeastern Pecan Growers' Handbook.

Wood, B. W., and C. C. Reilly. 1998. Susceptibility of pecan to black pecan aphids. Hortscience 33: 798-801.

Wood, B. W., W. L. Tedders, and J. D. Dutcher. 1987. Energy drain by three pecan aphid species (Homoptera: Aphididae) and their influence on in-shell pecan production. Environ. Entomol. 16: 1045-1056.

Wood, B. W., P. J. Conner, and R. E. Worley. 2003. Relationship of alternate bearing intensity in pecan to fruit and canopy characteristics. Hortscience 38: 361-366.

Ye, Z., I. M. Vollhardt, S. Girtler, C. Wallinger, Z. Tomanovic, and M. Traugott. 2017. An effective molecular approach for assessing cereal aphid-parasitoid-endosymbiont networks. Sci. Rep. 7: 1-12.

Zhou, Q. S., Y. Q. Xi, F. Yu, X. Zhang, X. J. Li, C. L. Liu, Z. Q. Niu, C. D. Zhu, G. X. Qiao, and Y. Z. Zhang. 2014. Application of dna barcoding to the identification of hymenoptera parasitoids from the soybean aphid (*Aphis glycines*) in China. Insect Sci. 21: 363-373.

Zhu, Y.-L., F. Yang, Z.-W. Yao, Y.-K. Wu, B. Liu, H.-B. Yuan, and Y.-H. Lu. 2019. A molecular detection approach for a cotton aphid-parasitoid complex in Northern China. Sci. Rep. 9: 1-8.

TABLES AND FIGURES

Table 1.1. Table of common pecan pests sorted by portion of tree primarily targeted.

Foliage Pests	Nut Pests	Bark and Root Pests
Phylloxera sp.	Pecan nut casebearer (*Acrobasis nuxvorella*)	Obscure scale (*Melanaspis obscura*)
Pecan bud moth (*Gretchina bolliana*)	Nut curculio (*Conotrachelus hicoriae*)	Prionus root borer (*Prionus sp.*)
Hickory shoot curculio (*Conotrachelus sp.*)	Hickory shuckworm (*Cydia caryana*)	Ambrosia beetle sp.
Yellow pecan aphid (*Monelliopsis pecanis*)	Pecan weevil (*Curculio caryae*)	
Blackmargined aphid (*Monellia caryella*)		
Black pecan aphid (*Melanocallis caryaefoliae*)		

Table 1.2. Parasitoid assemblage associated with each of the aphid species analyzed in de Menten 2011 and Cingolani and Greco 2018.

Aphid	Parasitoid Assemblage
Acyrtosiphon malvae	*Aphidius ervi, Praon volucre*
Aphis gossypii	*Aphidius colemani, A. ervi, Aphidius matricariae, P. volucre*
Aulacorthum solani	*Aphelinus abdominalis, A. ervi, Ephedrus cerasicola, P. volucre*
Macrosiphum euphoribiae	*A. abdominalis, A. ervi, P. volucre*
Macrosiphum rosae	*A. abdominalis, A. ervi, P. volucre*
Myzus persicae	*A. abdominalis, A. colemani, A. ervi, A. matricariae, P. volucre*
Rhodobium porosum	*A. abdominalis, A. ervi*

CHAPTER 2

MULTI-SITE SEASONAL MONITORING OF PECAN APHIDS AND THEIR PARASITOIDS IN COMMERCIAL PECAN ORCHARDS

***** Slusher, E. K., W. G. Hudson, P. L. Halliday, and A. L. Acebes-Doria. 2021. Multisite Seasonal Monitoring of Pecan Aphids and Their Parasitoid in Commercial Pecan Orchards. Environ. Entomol. Online ahead of Print. Reprinted here with permission of publisher, 11/12/2021.

ABSTRACT

Aphids are important pests of pecans in Georgia. While previous studies conducted seasonal monitoring of pecan aphids, these studies were done at a single experimental site. In addition, only a few seasonal monitoring studies have tracked pecan aphid mummies parasitized by the aphid parasitoid, *Aphelinus perpallidus* Gahan. The objective of this study was to assess the seasonal phenology of yellow pecan aphid (*Monelliopsis pecanis* Bissell), blackmargined aphid (*Monellia caryella* (Fitch)), black pecan aphid (*Melanocallis caryaefoliae* (Davis)), aphid mummies, and adult *A. perpallidus* in four Georgia commercial orchards, with varying aphid management regimes, in 2019 and 2020. Comparison of overall aphid and parasitoid numbers between sites revealed few consistent annual patterns in both years. Aphid seasonal trends were consistent among sites and followed the patterns seen in previous studies, with the yellow aphid complex peaking in May, June, September and October; and black pecan aphids peaking in late September and October. Despite varying levels of insecticide application between sites, aphid phenology followed a similar seasonal pattern and remained low, throughout both growing seasons. This may indicate that growers can apply low frequencies of insecticides and still achieve pecan aphid control. Parasitism numbers were highest in the low insecticide frequency site compared to the other three sites. Mummies varied in their correlation with yellow aphid complex and black pecan aphid numbers. Parasitoid numbers typically followed the cycle of their host throughout the season.

KEYWORDS**:** Yellow pecan aphid, blackmargined aphid, black pecan aphid, phenology, parasitism.

INTRODUCTION

Pecan, *Carya illinoinensis* (Wangenh.) K. Koch, (Fagales: Juglandaceae) is one of the most important agricultural commodities in Georgia with a farm gate value of $218,477,486 (Wolfe and Stubbs 2019). Similar to other crops, pecans are susceptible to attacks by a range of pest species that vary in their seasonality and vulnerability to pesticides (Wells and Conner 2007). Pecan aphids are among the most important pests of pecans in Georgia. Three species of pecan aphid (Hemiptera: Aphididae) feed on pecans in Georgia namely, yellow pecan aphid, *Monelliopsis pecanis* Bissell, blackmargined aphid, *Monellia caryella* (Fitch), and black pecan aphid, *Melanocallis caryaefoliae* (Davis) (Wood et al. 1987, Mizell III and Schiffhauer 1990, Wells and Conner 2007, Shapiro–Ilan et al. 2013). Yellow pecan aphid and blackmargined aphid are collectively referred to as the 'yellow aphid complex'. Yellow pecan aphids and black pecan aphids can cause both direct damage, by feeding on leaf sap robbing the leaf of nutrients, and indirect damage due to the secretion of honeydew as a byproduct of feeding. This honeydew can encourage the growth of sooty mold which covers the leaf and hinders photosynthetic ability (Tedders 1978, Cottrell et al. 2009, Paulsen et al. 2013). Black pecan aphid feeding elicits leaf chlorosis around the feeding site with persistent feeding eventually leading to leaf abscission (Cottrell et al. 2009). Estimates of reduction of nut yield by yellow pecan aphid and blackmargined aphid have been found to be roughly 2.41 kg and 18.13 kg, respectively, per individual aphid (Wood et al. 1987). The University of Georgia pecan spray guide (Acebes-Doria and Hudson 2020) discourages spraying of yellow aphid complex in the early season and only spraying when there are large amounts of honeydew present. Meanwhile, it is recommended to treat black pecan aphids if as few as one aphid is found after checking 10 terminals on 10 trees after the first of July (Acebes-Doria and Hudson 2020).

A few previous studies have assessed pecan aphid phenology in both experimental and commercial orchards (Tedders 1978, Bueno and Stone 1983, Dutcher et al. 2012). Assessing aphid phenology in commercial orchards is important as commercial orchards are often treated with insecticides regularly and often for more than one pest. This places aphids under insecticide pressure from varying management regimes across different orchards that they likely would not experience in an experimental orchard. Several previous studies have demonstrated the effects of insecticides on pecan aphid populations (Dutcher 2005, Dutcher et al. 2010, Shapiro-Ilan et al. 2013). For example, trees treated with Requiem 25EC® (terpene constituents of the extract of *Chenopodium ambrosioides* near ambrosioides as synthetically manufactured, IRAC Class: N/A, Bayer CropScience, Research Triangle Park, NC, USA) and Movento® (Spirotetramat, IRAC Class: 23, Bayer CropScience, Research Triangle Park, NC, USA) had significantly less yellow pecan aphid, blackmargined aphid, and black pecan aphid compared to the untreated control (Dutcher et al., 2010). Some phenology studies have been done in Georgia, but these have been done at a single experimental orchard with no insecticide applications (Tedders 1978, Dutcher et al. 2012). One of the few assessments of pecan aphid phenology in commercial orchards was by Bueno and Stone (1983) which assessed both blackmargined aphid and its parasitoid, *Aphelinus perpallidus* Gahan (Hymenoptera: Aphelinidae) at multiple commercial orchards in Texas. So far, it appears that no such study has been conducted in Georgia.

It is also important to assess aphid populations in commercial orchards as management tactics have changed over time. Most insecticides applied back in the 1970's primarily consisted of broad-range insecticides such as organophosphates and pyrethroids (Ball 1977, Harris and Cutler 1977), this contrasts with insecticides today which consist primarily of insecticides that primarily target piercing-sucking insects (Mulder et al. 2018, Acebes-Doria and Halliday 2020).

In addition, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), the multicolored Asian lady beetle, has become established in Georgia and since that time, it has become an effective biological control agent of pecan aphid (Mizell 2007).

Furthermore, little work has been done assessing the seasonal abundance of the pecan aphid parasitoid, *A. perpallidus,* and its relationship with aphid abundance in Georgia. Tedders (1978) documented attacks on all three pecan aphid species by *A. perpallidus*. However, previous sampling has shown that *A. perpallidus* parasitizes *M. caryella* at higher rates than *M. caryaefoliae* or *M. pecanis* (Tedders 1978). Only a single study, done in Texas, has assessed populations of pecan aphid and their parastioids across multiple sites and this study only looked at blackmargined aphids (Bueno Jr and Stone 1983). The impact of *A. perpallidus* on pecan aphid populations appears to fluctuate depending on the season as previous studies have reported parasitism rates anywhere from 6% to 30% (Bueno Jr and Stone 1983, Bueno Jr and Stone 1985). It is important to examine the seasonality of predators and parastioids as being able to detect natural enemy arrival and dispersal can help with development of management plans. For example, pecan aphid predators such as lacewings have populations that lag behind their prey by as much as one week. (Kunkel and Cottrell 2007).

Little work has also been done looking at hyperparasitoid emergence rates in Georgia pecan orchards. Hyperparasitoids are thought to play a major role in primary parasitoid mortality, so understanding their rates of emergence is important as well (Gómez-Marco et al. 2015, Lefort et al. 2017). Bueno and Stone (1983) assessed hyperparasitism rates in Texas and found rates between 1.2% to 3.3%. However, such rates have yet to be quantified in Georgia.

Regular seasonal assessments, therefore, are critical to reveal shifts in pest and parasitoid presence or abundance. During the 2019 and 2020 pecan growing season, we assessed the

populations of all three aphid species, aphid mummies, and adult *A. perpallidus* at commercial orchards with varying management regimes in southern Georgia. Three commercially managed sites were assessed in both 2019 and 2020 (Albany, Marshallville, and Nashville) with an additional site assessed in 2020 (Ray City). These sites were chosen because they were located in major pecan growing regions of Georgia. Changes in seasonal phenology were assessed at each site and overall aphid and parasitoid numbers were compared between sites for both growing seasons. In addition, the relationship between parasitoid and aphid populations was examined. The number of insecticide/acaricide applications was also compared between sites. We hypothesize that we will see seasonal differences in aphid and parasitoid abundance both between and among sites with different management regimes in these various pecan growing regions of Georgia.

MATERIALS AND METHODS

All sampling sites were commercial orchards in major pecan growing regions in southern Georgia, USA. Site locations, sampling period, climate data, and site characteristics are listed in Table 1. Insecticide application information at each site is provided in Table 2. During the 2019 and 2020 growing season, two 0.4 ha sampling areas (20.1 x 201.2 m each) were measured at each location prior to sampling. Within each sampling area, five mature 'Sumner' variety pecan trees were selected at random. From each tree, five leaves were collected from the lower canopy (~1-2 m from the ground) using a pole pruner and stored in labeled 3.79 L Ziploc® bags. To standardize the leaf samples, only the middle three pairs of leaflets from each compound leaf were collected and examined. During transit, leaf samples were stored in a cooler containing an ice block in order to mitigate aphid movement in the bag. Leaf samples were stored in a refrigerator and examined

within 48 hours of collection. The interior of all bags was examined to account for aphids/mummies that moved or fell off leaves during pre-examination.

Sampling was done every other week throughout the sampling period and sampling was ceased once growers began to harvest pecans (Table 2.1). Leaves were taken back to the lab to quantify the number of live aphids and parasitized aphids that were mummified (from here on referred to as mummies). Both emerged parasitoid mummies (*i.e.*, mummies from which adult wasps successfully emerged from) and non-emerged parasitoid mummies (*i.e.*, mummies on which no successful wasp emergence has occurred) were counted. In 2020, non-emerged parasitoid mummies were placed individually in plastic capsules (Size 0, 7.62 cm, Healthy Life Supply; Mound House, NV) and stored in an environmental chamber (25°C, 60% RH, 16:8 L: D, Percival© E36L2; Perry, IA). Parasitoids that emerged from the mummies were identified as either primary or hyperparasitoids using a reference collection identified by a parasitic hymenopteran taxonomist, James Woolley (Texas A&M University), and *Hymenoptera of the World* by Goulet and Huber (1993). This information was used to quantify the proportion of primary and hyperparasitoid emergence at each site. In addition to leaf sampling, one yellow sticky card (7.6 x 12.7cm, Olson Products Inc.; Medina, OH) was placed for one week in the lower canopy of five randomly selected trees in each sampling area (10 cards total) from July to September in 2019 and from May to October in 2020 to assess adult *A. perpallidus* populations.

For all analyses, adults and nymphs of yellow pecan aphid and blackmargined aphids were pooled together as the yellow aphid complex. Adults and nymphs of black pecan aphids were pooled together. Aphid mummy analysis was divided into emerged parasitoid mummies and non-emerged parasitoid mummies. Differences in seasonal mean aphid, mummy and adult parasitoid numbers across the sampling periods for each site and between sites were analyzed with a One-

way ANOVA. Subsequently, Tukey's HSD was used for post hoc analysis to separate means among sampling dates and between sites at $\alpha = 0.05$. Spearman's correlation was used to analyze the correlation between aphids and both mummies and adult parasitoids at $\alpha = 0.05$. Difference among insecticide application at each site were analyzed using a Pearson's chi-squared test at $\alpha = 0.05$. All analyses were conducted in JMP® Pro 14.1.0 (SAS Version 14.1.0, Cary, NC).

RESULTS

Comparison across sampling locations: abiotic conditions and management programs

Nashville had a significantly greater average maximum temperature throughout the sampling period in 2019 than in Marshallville or Nashville ($F = 6.40$, DF= 2, 477, $P = 0.0018$) (Table 1). In 2020, Marshallville had a significantly cooler average maximum temperature than the other three sites sampled ($F = 10.96$, DF= 3, 583, $P < 0.0001$) (Table 2.1). In 2019, Albany and Marshallville had significantly cooler average minimum temperatures than Nashville ($F = 13.7$, DF= 2, 477, $P < 0.0001$) (Table 2.1). In 2020, Marshallville had a significantly lower average minimum temperature than Albany ($F = 4.18$, DF= 3, 583, $P = 0.0060$) (Table 2.1). Rainfall was statistically equal across all sites sampled in both 2019 and 2020 (2019: $F = 0.11$, DF= 2, 477, $P = 0.8951$; 2020: $F = 0.25$, DF= 3, 583, $P = 0.8582$) (Table 2.1). Comparison of spray frequency at each site revealed that in 2019, Nashville sprayed significantly more times than Albany but not Marshallville (Fig. 1 and Table 3). In 2020, Ray City applied insecticides significantly more times than Albany or Marshallville (Fig. 2.1 and Table 2.3).

Comparison across sampling locations: aphid and parasitoid abundance

Mean densities of yellow aphid complex, black pecan aphids, and aphid mummies varied significantly across sites during the two years of sampling. Marshallville had significantly more

yellow aphid complex than Albany or Nashville in 2019 (F= 12.02, DF = 2, 367 P<.0001; Fig. 2.2). In 2020, significantly more yellow aphid complex were found in Albany than in Nashville or Ray City (F= 7.451, DF = 3, 456 P<.0001; Fig. 2.2). Albany had significantly greater numbers of black pecan aphids in 2019 compared to Marshallville or Nashville (F= 5.98, DF = 2, 367, P = 0.0028; Fig. 2.2). In 2020, black pecan aphid numbers were significantly greater in Marshallville than in the other sites (F= 10.07, DF= 3, 456 P<.0001; Fig. 2.2). Aphid mummies were significantly greater in Marshallville than the other sampling sites in both 2019 and 2020 (2019: F= 89.54, DF= 2, 367, P<.0001; 2020: F= 85.09, DF= 3, 456 P<.0001; Fig. 2.2). Marshallville also had a significantly greater number of adult parasitoids in 2019 among all the sites sampled (F= 5.5, DF= 2, 87, P= 0.0056; Fig. 2.2). In 2020, Ray City had significantly more adult parasitoids than Albany or Nashville (F= 4.33, DF = 3, 225 P= 0.0055; Fig. 2.2).

The majority (46% - 100%) of the unhatched mummies collected in 2020 did not emerge under lab conditions (Fig. 2.3). Of those that successfully hatched, the emergence rates of the primary parasitoid, *Aphelinus perpallidus*, were 21%, 26%, and 46% at Albany, Marshallville, and Ray City, respectively. Hyperparasitoid emergence was only observed in mummies collected from Marshallville and Ray City with 16% and 8%, respectively (Fig. 2.3). Preliminary morphological assessment has determined these parasitoids to be in the families Pteromalidae (Hymenoptera), Figitidae (Hymenoptera), and Signophoridae (Hymenoptera). However, a molecular assessment will need to be performed to confirm specimen identity.

Seasonal phenology of aphids, parasitized aphids and adult parasitoids at each location

Albany. In 2019, the yellow aphid complex numbers varied significantly throughout the sampling period (F= 6.92, DF= 12, 117 P<.0001; Fig. 2.4A), peaking in mid-June before falling and rising slightly again in late July before experiencing a second peak in October (Fig. 2.4A).

Black pecan aphid numbers were also significantly affected by seasonality (F= 4.99, DF = 12, 117, P<.0001; Fig. 2.4A). No black pecan aphid observations were made until late September, where numbers increased in mid-October and peaked in late October (Fig. 2.4A). Emerged parasitoid mummies represented 44.5% of total mummies, while non-emerged mummies represented 55.5%. Emerged parasitoid mummies varied significantly throughout the sampling period (F= 3.88, DF= 12, 117, P<.0001, Fig. 2.4A). Emerged parasitoid mummies were absent early in May and occurred in low numbers throughout the rest of the season (Fig. 2.4A). Emerged parasitoid mummies experienced small peaks in early-August and mid-October but remained similar throughout most of the sampling period (Fig. 2.4A). Non-emerged parasitoid mummies also significantly differed throughout the sampling period (F= 5.84, DF = 12, 117, P<.0001, Fig. 2.4A). Non-emerged parasitoid mummies were absent in May and low throughout most of the season (Fig. 2.4A). They experienced small rises and crashes throughout most of the season before increasing, starting in late September, peaking in mid-October (Fig. 2.4A). Adult parasitoid numbers were not significantly affected by sampling time (F= 0.04, DF= 2, 27, P= 0.96; Fig. 2.4C).

In 2020, yellow aphid complex numbers again differed significantly across time (F= 7.36, DF= 11, 108, P<.0001, Fig. 2.4B), peaking in June before dropping significantly in July (Fig. 2.4B). Numbers increased slightly in early September but decreased again going into mid-October (Fig. 2.3B). Black pecan aphid populations remained consistently low throughout the sampling period, and were not significantly affected by seasonality (F= 0.727, DF= 11, 108, P= 0.71; Fig. 2.4B). Emerged parasitoid mummies made up 56% of total mummies collected, while non-emerged parasitoid mummies made up 44% of total mummies collected. Emerged parasitoid mummies significantly differed throughout the sampling period (F= 3.12, DF= 11, 108, P=

0.0011; Fig. 2.4B). (Fig. 4B). Emerged parasitoid mummies were absent in May but began rising in mid-June before peaking in early July where they remained until decreasing in mid-August (Fig. 2.4B). Numbers increased again in early September before crashing again in mid-September (Fig. 2.4B). Emerged parasitoid mummy numbers then started rising again starting in late September reaching a seasonal high in mid-October (Fig. 2.4B). Non-emerged parasitoid mummies were not significantly affected by sampling time (F= 1.74, DF= 11, 108, P= 0.0732; Fig. 2.4B). Adult parasitoid numbers were significantly greater in October, compared to the populations in August and May (F= 2.77, DF= 5, 53, P= 0.0269; Fig. 2.4D).

Marshallville. In 2019, yellow aphid complex numbers were affected by seasonality (F= 15.90, DF= 11, 108, P<.0001(Fig. 2.5A). Yellow aphid complex numbers were significantly greater in early June than any other time of the growing season. Black pecan aphid numbers remained consistently low and did not significantly differ throughout the sampling period (F= 1.37, DF = 11, 108, P= 0.1953; Fig. 2.5A). Emerged parasitoid mummies made up 68% of total mummies collected, while non-emerged parasitoid mummies made up 32%. Emerged parasitoid mummies were affected significantly by seasonality (F= 9.43, DF = 11, 108, P<.0001, Fig. 2.5A). Emerged parasitoid mummies were absent in May and early June and were low in late June and early July before increasing in early August and peaking in mid-August (Fig. 2.5A). Emerged parasitoid mummy numbers crashed and rose throughout the rest of the sampling period with two more peaks in early September and early October. Non-emerged parasitoid mummies also significantly differed throughout the sampling period (F= 10.49, DF = 11, 108, P<.0001, Fig. 2.5A). Non-emerged parasitoid mummies were low in May, June, and early July, before rising and peaking in mid-August (Fig. 2.5A). Their numbers then dropped significantly going into late

August before steadily rising in September and October (Fig. 2.5A). Adult parasitoids had greater numbers in July compared to August or September (F= 7.04, DF= 2, 27, P= 0.004; Fig. 2.4C).

In the 2020 field season, yellow aphid complex numbers were significantly higher in late June than at any other point in the season (F= 6.90, DF= 11, 108, P<.0001; Fig. 2.5B). Black pecan aphid numbers were significantly greater in late September than any other time in the sampling period except mid-October (F= 4.46, DF= 11, 108, P<.0001; Fig. 2.5B). Total mummies collected consisted of 71% emerged parasitoid mummies and 29% non-emerged parasitoid mummies. Emerged parasitoid mummies differed significantly throughout the season (F= 23.1, DF= 11, 108, P<.0001; Fig. 2.5B). Emerged parasitoid mummies were absent during May and were low in June and July before rising significantly in mid-September after which numbers continued to rise throughout the rest of the sampling period (Fig. 2.5B). Non-emerged parasitoid mummies significantly differed throughout the sampling period (F= 9.47, DF= 11, 108, P<.0001; Fig. 2.5B). Non-emerged parasitoid mummies were low throughout the sampling period before peaking in early September where they increased in number before dropping slightly in early October (Fig. 2.5B). Adult parasitoid numbers were significantly greater in September compared to May, June, August, and October. Numbers in May and June were significantly less than July (F= 6.03, DF= 5, 54, P= 0.0002; Fig. 2.5D).

Nashville. In 2019, for the yellow aphid complex, late May had the greatest number of aphids compared to the rest of the growing season (F= 22.50, DF= 11, 108, P<.0001, Fig. 2.6A). The rest of the season had very low to no aphid numbers and there was no significant difference among the rest of the sampling dates (Fig. 2.6A). Black pecan aphid numbers were low and did not significantly differ at any point in the sampling period (F= 1.80, DF= 11, 108, P= 0.06; Fig. 2.6A). 75% of total mummies collected during the season were emerged parasitoid mummies,

while 25% were non-emerged parasitoid mummies. Emerged parasitoid mummies were statistically equal throughout the sampling period (F= 0.7567, DF= 11, 108, P= 0.6819, Fig. 2.6A). Sampling period had a significant effect on non-emerged parasitoid mummies (F= 2.74, DF= 11, 108, P= 0.0037, Fig. 2.6A). Non-emerged parasitoid mummies were low throughout most of the season with the highest numbers being recorded in late May (Fig. 2.6A). Non-emerged parasitoid mummy numbers increased slightly in September before crashing again then increasing again at the end of the sampling period in late-October (Fig. 2.6A). Adult parasitoid numbers were significantly greater in July compared to the August and September (F= 32.1, DF= 2, 27, P<.0001; Fig. 2.5C).

In 2020, yellow aphid complex numbers were significantly greater in late May compared to all other sampling periods with few or no aphids found (F= 9.58, DF= 11, 108, P<.0001; Fig. 2.6B). No significant difference was found in black pecan aphid numbers during the sampling period (F= 1, DF= 11, 108, P= 0.4513; Fig. 2.6B), with no black pecan aphids collected except in early August. Of the total mummies collected, 82% were emerged and 17% were non-emerged. Emerged parasitoid mummies significantly differed throughout the sampling period (F= 3.4, DF= 11, 108, P= 0.0004; Fig. 2.6B). Emerged parasitoid mummies were absent during most of the sampling period with mid-August having significantly higher numbers of emerged parasitoid mummies compared to most of the sampling period except for early October and mid-September (Fig. 2.6B). Non-emerged parasitoid mummies were low to absent during most of the sampling period and did not significantly differ throughout sampling (F= 0.85, DF= 11, 108, P= 0.5925; Fig. 2.6B). Adult parasitoid numbers were statistically equal throughout the sampling period (F= 2.07, DF= 5, 54, P= 0.082; Fig. 2.6D).

Ray City. The Ray City orchard was only sampled in 2020. Yellow aphid complex numbers in Ray City were low throughout the growing season with early October having significantly higher numbers compared to the rest of the sampling period except early June and mid-October (F= 9.45, DF= 9, 90, P<.0001; Fig. 2.7A). Black pecan aphid numbers did not significantly differ throughout the sampling period (F= 0.890, DF= 9, 90, P= 0.538; Fig. 2.7A), with populations occurring only in early June and late July. Out of the total mummies collected throughout the season, emerged aphid parasitoid mummies made up 75% and non-emerged parasitoid mummies made up 24%. Emerged parasitoid mummies were statistically equal throughout the sampling period (F= 1.16, DF = 9, 90, P= 0.3325; Fig. 2.7A). Non-emerged parasitoid mummies statistically differed throughout the sampling period (F= 4.66, DF= 9, 90, P<.0001; Fig. 2.7A) but were absent through most of the season. Non-emerged parasitoid mummies were significantly higher in early October compared to the rest of the sampling period except early June and late October (Fig. 2.7A). Adult parasitoid numbers did not significantly differ across the sampling period (F= 1.21, DF= 4, 45, P= 0.321; Fig. 2.7B).

Relationship between aphid populations and parasitoids

In Albany in 2019, there was a significant positive correlation between emerged parasitoid mummies and black pecan aphids (Spearman's ρ= 0.1437, P= 0.0051). Non-emerged parasitoid mummies had a significant positive correlation with both the yellow aphid complex and black pecan aphid (yellow aphid complex: Spearman's ρ= 0.1158, P= 0.0031; black pecan aphid: Spearman's ρ= 0.1750, P<.0001). In Marshallville, emerged parasitoid mummies had a significant negative correlation with the yellow aphid complex (Spearman's ρ= -.2340, P<0.0001) and positive correlation with black pecan aphid (Spearman's ρ= 0.0891, P= 0.0291). Non-emerged parasitoid mummies had a negative correlation with the yellow aphid complex (Spearman's ρ= -

0.1000, P= 0.0143). In Nashville, there was a significant positive correlation between both emerged parasitoid mummies and non-emerged parasitoid mummies and the yellow aphid complex (emerged parasitoid mummies: Spearman's ρ= 0.0899, P= 0.0276; non-emerged parasitoid mummies: Spearman's ρ= 0.1510, P= 0.0002). In Marshallville, adult parasitoid populations were positively correlated with yellow aphid complex populations (Spearman ρ= 0.5126, P= 0.0038).

In Marshallville in 2020, there was a positive correlation between black pecan aphid and emerged parasitoid mummies (Spearman's ρ= 0.2111, P<.0001). Non-emerged parasitoid mummies had a positive correlation with yellow aphid complex and black pecan aphid (yellow aphid complex: Spearman's ρ= 0.2111, P<.0001; black pecan aphid: Spearman's ρ= 0.1661, P<.0001). In Ray City, adult parasitoids were positively correlated with the yellow aphid complex (Spearman ρ= 0.3011, P= 0.0336).

DICUSSION

Analysis of spray frequency between each site revealed differences in the number of insecticide applications between sites. In 2019, Nashville applied insecticides significantly more than Albany (Fig. 2.1). This may explain the low aphid numbers in the Nashville orchard (Fig. 2.6A). However, aphid numbers were still very low at the Nashville orchard in 2020 despite a reduced spray schedule indicating that the growers there may be able to spray less and achieve the same results (Fig. 2.6B). Interestingly, Albany and Nashville had statistically similar numbers of yellow aphid complex in 2019 but not in 2020 when Nashville had less yellow aphid complex despite spraying less that season (Fig. 2.2). Ray City sprayed more than Albany and Marshallville in 2020 (Fig. 2.1). Interestingly, Ray City had significantly more overall adult *A. perpallidus* in

2020 indicating that despite the more intensive spray schedule, Ray City was still able to maintain a high adult parasitoid population (Fig. 2.2). Albany, despite its low spray frequency, had low total mummy numbers and parasitoid adult numbers during both years of the study indicating that some other factor may be responsible for lower natural enemy numbers at that site (Fig. 2.2).

Comparison of aphids and parasitoids at each site in both years revealed variation among sites across both years of the study with little consistency from year to year. The only consistent trend among the sites was that Marshallville had the highest number of parasitoids during both years of the study. One interesting aspect of this analysis is that it suggests that intensive spraying may not be necessary to achieve low aphid density.

Regardless of site, yellow aphid complex numbers typically followed a similar pattern of rising and crashing throughout the season with peaks usually occurring in May and June followed by another peak in late September and early October. Black pecan aphids were rarely collected throughout the growing season usually being found in September and October. The population trends in our data are similar to previous studies on aphid phenology and life history (Tedders 1978, Dutcher et al. 2012). Interestingly, despite insecticide application at the sites in our study, aphid numbers still peaked at similar times as peaks at the unsprayed experimental orchards of other studies (Tedders 1978, Dutcher et al. 2012). However, the growth of these peaks does not appear to be as great. The yellow aphid complex collected during our study seem to achieve their highest abundance between May and June and September and October. This lines up with the recommendations of the UGA spray guide which discourages spraying for the yellow aphid complex in the early season (May and June) and when growers ceased treating and turn their focus to harvest (September and October) (Table 2.2). Thus, these peaks fall outside what is often a normal yellow aphid complex treatment window for most Georgia pecan growers. However,

treatment for black pecan aphid appears to differ from the recommendations of the UGA spray guide, at least at some sites. For example, growers at the Marshallville and Nashville orchard treated for black pecan aphid despite black pecan aphid numbers being low throughout. Thus, there is potential for growers to reduce costs by using monitoring information to determine if black aphid numbers are at threshold values recommended in the spray guide.

While it appears that aphid phenology in commercial orchards is not too different from previous studies in experimental orchards, it does appear that aphid abundance is lower in commercial orchards. Tedders (1978) found an average number of 100 aphids per 25 leaves during seasonal peaks with blackmargined aphid averaging around 900 per 20 leaves around October and December. Dutcher et al. (2012) found numbers closer to ours, but still found that during the peak aphid numbers could get as high as 40-100 aphids per shoot. This is much higher than our highest average peak of 10 yellow pecan aphids per leaf which was in June of 2019 in Marshallville, GA. Insecticide application pressure could be the likely explanation for this. The type of insecticide used may also be a cause of low aphid numbers, as the shift from broad-spectrum insecticides in the 1970's such as organophosphates and pyrethroids (Ball 1977, Harris and Cutler 1977) to products which are designed to primarily target piercing-sucking insects (Mulder et al. 2018, Acebes-Doria and Halliday 2020) may help to conserve natural enemies such as *H. axyridis* and *A. perpallidus* that can put pressure on aphid populations.

The adult parasitoid phenology typically exhibited a similar trend to the aphids in terms of peaks and crashes. This suggests that adult parasitoid numbers rise and fall with that of their host. This can be supported by the positive correlation we found between adult parastioids and aphids in Marshallville in 2019 and Ray City in 2020. Aphid mummies differed in their correlation with aphid numbers regardless of whether they were emerged or non-emerged. Interestingly, there was

a negative correlation between both hatch and unhatched mummies and the yellow aphid complex at Marshallville in 2019. This can possibly be explained by the persistence of mummies on the leaf when other factors, such as insecticide application, rainfall, or natural population crashes eliminate the aphids in an area. Even after the parasitoid has hatched, the remaining mummy can be found on the leaf afterwards. Bueno and Stone (1985) saw a similar trend when blackmargined aphids were sprayed. They attributed this to having an ample number of aphids available to sustain *A. perpallidus* populations even after treatment. In addition, we observed several positive correlations between black pecan aphid and both hatch and unhatched mummies at several sites. This is interesting as black pecan aphid mummies were not found in this study and are noted to be rarely parasitized by *A. perpallidus* (Tedders 1978). Several previous studies have assessed aphid mummies and parasitism rates in correlation with pecan aphid numbers, but assessment of adult numbers is lacking (Bueno Jr and Stone 1983, Bueno Jr and Stone 1985). Our study is one of the first studies to look at the population density of adult *A. perpallidus* in the field.

The proportion of primary parasitoids that emerged from the collected mummies was lower than those of a previous study done in Texas which reported *A. perpallidus* emergence of 48.3%, 60.3%, and 60.3% across three sites (Bueno Jr and Stone 1983). Interestingly, hyperparasitism was detected at the two sites with the highest number of parasitoid adults captured in 2019 (Marshallville) and 2020 (Ray City). This may indicate some sort of relationship between parasitoid abundance and the presence of hyperparasitoids. Hyperparasitoid emergence was much higher in this study at 8% and 16% compared to 1.2%, 1.7%, and 3.3% emergence rates in the Texas study. It should also be noted that Bueno and Stone (1983) acquired many more mummies during their study (1328) than in this one (376). Collection method likely explains these differences as Bueno and Stone (1983) collected aphids and observed them for mummification in the lab

whereas the mummies in this study were collected directly from the field. This likely allowed for the authors to collect mummies and take them to the lab thus decreasing exposure to environmental conditions and hyperparasitoids. Aphid hyperparasitoids have been found to prefer attacking aphid mummies rather than non-mummified parasitized aphids (Buitenhuis et al. 2004).

Overall, this study was one of first to perform a multi-site assessment of pecan aphid and *A. perpallidus* populations in commercial pecan orchards. The findings on aphid seasonal activity confirmed that of previous studies by Tedders (1978) and Dutcher et al. (2012). While the study did not find any drastic differences in aphid phenology compared to experimental orchards it did reveal that aphid numbers are lower in commercial orchards than in experimental orchards. Additional years of study may be necessary to see if this is a due to management pressure or simply a temporary lull in populations. This study was also among the first to plot a seasonal phenology for adult *A. perpallidus*. Future studies could help expand upon this and help reveal long-term population trends. The literature available on this topic is currently quite limited and many of the points analyzed in this study could be further examined to reveal more information. Further regular and continued assessment is important to help growers develop pecan aphid management plans.

LITERATURE CITED

Acebes-Doria, A. L., and W. G. Hudson. 2019. 2020 Commercial Pecan Spray Guide. Univ. Georgia Coop. Ext. Pub. 841. Tifton, GA.

Acebes-Doria, A. L., and P. L. Halliday. 2020. Insecticide efficacy against pecan aphids and pecan leaf scorch mites, 2018. Arthropod Manag. Tests. 45: 1-2.

Ball, J. C. 1977. Aphid control on pecans, 1976. Insecticide and Acaricide Tests. 44: 1-2

Bueno Jr, R., and J. D. Stone. 1983. Phenology of a parasite of the blackmargined aphid in west Texas [*Aphelinus perpallidus, Monellia caryella*]. Southwest. Entomol. 8: 73-79.

Bueno Jr, R., and J. D. Stone. 1985. *Aphelinus perpallidus* parasitism of *Monellia caryella* populations in far west Texas. J. Entomol. Sci. 20: 325-330.

Buitenhuis, R., G. Boivin, L. Vet, and J. Brodeur. 2004. Preference and performance of the hyperparasitoid *Syrphophagus aphidivorus* (Hymenoptera: Encyrtidae): fitness consequences of selecting hosts in live aphids or aphid mummies. Ecol. Entomol. 29: 648-656.

Cottrell, T. E., B. W. Wood, and X. Ni. 2009. Chlorotic feeding injury by the black pecan aphid (Hemiptera: Aphididae) to pecan foliage promotes aphid settling and nymphal development. Environ. Entomol. 38: 411-416.

Dutcher, J. D. 2005. Chemical control of aphids on pecan foliage. Arthropod Manag. Tests. 30: 1-2.

Dutcher, J. D., H. Karar, and M. G. Abbas. 2010. Effectiveness of two insecticides for control of foliage feeding pecan aphids. Arthropod Manag. Tests. 35: 1-2.

Dutcher, J. D., H. Karar, and G. Abbas. 2012. Seasonal abundance of aphids and aphidophagous insects in pecan. Insects 3: 1257-1270.

Forrest, J. R. 2016. Complex responses of insect phenology to climate change. Curr. Opin. Insect Sci. 17: 49-54.

Gómez-Marco, F., A. Urbaneja, J. A. Jaques, P. Rugman-Jones, R. Stouthamer, and A. Tena. 2015. Untangling the aphid-parasitoid food web in citrus: Can hyperparasitoids disrupt biological control? Biol. Control 81: 111-121.

Goulet, H., and J. T. Huber. 1993. Hymenoptera of the world: an identification guide to families. Agriculture Canada, Ottawa, Canada.

Harris, M. K., and B. L. Cutler. 1978. Pecan, pecan weevil and hickory shuckworm management, 1977. Insecticide and Acaricide Tests 3: 59-60.

Kistner, E. J. 2017. Climate change impacts on the potential distribution and abundance of the brown marmorated stink bug (Hemiptera: Pentatomidae) with special reference to North America and Europe. Environ. Entomol. 46: 1212-1224.

Kunkel, B. A., and T. E. Cottrell. 2007. Oviposition response of green lacewings (Neuroptera: Chrysopidae) to aphids (Hemiptera: Aphididae) and potential attractants on pecan. Environ. Entomol. 36: 577-583.

Lefort, M. C., S. Wratten, A. Cusumano, Y. D. Varennes, and S. Boyer. 2017. Disentangling higher trophic level interactions in the cabbage aphid food web using high-throughput DNA sequencing. MBMG. 1: e13709.

Mizell III, R. F., and D. E. Schiffhauer. 1990. Effects of pesticides on pecan aphid predators *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), *Hippodamia convergens*, *Cycloneda sanguinea* (L.), *Olla v-nigrum* (Coleoptera: Coccinellidae), and *Aphelinus perpallidus* (Hymenoptera: Encyrtidae). J. Econ. Entomol. 83: 1806-1812.

Mizell, R. F. 2007. Impact of *Harmonia axyridis* (Coleoptera: Coccinellidae) on native arthropod predators in pecan and crape myrtle. Fla. Entomol. 90: 524-536.

Mulder, P. G., S. K. Seuhs, M. E. Payton. 2020. Insecticide efficacy for controlling pecan aphids, 2018. Arthropod Manag. Tests. 45:1-2.

Paulsen, C., T. Cottrell, and J. Ruberson. 2013. Distribution of the black pecan aphid, *Melanocallis caryaefoliae,* on the upper and lower surface of pecan foliage. Entomol. Exp. Appl. 146: 252-260.

Shapiro–Ilan, D. I., T. E. Cottrell, M. A. Jackson, and B. W. Wood. 2013. Control of key pecan insect pests using biorational pesticides. J. Econ. Entomol. 106: 257-266.

Tedders, W. 1978. Important biological and morphological characteristics of the foliar-feeding aphids of pecan. USDA Technical Bulletin. 1579. Hyattsville, MD.

(USDA-NASS) U.S. Department of Agriculture National Agricultural Statistics Service. 2020. Pecan production (January 2020). USDA, NASS. Washington, D.C.

Wells, L., P. Conner, B. Goff, M. Nesbitt, B. Wood, P. Bertrand, T. Brenneman, J. Brock, M. Hotchkiss, C. Reilly, K. Stevenson, J. Dutcher, W. Hudson, H. Ellis, J. Payne, L. Tedders, K. Harrison, and P. Sumner. 2007. *In Southeastern Pecan Growers Handbook*. Univ. Georgia Coop. Ext. Pub. 1327. Tifton, GA.

Wolfe, K., and K. Stubbs. 2019. Georgia Farm Gate Value Report 2018. Univ. Georgia Coop. Ext. Pub. 17. Tifton, GA.

Wood, B. W., W. L. Tedders, and J. D. Dutcher. 1987. Energy drain by three pecan aphid species (Homoptera: Aphididae) and their influence on in-shell pecan production. Environ. Entomol. 16: 1045-1056

TABLES AND FIGURES

Table 2.1. GPS coordinates, tree age and spacing, climate data, and the corresponding sampling periods for each site sampled in Georgia, USA.

Location	GPS Coordinates	Tree Age (Yrs.)	Tree Spacing (Between Row/ Within Row) (m)	Sampling Period		Average Temperature (Max/Min) (°C)		Rainfall (mm)	
				2019	2020	2019	2020	2019	2020
Albany	31°36'09.6"N 84°02'30.3"W	20	6/14	17 May - 1 Nov	14 May - 15 Oct	32.5[b]/20.4[b]	31.4[a]/20.6[a]	3.6[a]	3.9[a]
Marshallville	32°30'00.0"N 83°55'46.3"W	24-27	16/14	21 May - 21 Oct	11 May – 15 Oct	32.3[b]/20.2[b]	30.1[b]/19.6[b]	3.2[a]	4.6[a]
Nashville	31°04'01.3"N 83°11'07.7"W	30-35	5/23	30 May – 25 Oct	15 May – 16 Oct	33.5[a]/21.9°[a]	31.6[a]/19.7[ab]	3.8[a]	4.4[a]
Ray City	31°01'16.7"N 83°14'35.8"W	13	9/7	N/A	9 June – 2 Oct	N/A	32.3[a]/20.6[ab]	N/A	5.2[a]

Table 2.2. Dates of 2019 and 2020 insecticide applications per site based on each grower's personal management program. Differing letters next to average temperature and rainfall values designate a significant difference between column values at $\alpha = 0.05$.

	2019			2020		
Location	Date	Insecticide (Rate/ha)	Active Ingredient (IRAC Classification)	Date	Insecticide (Rate/ha)	Active Ingredient (IRAC Classification)
Albany	1-Aug	Carbine (204.6 ml)[1]	Flonicamid (29) *	1-Aug	Carbine (204.6 ml)[1]	Flonicamid (29) *
Marshallville	24-June	Imidacloprid (876.9 ml)[2]	Imidacloprid (4A) *	30-July	Transform (109.6 ml)[4]	Sulfoxaflor (4C) *
	19-Aug	Transform (109.6 ml)[1]	Sulfoxaflor (4C) *			
Nashville	6-May	Dimilin (584.6 ml)[3]	Diflubenzuron (15) *	27-May	Intrepid Edge (467.7 ml)[3]	Methoxyfenozide (18)/ Spinetoram (5) *
	22-May	Intrepid (467.7 ml)[3]	Methoxyfenozide (18)	8-Jun	Sefina (438.6 ml)[1]	Afidopyropen (9) *
	5-June	Carbine (204.6 ml)[4]	Flonicamid (29) *	4-Aug	Intrepid (467.7 ml)[5]	Methoxyfenozide (18)
		Imidacloprid (233.8 ml)[4]	Imidacloprid (4A) *	12-Aug	Intrepid (467.7 ml)[5]	Methoxyfenozide (18)
		Intrepid Edge (467.7 ml)[3]	Methoxyfenozide (18)/ Spinetoram (5) *		Transform (146.2 ml)[1]	Sulfoxaflor (4C) *
	18-June	Intrepid (467.7 ml)[5]	Methoxyfenozide (18)			
	8-July	Intrepid (467.7 ml)[5]	Methoxyfenozide (18)			
	4-Aug	Intrepid (467.7 ml)[5]	Methoxyfenozide (18)			
		Transform (146.2 ml)[1]	Sulfoxaflor (4C) *			
	20-Aug	Dimilin (584.6 ml)[5]	Diflubenzuron (15) *			
	13-Sept	Nexter (935.4 ml)[6]	Pyridaben (21A) *			
Ray City				12-May	Intrepid Edge (292.3 ml)[3]	Methoxyfenozide (18)/ Spinetoram (5) *
				8-June	Sefina (219.2 ml)[1]	Afidopyropen (9) *
				21-July	Durant (584.6 ml)[5]	Diflubenzuron (15) *
				29-July	Sefina (219.2 ml)[1]	Afidopyropen (9) *
					Durant (584.6 ml)[5]	Diflubenzuron (15) *
				11-Aug	Intrepid Edge (292.3 ml)[5]	Methoxyfenozide (18)/ Spinetoram (5) *
					Transform (87.7 ml)[1]	Sulfoxaflor (4C) *
				21-Aug	Transform (87.7 ml)[1]	Sulfoxaflor (4C) *
					Abamex (1,3195.4 ml)[6]	Abamectin (6) *

*Designates active ingredient that is effective against aphids.
[1]Pecan Aphid (Hemiptera: Aphididae)
[2]Pecan Spittlebug, *Clastoptera achatina*, (Hemiptera: Clastopteridae)
[3]Pecan Nut Casebearer, *Acrobasis nuxvorella*, (Lepidoptera: Pyralidae)
[4]Black Pecan Aphid, *Melanocallis caryaefoliae*, (Hemiptera: Aphididae)
[5]Hickory Shuckworm, *Cydia caryana*, (Lepidoptera: Tortricidae)
[6]Mites (Acari: Tetranychidae)

Table 2.3. Pearson's chi-squared statistical comparison of the number of insecticide applications at each site during the sampling period.

2019				2020			
Site Comparison	χ^2	DF	*P*	Site Comparison	χ^2	DF	*P*
Albany vs Marshallville	0.3333	1	0.5637	Albany vs Marshallville	0	1	1
Albany vs Nashville	4.5	1	0.0339*	Albany vs Nashville	1	1	0.3173
Marshallville vs Nashville	2.7778	1	0.0956	Albany vs Ray City	6.4	1	0.0114*
				Marshallville vs Nashville	1	1	0.3173
				Marshallville vs Ray City	6.4	1	0.0114*
				Nashville vs Ray City	3	1	0.0833

*Designs a statistically significant difference between sites at $\alpha = 0.05$

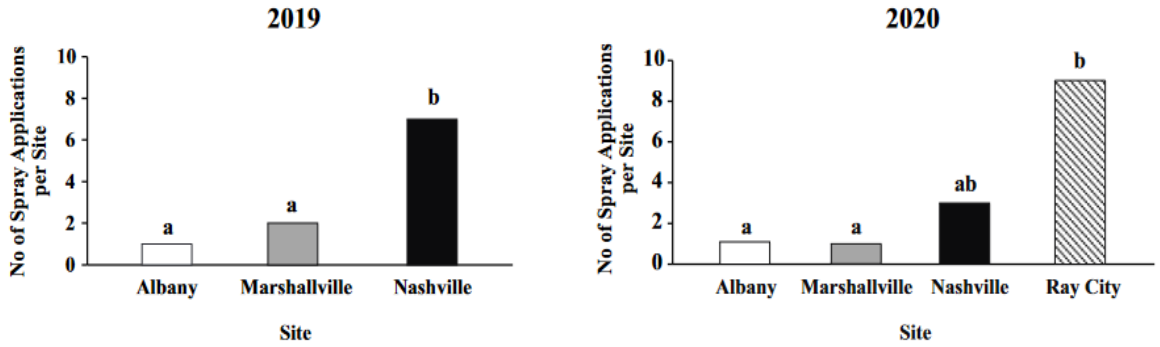

Figure 2.1. Number of insecticide/acaricide applications at each site during each sampling period. Different letters above each bar signify a significant difference using Pearson's chi-squared test. Only insecticides with active ingredients effective against aphids were counted for each site.

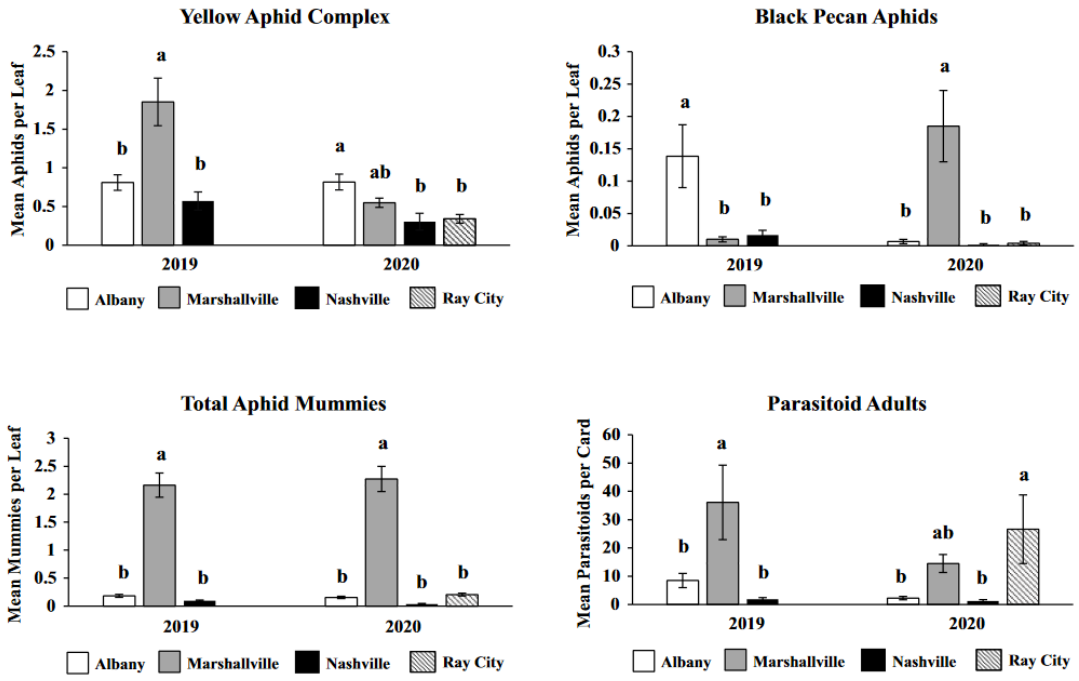

Figure 2.2. Mean ± SEM comparison of yellow aphid complex, black pecan aphids, total aphid mummies and adult parasitoids between collection sites in 2019 and 2020. Different letters above each bar signify a significant difference using Tukey's HSD at $\alpha = 0.05$.

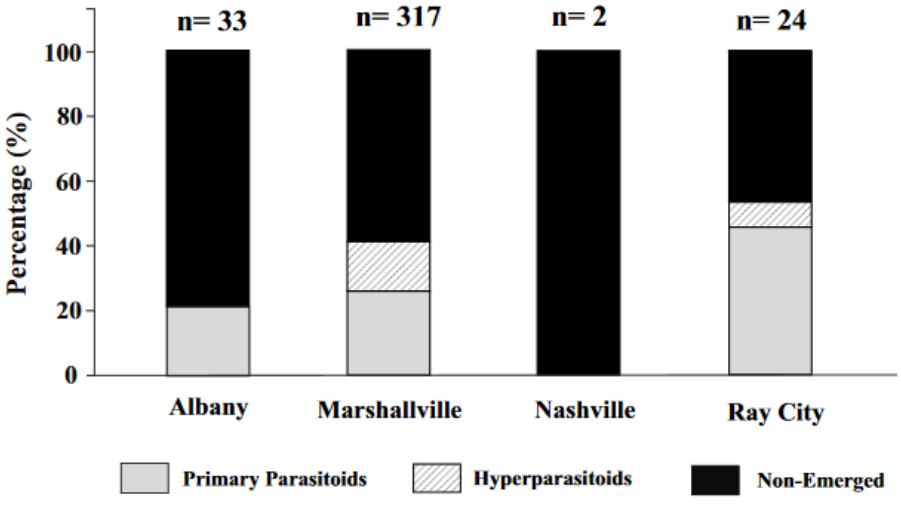

Figure 2.3. Proportion of primary and hyperparasitoids that emerged from unhatched mummies collected and reared from leaf samples in 2020. The proportions of parasitized aphids that did not emerge are also reported.

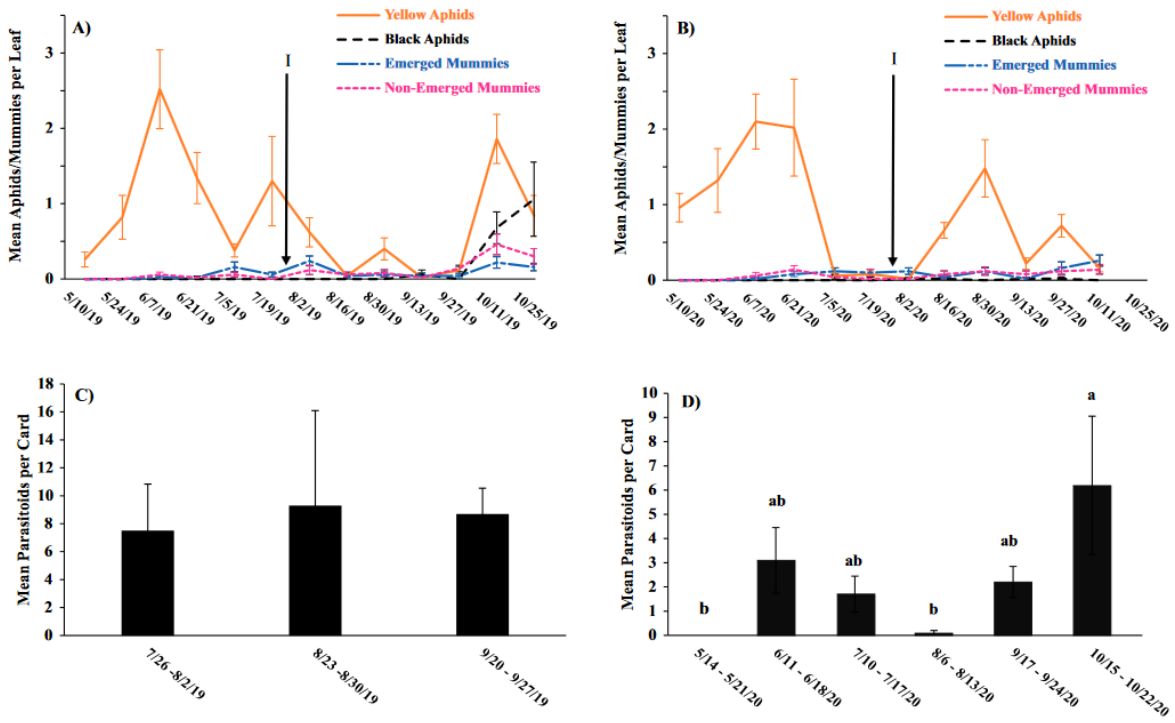

Figure 2.4. Mean ± SEM of Albany aphid and mummy numbers per leaf across the (A) 2019 and (B) 2020 sampling period and Mean ± SEM of Albany adult parasitoid numbers per card across the (C) 2019 and (D) 2020 sampling period. Different letters above each bar signify a significant difference using Tukey's HSD at $\alpha = 0.05$. **I** signifies when growers applied insecticides.

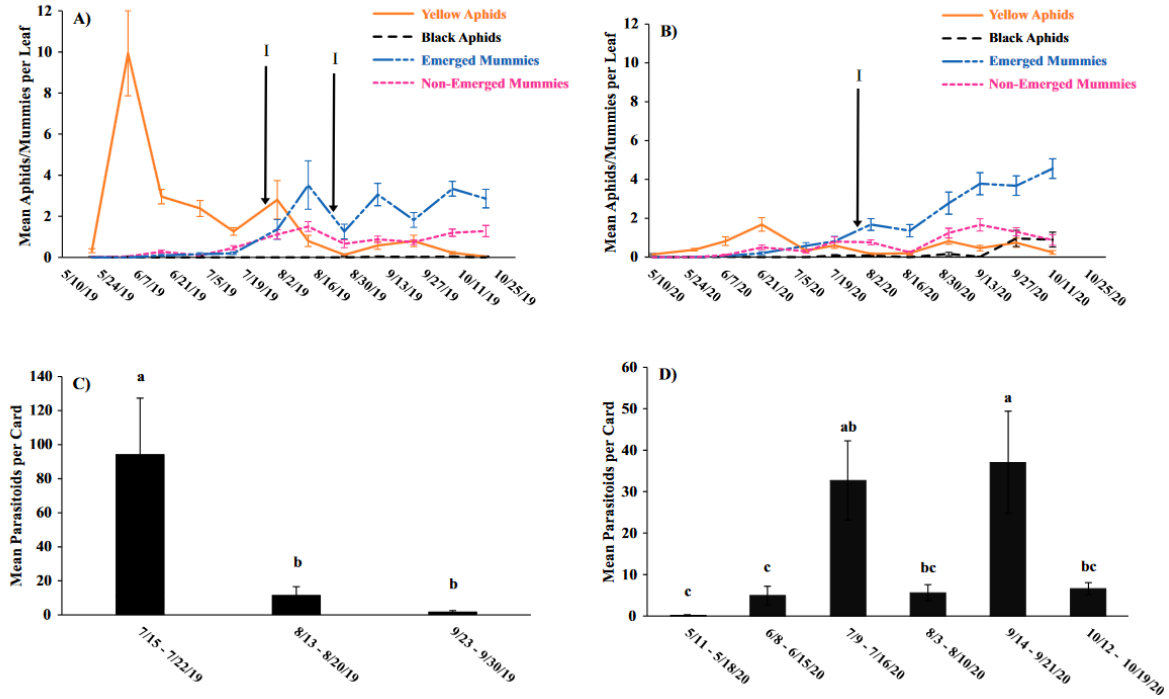

Figure 2.5. Mean ± SEM of Marshallville aphid and mummy numbers per leaf across the (A) 2019 and (B) 2020 sampling period and Mean ± SEM of Marshallville adult parasitoid numbers per card across the (C) 2019 and (D) 2020 sampling period. Different letters above each bar signify a significant difference using Tukey's HSD at $\alpha = 0.05$. **I** signifies when growers applied insecticides.

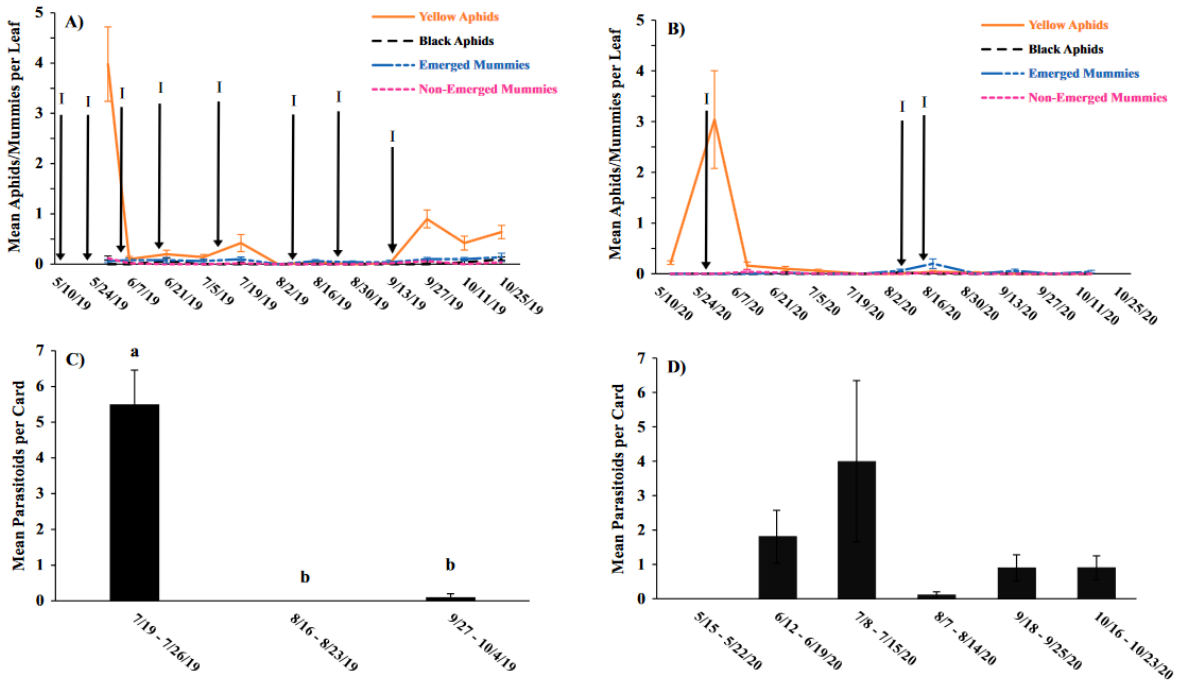

Figure 2.6. Mean ± SEM of Nashville aphid and mummy numbers per leaf across the (A) 2019 and (B) 2020 sampling period and Mean ± SEM of Nashville adult parasitoid numbers per card across the (C) 2019 and (D) 2020 sampling period. Different letters above each bar signify a significant difference using Tukey's HSD at $\alpha = 0.05$. **I** signifies when growers applied insecticides.

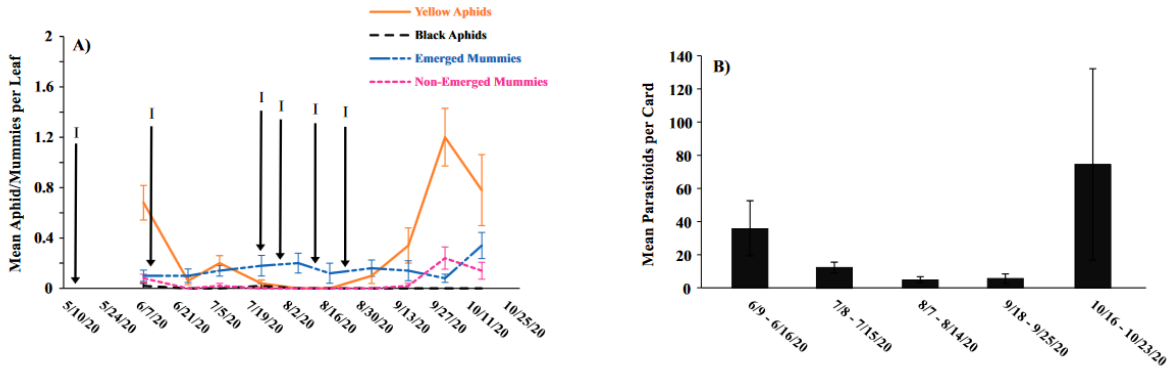

Figure 2.7. Mean ± SEM of Ray City aphid and mummy numbers per leaf across the 2020 sampling period (A) and Mean ± SEM of Ray City adult parasitoid numbers per card across the 2020 sampling (B). Different letters above each bar signify a significant difference using Tukey's HSD at $\alpha = 0.05$. **I** signifies when growers applied insecticides.

CHAPTER 3

MOLECULAR UNRAVELING OF PARASITOIDS AND HYPERPARASITOIDS ASSOCIATED WITH PECAN APHIDS

***** Slusher, E. K., T. Cottrell, T. Gariepy, J. Schmidt, and A. L. Acebes-Doria. 2021. Molecular Unraveling of Parasitoids and Hyperparasitoids Associated with Pecan Aphids. To be submitted to Bulletin of Entomological Research.

ABSTRACT

Advances in molecular ecology can overcome many challenges in understanding parasitoid-host interactions in the agro-ecosystem; however, relatively few cropping systems have been explored with this technology. An initial genetic characterization of the key-players in a given system is critical in order to take advantage of the increased resolution provided by the molecular analysis of trophic interactions, which requires extensive surveys and DNA-barcoding of voucher specimens. The pecan system contains several herbivores and parasitoids, but the role of natural enemies in this foodweb, and their ability to suppress key pest populations remains to be defined. As a first step towards characterizing pecan food webs, we collected aphid mummy samples from pecan orchards across three years as a proof-of-concept approach to defining trophic interactions in this crop and their potential impact in a biological control context. The DNA from aphids, parasitoids and hyperparasitoids was extracted, and amplified using COI primers targeting a 658-bp fragment for the DNA barcode region. DNA sequence data were generated for each trophic level to develop a DNA barcode library for species associated with commercial pecan production in Georgia. Based on DNA barcoding results, three species of aphid, one primary parasitoid family, and five species of hyperparasitoids were identified. Our initial molecular analysis of the pecan-aphid-parasitoid-hyperparasitoid system shows multiple hyperparasitoid species within the pecan aphid-parasitoid complex. Although further research is needed on a broader spatial scale, our results suggest multiple hyperparasitoids may interfere with the biological control potential by

primary parasitoids on aphids in commercial pecan orchards. This was the first time that many of these species have been genetically characterized, and represents a novel approach to analyses the pecan aphid-parasitoid food web in both pecans and the tree nut system in general.

KEYWORDS**:** Aphid-parasitoid interactions, black pecan aphid, yellow pecan aphid, blackmargined aphid, *Aphelinus perpallidus*, food webs, DNA barcoding, parasitism

INTRODUCTION

Elucidating trophic interactions between herbivorous insects and their parasitoids is important for understanding biodiversity and for developing successful biological control strategies. Traditionally, rearing and morphological assessments have been the primary methods of identifying parasitoids associated with certain hosts (Memmott et al. 1994, Hrcek et al. 2013). However, there are numerous challenges that complicate the effectiveness of these techniques. There is the possibility of interspecific interactions within the same host (e.g., multiparasitism, hyperparasitism), and traditional rearing only reveals the 'winner' of these interactions (Gómez-Marco et al. 2015, Lefort et al. 2017). Furthermore, rearing does not always guarantee successful emergence as a result of host and/or parasitoid mortality, which can lead to an underestimation of the diversity, abundance, and impact of the parasitoids present in a system (Gómez-Marco et al. 2015, Lefort et al. 2017, Šigut et al. 2017, Kitson et al. 2019, Sow et al. 2019). Even if rearing is successful, a majority of parasitoids can be taxonomically challenging to identify due to their small

size, diversity, intraspecific morphological plasticity, and complicated lifecycles, with many specimens only identified to the Genus level (Šigut et al. 2017, Yang et al. 2017). The use of molecular analysis can facilitate identification provided that reliable sequence data are available on public platforms (Hajibabaei et al., 2006, Moritz and Cicero 2004, Smith et al. 2008, Taberlet et al. 2018). In addition, there is a need for reliable and accurate DNA sequences for voucher specimens and publicly available DNA barcode databases. Thus, the development of DNA barcode libraries can be useful to identify and catalog species involved across all trophic levels in systems where morphological assessments are challenging (Taberlet et al. 2018). As such, the use of DNA barcoding can help unravel complex food-web interactions between parasitoids and their hosts, which may be particularly valuable for economically-important crop pests (Traugott et al. 2006, Heraty et al. 2007, Bon et al. 2008, Traugott et al. 2008, Desneux et al. 2009)

Aphids are an example of economically important crop pest that had their parasitoid tropic interactions characterized using molecular approaches in a number of different systems especially in cereal and row crops (Traugott et al. 2008, Gariepy and Messing 2012, Zhou et al. 2014, Gómez-Marco et al. 2015, Lefort et al. 2017, Ye et al. 2017b, Ye et al. 2017a, Zhu et al. 2019). One area that has been underrepresented using these approaches is tree nut crops such as pecans, where aphids are of economic concern (Wood et al. 1987, Honaker et al. 2013). The pecan system in Georgia uniquely hosts three species of pestiferous foliar feeding aphid that are present throughout the growing season: yellow pecan aphid, *Monelliopsis pecanis* Bissell, blackmargined aphid,

Monellia caryella (Fitch) (Hemiptera: Aphididae), and black pecan aphid, *Melanocallis caryaefoliae* (Davis), (Hemiptera: Aphididae) (Tedders 1978, Cottrell et al. 2009, Paulsen et al. 2013). These three species can cause economic damage both directly (i.e. leaf damage and defoliation) and indirectly (i.e. honeydew secretion promoting sooty mold) (Tedders 1978, Cottrell et al. 2009, Paulsen et al. 2013). While insecticide regimes are the commonly used method of control for these aphids, the threat of resistance has led to consideration of alternative control methods such as biological control (Shapiro–Ilan et al. 2013, Acebes-Doria and Hudson 2019, Slusher et al. 2021). Despite this, there hasn't been a concerted effort to characterize pecan aphid-parasitoid interactions.

At least one primary parasitoid species, *Aphelinus perpallidus*, specializes on the pecan aphid complex (Bueno Jr and Stone 1983, Bueno Jr and Stone 1985). Female *A. perpallidus* lay a single egg inside of an aphid, producing a mummy that hatches into a larva (Tedders 1978). *Aphelinus perpallidus* targets first and second instar aphids and aphids appear to live until their third or fourth instar with *A. perpallidus* emerging 7-10 days after oviposition (Tedders 1978). Previous studies document parasitism rates ranging from 6% to as high as 52% (Bueno Jr and Stone 1983, Bueno Jr and Stone 1985, Mansour et al. 1988). However, there is still a substantial lack of information on the ecology and life history of *A. perpallidus*, with intensive studies dropping off in the 1980's (Bueno Jr and Stone 1983, Bueno Jr and Stone 1985, Bueno Jr and Stone 1987). Furthermore, the occurrence of other aphid parasitoid and hyperparasitoid species in

the pecan system has not been well-documented. Although Bueno Jr. and Stone (1983) assessed hyperparasitism rates of *A. perpallidus* in Texas and found an average rate of 1.7%, Slusher et. al (2021b) observed hyperparasitism rates of upwards of 16% in Georgia, and it is unclear what impact the different species assemblages may have on intraguild interactions and biological control. Understanding parasitoid-hyperparasitoid interactions is important, as hyperparasitoids are regarded as a major cause of primary parasitoid mortality (Gariepy & Messing 2012; Gómez-Marco et al. 2015, Lefort et al. 2017).

In this study, we used DNA barcoding to generate the initial molecular data needed to unravel the structure of the pecan aphid-parasitoid-hyperparasitoid food web. Two of the aphids, *M. pecanis* and *M. caryaefoliae*, and *A. perpallidus*, do not currently have sequence data in public databases (BOLD and GenBank), and the identity of the hyperparasitoids is currently unresolved. Understanding the hyperparasitoid assemblage associated with *A. perpallidus* will provide additional support to determine the biological control potential of *A. perpallidus* in pecan orchards. In addition, this study will characterize the diversity of hyperparasitoids associated with *A. perpallidus* in natural field conditions. This will contribute to an improved understanding of parasitoid and hyperparasitoid ecology by identifying the key players that contribute to the natural enemy community of aphid species in pecan. Further, an improved understanding of the occurrence and role of each member of parasitoid and hyperparasitoid community may provide insight on the potential of biological control in regulating aphid pests in this agroecosystem.

MATERIALS AND METHODS

Aphid Acquisition

The three species of pecan aphid: *M. pecanis*, *M. caryella*, and *M. caryaefoliae* were collected from specimens that were lab-reared on Sumner variety pecan saplings in Tift County, Georgia, USA. Twenty adult stage aphids of each species were then collected and placed in 70% ethanol for preservation. Morphological identification of each aphid species was completed by Ted Cottrell.

Parasitoid Acquisition

Sampling was conducted during the 2018, 2019, and 2020 growing seasons in major pecan growing regions of southern Georgia, USA. Commercial pecan orchards were sampled from each of the following counties: Dougherty (31°36'12.1"N, 84°02'34.0"W) (2019), Macon (32°30'00.8"N, 83°55'46.8"W) (2018, 2019, & 2020), Berrien (31°04'01.3"N 83°11'08.8"W) (2020), Lowndes (31°01'18.2"N 83°14'40.8"W) (2019), and Brooks (31°0'26.40"N, 83°28'58.72"W) (2018). All trees were 'Sumner' variety mature pecan trees between the ages of 13-35 years old. The Berrien and Lowndes County orchards were sprayed for aphids at a high frequency (5-11 insecticide applications a year), while the Macon and Dougherty County orchards were treated for aphids at a low frequency (1-2 insecticide applications a year). Additional site characteristics including temperature, rainfall and detailed spray records can be seen in Table 1 and Supplemental Table in Slusher et al. (2021b). We attempted to collect at least twenty aphid

mummies from each site. Sampling effort was based on the proportion of mummies collected on leaf samples in Slusher et al. (2021b).

At each site, leaves were examined in the lower canopy (~1-2 m from the ground) of mature pecan trees for aphids and parasitized aphids (hereafter referred to as mummies). Leaves with mummies were collected by hand and stored in labeled 3.79 L Ziploc® bags. Leaves were taken back to the lab and mummies were examined to determine if they were emerged (*i.e.*, mummies from which adult wasps successfully emerged from) or unemerged (*i.e.*, mummies on which no successful wasp emergence occurred). Unemerged mummies were gently removed from the leaf, placed individually in plastic capsules (Size 0, 7.62cm, Healthy Life Supply; Mound House, NV), placed on labeled 7.62cm x 12.7cm index cards according to site and collection date and stored in an environmental chamber (25°C, 60% RH, 16:8 L: D, Percival© E36L2; Perry, IA). Mummies were checked daily for parasitoid emergence. Date and type of parasitoid (primary or hyperparasitoid) was noted for each emerged parasitoid. Mummies in capsules on cards were left in the environmental chamber for three weeks before removal. Mummies in capsules on cards were removed from the chamber were stored in labeled 3.79 L Ziploc® bags at room temperature based on year of collection.

DNA Isolation, sequencing, and bioinformatics

Genomic DNA of colony aphids and parasitoids collected from pecan orchards was extracted using Qiagen DNeasy® Blood and Tissue 96-well kits (including a negative control

containing all buffers and solutions, but no insect tissue) following manufacturer protocol (Qiagen, Chatsworth, CA, USA) or following Chelex method (Walsh et al. 1991, Cutler et al. 2015). Extracted DNA was stored at -20 °C. DNA extractions were amplified using published primers following standard protocols (Folmer et al. 1994). PCR reactions (10 µl) contained 5 µl/rxn 2x Qiagen multiplex master mix, 0.1 µl/rxn BSA, 1.9 µl/rxn PCR grade H_2O, and 0.5 µl/rxn of either LCO1490 or HCO2198 primers. PCR reactions were run using a Bio-Rad C1000 Touch™ Thermal Cycler. PCR protocol was performed as follows: *95*°C for 15 min, *94*°C for 30 s, *53.3*°C for 40 s, 73°C for 1 min, 94°C for 30 s, 46.6°C for 1 min, 73°C for 1:30 min, and a final extension of 72°C for 10 min. The PCR products were bi-directionally sequenced on an ABI 3730 DNA Analyzer (Eurofins Genomics LLC). Following sequencing, forward and reverse sequences were assembled, aligned, and edited using Codon Code Aligner program, version 4.0.4. Resulting sequences were screened against all barcode records in the Barcode of Life Datasystems (BOLD) and NCBI to identify the genus and/or species of collected parasitoids. Only high-quality assembled COI sequences were retained for analysis.

RESULTS

In total, 196 samples were extracted and analyzed for COI sequences. The samples included 60 whole pecan aphid specimens, 58 primary parasitoids reared from mummies, 43 hyperparasitoids reared from mummies, and 18 mummies. 43 of the 63 voucher specimens of the

aphid species known from pecan were successfully extracted and amplified to provide high quality COI sequences of *M. caryaefoliae* (N=14), *M. pecanis* (N=13), *M. caryella* (N=16). Of the 18 mummies extracted, only 44% yielded high quality COI sequences, hence identification (N=8).

Sequence comparisons in GenBank and BOLD found evidence of host (*M. caryella*) DNA, primary parasitoid (*A. perpallidus*) DNA, one Pteromalidae (Hymenoptera) species (*Pachyneuron spp.),* and Figitidae (Hymenoptera) species in the subfamily Charipinae. For whole emerged primary parasitoids, 50 of 58 specimens were successfully extracted and amplified. All specimens identified by GenBank were identified as members of the species *A. perpallidus*. Of the 43 whole hyperparasitoid specimens extracted and analyzed, only 15 were successfully extracted and analyzed. Based on sequence comparisons in GenBank and BOLD, specimens analyzed consisted of two pteromalid species (*Asaphes vulgaris* Walker (Hymenoptera: Pteromalidae), *Pachyneuron* sp.), two figitid species in the subfamily Charipinae, and one species in the family Signophoridae (Hymenoptera) (Table 3.1).

For the aphid sequences and the parasitoid sequences, we conducted a crude cluster analysis to view relatedness of samples based on the COI barcodes prepared. The three aphid species we identified morphologically grouped together consistently with the COI sequence data. The primary parasitoid sequences were not consistently resolved at the species level, but all appeared to group with samples that could be resolved to the species level, suggesting one primary parasitoid, *A. perpallidus* present in this pecan system. Based on the COI sequences generated,

<2% sequence variation was observed, and all were categorized with the same Barcode Index Number (BIN) suggesting a single species.

DICUSSION

This study is one of the first to provide a molecular characterization of the pecan aphid-parasitoid system. Our study provides the first molecular data to differentiate the three pecan aphid species. COI sequence data suggest that aphids reared in our colonies over the three years of this study constitute three separate species. This supports the morphological assessment of the three aphids detailed in Tedders (1978). Studies by Dickey and Medina (2011) on yellow pecan aphid found two genetically distinct populations that feed on pecan and water hickory, *Carya aquatica* (Michx. F.) Nutt. Thus, there is potential for the existence of several different strains of pecan aphid, however, we did not find this in our study.

We generated new sequence data for three species of aphid, one primary parasitoid, and five species of hyperparasitoids (Figure 3.1). The only primary parasitoid revealed in this study was *A. perpallidus* a known member of Aphelinidae that parasitizes pecan aphids (Tedders 1978, Bueno and Stone 1985). However, it is unknown if more species are present in this system due to lack of sequences for this family. James Wooley, a parasitoid specialist formerly of Texas A&M, identified some specimens of primary pecan aphid parasitoids submitted by the authors and revealed most to be *A. perpallidus*. It was noted, however, that some specimens were lighter in

color and were possibly a different species. Dickey and Medina (2011) found three distinct populations consisting of unique phenotypes of *A. perpallidus*. However, based on the DNA results provided by this study it appears that the primary parasitoid of pecan aphids in Georgia consists of a single species.

Our study is the first to characterize hyperparasitoids associated with *A. perpallidus* in the southeast. Bueno and Stone (1985) previously identified *Alloxysta schlingeri* (Andrews) (Hymenoptera: Charipidae), Signophora spp. (Hymenoptera: Signophoridae), and *Aphidencyrtus* spp. (Hymenoptera: Encyrtidae) as hyperparasitoids of *A. perpallidus* in Texas using morphological methods. Similarly, our barcoding of parasitoids identified samples as Signophoridae and Charipidae. While our study was unable to conclusively use sequence data to identify the species of Charipinae, it is possible that *A. schlingeri* could be one member of this subfamily associated with *A. perpallidus* in the southeast. *A. vulgaris* was one specimen that was not found in the west, while *Aphidencyrtus spp*. were not found in our samples. Although there may be more hyperparasitoids in this system, we currently uncovered a minimum of five species targeting one primary parasitoid of the pecan aphid complex (Figure 3.1).

A developing theme from Bueno and Stone (1985) and our study, for the pecan aphid-parasitoid system, is both feature a single primary parasitoid that is experiencing potential top-down pressure from multiple hyperparasitoids. All five hyperparasitoids identified in our system are generalist hyperparasitoids of aphids and other sternorrhyncans in a number of different field

and greenhouse systems (Specht 1969, Dean et al. 1981, Powell 1982, Ferrer-Suay et al. 2014, Zamora-Mejías and Hanson 2016, Martens 2018, Taberlet et al. 2018, Bandyan et al. 2021). Therefore, it is possible for these hyperparasitoids to build up in other cropping systems and spill over into the pecan system and put pressure on the specialist *A. perpallidus*. Therefore, it is possible that the pecan aphid-parasitoid assemblage may represent a very fragile system. Heavy pressure from multiple hyperparasitoids has been suggested as a possible explanation for the low success of biological control in several other aphid-parasitoid systems (Gómez-Marco et al. 2015, Lefort et al. 2017, Dong et al. 2019). For example, Lefort et al. (2017) found that in the cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), food-web, there was a high top down hyperparasitoid pressure on the primary parasitoid, *Diaretiella rapae* (McIntosh) (Hymenoptera: Braconidae), in the cabbage aphids invaded range of New Zealand but not in the aphid's native range in the United Kingdom. This potentially led to the failure of *D. rapae* as a biological control agent in its introduced range. *Binodoxys angelicae* Haliday (Hymenoptera: Braconidae), a primary parasitoid of *Aphis spiraecola* Patch (Hemiptera: Aphididae), was also found to experience heavy top-down pressure from six different hyperparasitoids (Gómez-Marco et al. 2015). The assemblage present in our data presents a similar potential issue for *A. perpallidus* in the pecan system. While studies commonly analyze primary parasitoid-host interactions, the understanding of hyperparasitoid assemblages is largely unknown (Franck et al. 2017, Kitson et al. 2019, Sow et al. 2019). Lefort et al. (2017) encountered a similar problem, as they were only able to assume

hyperparasitism based off data trends and prior knowledge of the taxa found. In addition, we don't know what role each of these potential hyperparasitoids plays in this assemblage. Lefort et al. (2017) brought up the idea of a 5th tropic level of hyperparasitism, which could very well be present in our system given the diversity of hyperparasitoids.

This study developed a more complete picture of the pecan aphid-parasitoid-hyperparasitoid food web (Figure. 3.1). Based on the results of this research, the next step would be to examine the effects of hyperparasitoids on *A. perpallidus* populations and the probable result of biocontrol failure. In addition, our study didn't examine additional factors such as the presence of predators and intraguild predation. Previous work by Slusher et al. (2021) found the highest hyperparasitoid rates at sites with the highest *A. perpallidus* abundance, indicating a potential relationship between primary parasitoid abundance and hyperparasitoid rates. However, the true effect of hyperparasitoids on *A. perpallidus* remains to be examined. Bueno and Stone (1983) claimed to not observe a significant impact of hyperparasitism on *A. perpallidus*, however, more research will need to be done to support this lone account. Further examination of hyperparasitoids should be completed and our work provides a foundation for future analysis of the pecan aphid-parasitoid foodweb.

LITERATURE CITED

Abram, P. K., J. Brodeur, A. Urbaneja, and A. Tena. 2019. Nonreproductive effects of insect parasitoids on their hosts. Annu. Rev. Entomol. 64: 259-276.

Acebes-Doria, A. L., and W. G. Hudson. 2019. University of Georgia commercial pecan spray Guide. University of Georgia.

Bandyan, S. K., R. S. Peters, N. B. Kadir, M. Ferrer-Suay, and W. H. Kirchner. 2021. A survey of aphid parasitoids and hyperparasitoids (Hymenoptera) on six crops in the Kurdistan Region of Iraq. J. Hymenopt. Res. 81: 9.

Bon, M., K. Hoelmer, D. Coutinot, and N. Ramualde. 2008. PCR-based, rapid diagnosis of parasitism of Lygus spp.(Hemiptera: Miridae) by *Peristenus relictus* (Hymenoptera: Braconidae). Biocontrol Sci. Technol. 18: 505-516.

Bueno Jr, R., and J. Stone. 1983. Phenology of a Parasite of the Blackmargined Aphid in West Texas [*Aphelinus perpallidus, Monellia caryella*]. Southwest. Entomol.

Bueno Jr, R., and J. D. Stone. 1985. *Aphelinus perpallidus* parasitism of *Monellia caryella* populations in far West Texas. J. Entomol. Sci. 20: 325-330.

Bueno Jr, R., and J. Stone. 1987. Reproductive response of *Aphelinus perpallidus* (Hymenoptera: Aphelinidae) to age of its parent and density of its host, *Monellia caryella* (Homoptera: Aphidae). Environ. Entomol. 16: 877-880.

Cottrell, T. E., B. W. Wood, and X. Ni. 2009. Chlorotic feeding injury by the black pecan aphid (Hemiptera: Aphididae) to pecan foliage promotes aphid settling and nymphal development. Environ. Entomol. 38: 411-416.

Cutler, G., T. Gariepy, E. De Silva, and N. Hillier. 2015. High rates of parasitism of blueberry spanworm (Lepidoptera: Geometridae) by Ichneumonidae and Tachinidae in commercial lowbush blueberry fields. J. Pest Sci. 88: 219-223.

Dean, G., M. G. Jones, and W. Powell. 1981. The relative abundance of the hymenopterous parasites attacking *Metopolophium dirhodum* (Walker) and *Sitobion avenae* (F.)(Hemiptera: Aphididae) on cereals during 1973–79 in southern England. Bull. Entomol 71: 307-315.

Desneux, N., P. Starý, C. J. Delebecque, T. D. Gariepy, R. J. Barta, K. A. Hoelmer, and G. E. Heimpel. 2009. Cryptic species of parasitoids attacking the soybean aphid (Hemiptera: Aphididae) in Asia: *Binodoxys communis* and *Binodoxys koreanus* (Hymenoptera: Braconidae: Aphidiinae). Ann. Entomol. Soc. Am. 102: 925-936.

Dickey, A. M., and R. F. Medina. 2011. Lack of sequential radiation in a parasitoid of a host-associated aphid. Entomol. Exp. Appl. 139: 154-160.

Dong, Z., X. Men, S. Liu, and Z. Zhang. 2019. Food web structure of parasitoids in greenhouses is affected by surrounding landscape at different spatial scales. Sci. Rep. 9: 1-8.

Ferrer-Suay, M., M. Janković, J. Selfa, F. F. Van Veen, Ž. Tomanović, K. Kos, E. Rakhshani, and J. Pujade-Villar. 2014. Qualitative analysis of aphid and primary parasitoid trophic relations of genus Alloxysta (Hymenoptera: Cynipoidea: Figitidae: Charipinae). Environ. Entomol. 43: 1485-1495.

Franck, P., M. Maalouly-Matar, and J. Olivares. 2017. Molecular tools for the detection and the identification of Hymenoptera parasitoids in tortricid fruit pests. Inter. J. Mol. Sci. 18: 2031.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3(5):294-9

Gariepy, T., and R. Messing. 2012. Development and use of molecular diagnostic tools to determine trophic links and interspecific interactions in aphid–parasitoid communities in Hawaii. Biol. Control 60: 26-38.

Gariepy, T. D., A. Bruin, J. Konopka, C. Scott-Dupree, H. Fraser, M. C. Bon, and E. Talamas. 2019. A modified dna barcode approach to define trophic interactions between native and exotic pentatomids and their parasitoids. Mol. Ecol. 28: 456-470.

Gómez-Marco, F., A. Urbaneja, J. A. Jaques, P. Rugman-Jones, R. Stouthamer, and A. Tena. 2015. Untangling the aphid-parasitoid food web in citrus: Can hyperparasitoids disrupt biological control? Biol. Control 81: 111-121.

Hajibabaei, M., D. H. Janzen, J. M. Burns, W. Hallwachs, and P. D. Hebert. 2006. DNA barcodes distinguish species of tropical Lepidoptera. PNAS 103: 968-971.

Heraty, J. M., J. B. Woolley, K. R. Hopper, D. L. Hawks, J.-W. Kim, and M. Buffington. 2007. Molecular phylogenetics and reproductive incompatibility in a complex of cryptic species of aphid parasitoids. Mol. Phylogenet. Evol. 45: 480-493.

Honaker, J., S. Skrivanek, J. Lopez, D. Martin, L. Lombardini, L. Grauke, and M. Harris. 2013. Blackmargined aphid (*Monellia caryella* (Fitch); Hemiptera: Aphididae) honeydew production in pecan and implications for managing the pecan aphid complex in Texas. Southwest. Entomol. 38: 19-32.

Hrcek, J., S. E. Miller, J. B. Whitfield, H. Shima, and V. Novotny. 2013. Parasitism rate, parasitoid community composition and host specificity on exposed and semi-concealed caterpillars from a tropical rainforest. Oecologia 173: 521-532.

Kanturski, M., Y. Lee, J. Choi, and S. Lee. 2018. DNA barcoding and a precise morphological comparison revealed a cryptic species in the *Nippolachnus piri* complex (Hemiptera: Aphididae: Lachninae). Sci. Rep. 8: 1-16.

Kitson, J. J., C. Hahn, R. J. Sands, N. A. Straw, D. M. Evans, and D. H. Lunt. 2019. Detecting host–parasitoid interactions in an invasive lepidopteran using nested tagging dna metabarcoding. Mol. Ecol. 28: 471-483.

Lee, Y., W. Lee, M. Kanturski, R. G. Foottit, S.-I. Akimoto, and S. Lee. 2017. Cryptic diversity of the subfamily Calaphidinae (Hemiptera: Aphididae) revealed by comprehensive DNA barcoding. PLOS One 12: e0176582.

Lefort, M. C., S. Wratten, A. Cusumano, Y.-D. Varennes, and S. Boyer. 2017. Disentangling higher trophic level interactions in the cabbage aphid food web using high-throughput dna sequencing. MBMG: e13709.

Mansour, F., M. Harris, J. Stone, and R. Bueno. 1988. Releases in Israel of *Aphelinus perpallidus*, a recently introduced parasite of the pecan blackmargined aphid, *Monellia caryella*. Phytoparasitica 16: 171-174.

Martens, A. P. Year. Published. Aphid-parasitoid (Insecta) diversity and trophic interactions in South Dakota, p. 83. *In*, *Proceedings of the South Dakota Academy of Science*, 2018.

Memmott, J., H. Godfray, and I. D. Gauld. 1994. The structure of a tropical host-parasitoid community. J. Anim. Ecol: 521-540.

Moritz, C., C. Cicero, and C. Godfray. 2004. DNA barcoding: promise and pitfalls. PLoS biology 2: e354.

Paulsen, C., T. Cottrell, and J. Ruberson. 2013. Distribution of the black pecan aphid, *Melanocallis caryaefoliae*, on the upper and lower surface of pecan foliage. Entomol. Exp. Appl. 146: 252-260.

Powell, W. 1982. The identification of hymenopterous parasitoids attacking cereal aphids in Britain. Syst. Entomol. 7: 465-473.

Shapiro–Ilan, D. I., T. E. Cottrell, M. A. Jackson, and B. W. Wood. 2013. Control of key pecan insect pests using biorational pesticides. J. Econ. Entomol. 106: 257-266.

Šigut, M., M. Kostovčík, H. Šigutová, J. Hulcr, P. Drozd, and J. Hrček. 2017. Performance of DNA metabarcoding, standard barcoding, and morphological approach in the identification of host–parasitoid interactions. PLOS One 12: e0187803.

Slusher, E. K., T. Cottrell, and A. L. Acebes-Doria. 2021a. Effects of aphicides on pecan aphids and their parasitoids in pecan orchards. Insects 12: 241.

Slusher, E. K., W. G. Hudson, P. L. Halliday, and A. L. Acebes-Doria. 2021b. Multisite seasonal monitoring of pecan aphids and their parasitoid in commercial pecan orchards. Environ. Entomol. Nvab069

Smith, M. A., J. J. Rodriguez, J. B. Whitfield, A. R. Deans, D. H. Janzen, W. Hallwachs, and P. D. Hebert. 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. PNAS 105: 12359-12364.

Specht, H. 1969. Hyperparasitism of the pea aphid parasite *Aphelinus semiflavus* by *Asaphes vulgaris* in a greenhouse. Ann. Entomol. Soc. Am. 62: 1207-1207.

Sow, A., T. Brévault, L. Benoit, M.-P. Chapuis, M. Galan, A. Coeur d'acier, G. Delvare, M. Sembène, and J. Haran. 2019. Deciphering host-parasitoid interactions and parasitism rates of crop pests using DNA metabarcoding. Sci. Rep. 9: 1-12.

Taberlet, P., A. Bonin, L. Zinger, and E. Coissac. 2018. Environmental DNA: For biodiversity research and monitoring, Oxford University Press.

Tedders, W. L. 1978. Important biological and morphological characteristics of the foliar-feeding aphids of pecan. Department of Agriculture, Science and Education Administration: 1578-1587.

Traugott, M., P. Zangerl, A. Juen, N. Schallhart, and L. Pfiffner. 2006. Detecting key parasitoids of lepidopteran pests by multiplex PCR. Biol. Control 39: 39-46.

Traugott, M., J. Bell, G. Broad, W. Powell, F. Van Veen, I. Vollhardt, and W. O. C. Symondson. 2008. Endoparasitism in cereal aphids: molecular analysis of a whole parasitoid community. Mol. Ecol. 17: 3928-3938.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506-513.

Watterson, G. P., and J. D. Stone. 1982. Parasites of Blackmargined Aphids and their Effect on Aphid Populations in Far-West Texas. Environ. Entomol. 11: 667-669.

Wood, B. W., W. L. Tedders, and J. D. Dutcher. 1987. Energy Drain by Three Pecan Aphid Species (Homoptera: Aphididae) and Their Influence on In-Shell Pecan Production. Environ. Entomol. 16: 1045-1056.

Yang, F., L. Xu, Y.-K. Wu, Q. Wang, Z.-W. Yao, V. Žikić, Ž. Tomanović, M. Ferrer-Suay, J. Selfa, and J. Pujade-Villar. 2017. Species composition and seasonal dynamics of aphid parasitoids and hyperparasitoids in wheat fields in northern China. Sci. Rep. 7: 1-9.

Ye, Z., I. M. Vollhardt, Z. Tomanovic, and M. Traugott. 2017a. Evaluation of three molecular markers for identification of european primary parasitoids of cereal aphids and their hyperparasitoids. PLOS One 12: e0177376.

Ye, Z., I. M. Vollhardt, S. Girtler, C. Wallinger, Z. Tomanovic, and M. Traugott. 2017b. An effective molecular approach for assessing cereal aphid-parasitoid-endosymbiont networks. Sci. Rep. 7: 1-12.

Zamora-Mejías, D., and P. Hanson. 2016. Trophic relationships of aphid hyperparasitoids (Hymenoptera) in Costa Rica. J. Entomol. Res. Soc. 18: 35-44.

Zhou, Q. S., Y. Q. Xi, F. Yu, X. Zhang, X. J. Li, C. L. Liu, Z. Q. Niu, C. D. Zhu, G. X. Qiao, and Y. Z. Zhang. 2014. Application of DNA barcoding to the identification of Hymenoptera parasitoids from the soybean aphid (Aphis glycines) in China. Insect Sci. 21: 363-373.

Zhu, Y.-L., F. Yang, Z.-W. Yao, Y.-K. Wu, B. Liu, H.-B. Yuan, and Y.-H. Lu. 2019. A molecular detection approach for a cotton aphid-parasitoid complex in northern China. Sci. Rep. 9: 1-8.

TABLES AND FIGURES

Table 3.1. Summary of taxa richness and specimens identified at each trophic level. The proportion of successfully identified primary and hyperparasitoids relative to each other is also included.

***Available upon publication.**

Trophic Level	Taxa Richness	Taxa Represented (Proportion of Tropic Level)	GenBank® number*
Hyperparasitoid	5	Signiphoridae sp. (19%) *Asaphes vulgaris* (19%) Pachyneuron sp. (19%) 2x Charipinae sp. (43.7%)	
Primary Parasitoid	1	*Aphelinidae* (100%)	
Aphid	3	Yellow Pecan Aphid (*Monelliopsis pecanis*) Blackmargined Aphid (*Monellia caryella*) Black Pecan Aphid (*Melanocallis caryaefoliae*)	
Plant	1	Pecan (*Carya illinoinensis*)	

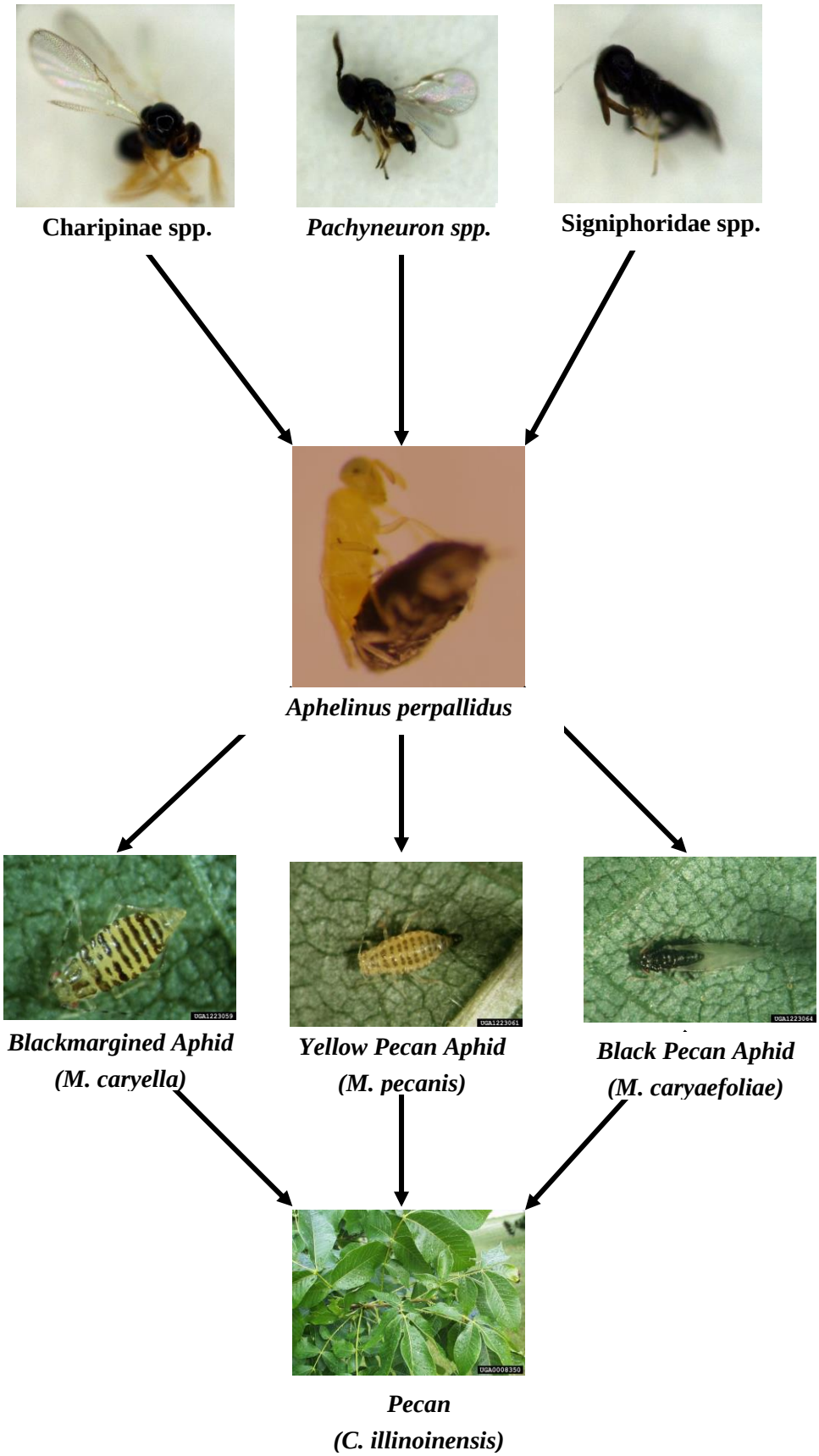

Figure 3.1. Current predicted aphid-parasitoid-hyperparasitoid food web in this study.

CHAPTER 4

EFFECTS OF APHICIDES ON PECAN APHIDS AND THEIR PARASITOIDS IN PECAN ORCHARDS

* Slusher, E. K., T. Cottrell, and A. L. Acebes-Doria. 2021. Effects of Aphicides on Pecan Aphids and Their Parasitoids in Pecan Orchards. Insects 12: 241. Reprinted here with permission of the publisher.

ABSTRACT

Aphids are important pests of pecans. Traditionally, insecticides have been the primary method of management. However, over-reliance and non-judicious use has led to resistance and damage to natural enemy populations. Therefore, frequent assessment of insecticides is necessary in order to monitor resistance development and non-target impacts. Aphicides, flonicamid, sulfoxaflor, and afidopyropen were assessed for their effects on pecan aphids and parasitoid, *Aphelinus perpallidus*, in a mature pecan orchard in 2019 and 2020. Post-application assessments were performed 7-, 14-, and 21-days-post application. Leaf samples from non-treated trees had greater aphid numbers than treated trees 7-days post application with differences diminishing throughout the other two treatment periods in 2019. In 2020, aphid numbers were lower but leaf samples from non-treated trees had more aphids than treated trees 7-days post application in the lower canopy. These differences again diminished 14- and 21-days-post application. There was no difference among treatments in number of parasitoid adults or mummies. These findings indicate that pecan growers have multiple potential options available for aphid management that do not negatively impact the primary pecan aphid parasitoid. Implications of the results on pecan aphid management are discussed.

KEYWORDS: Carbine, Closer Insecticide Resistance, Parasitism, Sefina

INTRODUCTION

Pecan, *Carya illinoensis* (Wangenh.) K. Koch, (Fagales: Juglandaceae) is a native nut crop and ranks as one of the ten most important agricultural commodities in Georgia. In 2019, Georgia produced 52,204.4 bearing hectares with an average yield of 599.4 kg per hectare (NASS 2020). In 2019, pecan production in the state amounted to $263,359,174 in farm gate value (Wolfe and Stubbs 2019). Like most agricultural commodities, pecan is attacked by an assemblage of pests such as aphids (Hemiptera: Aphididae), mites (Acari: Tetranychidae), pecan weevil (Coleoptera: Curculionidae), hickory shuckworm (Lepidoptera: Tortricidae), pecan nut casebearer (Lepidoptera: Pyralidae), ambrosia beetles (Coleoptera: Curculionidae), and prionus rootborers (Coleoptera: Cerambycidae). The foliage, nuts, trunk, and roots of the tree are all targets of pest pressure (Wells and Conner 2007). The number of pests and the different parts of the tree being attacked causes issues for growers who must rely on a wide variety of pest management tactics.

Aphids are serious pests whose feeding can compromise tree health in addition to negatively impacting nut quality and yield (Mizell III and Schiffhauer 1990, Wells and Conner 2007, Shapiro–Ilan et al. 2013). Three species of aphids feed on pecans: the yellow pecan aphid, *Monelliopsis pecanis* Bissell (Hemiptera: Aphididae), the blackmargined aphid, *Monellia caryella* (Fitch) (Hemiptera: Aphididae), and the black pecan aphid, *Melanocallis caryaefoliae* (Davis) (Hemiptera: Aphididae). Yellow pecan aphid and blackmargined aphid are collectively referred to as the ‘yellow aphid’ complex. All three species feed on the leaves but cause different types of damage. Both the yellow aphid complex and black pecan aphid excrete honeydew as a by-product of feeding. The honeydew coats the leaf causing the leaf to turn glossy and sticky and, over time, leads to the development of sooty mold. Sooty mold–coated leaves have lower photosynthetic capabilities. In addition, black pecan aphid feeding elicits localized leaf chlorosis around the

feeding area. Severe infestations of black pecan aphid can lead to defoliation of the tree. Insecticide application is the primary and often necessary method for aphid management in various systems. However, the reliance on insecticides, coupled with only a recent understanding of some of the resistance mechanisms present in many aphid species, has led to increased difficulty in managing aphids as insecticide resistance emerges across various systems (Hanson et al. 2017, Kandil et al. 2017, Tang et al. 2017, Koch et al. 2018, Mingeot et al. 2020, Ullah et al. 2020). Studies done on green peach aphid (*Myzus persicae* Sulzer; Hemiptera: Aphididae) in the late 1990's found that carboxylesterases in the aphid's body provided resistance to organophosphates, pyrethroids, and carbamates. This was only found years after the first case of aphicide resistance was reported in this species in 1955 (Tang et al. 2017). Therefore, testing and assessment of numerous aphicides is important in order to evaluate effectiveness. In addition, evaluation of the non-target effects on beneficial insects is also critical. Non-judicious application of aphicides can destroy natural enemy populations such as parasitoids and predators leading to secondary outbreaks of other pests or a resurgence of the target pest (Dutcher et al. 1985, Mizell III and Schiffhauer 1990, Shapiro–Ilan et al. 2013, Jiang et al. 2019, Joseph 2020).

One important organism that may be affected is *Aphelinus perpallidus* Gahan (Hymenoptera: Aphelinidae). They are known to parasitize both the nymphal and adult stages [14-18]. While little is known about their biology and life history, they are an important natural enemy of pecan aphid (Bueno Jr and Stone 1983, Bueno Jr and Stone 1985, Bueno Jr and Stone 1987, Bueno Jr and Van Cleve 1997a, b). Their specialization on pecan aphids contrasts with most aphid predators, such as lacewings and ladybeetles, that are more generalist in nature and leave the host plant when prey is limited (Kunkel and Cottrell 2007).

Another aspect of insecticide application in pecan that is poorly studied is the effects of insecticide application on pests and beneficial insects at different canopy heights. Mature pecan trees in orchards can vary in height from 4–20 m with exceptionally tall wild individuals exceeding 30 m (Wells 2017). Both previous research and unpublished research by the authors indicates that pest and predators vary in their usage of the upper and lower canopy of pecan trees (Cottrell 2017). In addition, previous research has shown that spray coverage decreases significantly as canopy height increases (Bock et al. 2015). However, research that examines the effects of insecticide application on both pest and beneficials at different canopy heights is currently lacking.

The objective of this study was to assess the effects of flonicamid, sulfoxaflor, and two concentrations of afidopyropen. The aphicides used in this study can be used as rotational chemistries for aphid management because they have different modes of action per the Insecticide Resistance Action Committee (IRAC)(Nauen et al. 2019). Flonicamid is a group 29 insecticide that targets the chordotonal organ modulators causing the target species to cease feeding after 30 min as well as reducing honeydew production (Nauen et al. 2019). Additional behavioral effects include light sensitivity and irregular, erratic movement (Nauen et al. 2019). The only evidence of resistance to flonicamid thus far has been found in the cotton aphid (*Aphis gossypii* Glover) attacking fruiting vegetables in Korea (Koo et al. 2014). Sulfoxaflor is a group 4C insecticide targeting the nicotinic acetylcholine receptor competitive modulators. Green peach aphids treated with sulfoxaflor experience tremors and eventual paralysis. Due to its novel chemistry, there is little to no evidence of cross-resistance to sulfoxaflor (Sparks and Nauen 2015). Afidopyropen is a group 9D insecticide targeting the chordotonal organ transient receptor potential cation channel (TRPV) modulators. This action affects movement and feeding activity in the target pest leading to starvation (Joseph 2020).

We assessed the effects of these products on the three pecan aphid species, aphid mummies and adults of the primary pecan aphid parasitoid, *A. perpallidus*. In addition, we assessed effects of these treatments on aphids and parasitoids in the upper and lower canopy of tall, mature pecan trees.

MATERIALS AND METHODS

This study was conducted during the 2019 and 2020 growing seasons in Ty Ty, Georgia, USA on a 3.64-ha research orchard planted with mature 'Desirable' pecan trees. All treatment applications were made using an airblast sprayer (CDP20P150P Air 32, Durand-Wayland, Inc., LaGrange, GA) calibrated to deliver 935 L/ha. Trees were sprayed with fungicides every two weeks from May to August each year. In 2020, carbaryl (Carbaryl 4L©, 479.9 g a.i./liter, Drexel Chemical Company, Memphis, TN) and zeta-cypermethrin (Mustang Maxx©, 95 g a.i./liter, FMC Corporation, Philadelphia, PA, USA) were applied at 4,731.8 and 22.7 g a.i./ha, respectively, once a week for two weeks in order help increase aphid numbers for the study. Carbaryl and zeta-cypermethrin are members of the carbamate and pyrethroid insecticide families. Carbamates and pyrethroids are often not recommended for aphid management due to resistance problems and non-target effects on natural enemies. Spraying these insecticides often allows increases in aphid abundance due to decreases in natural enemies (Shapiro–Ilan et al. 2013, Acebes-Doria and Hudson 2019). This was not done during the 2019 study when yellow and black aphids were more abundant. Our experimental design was a randomized complete block design consisting of four blocks. Treatments were randomly assigned to their own pair of pecan trees in each block. Pre-treatment aphid sampling was done on all pre-selected trees for the experiment on 9 September 2019 and 14 August 2020. On 10 September 2019 and 17 August 2020, each replicate pair within

each block was treated with one of five treatments: flonicamid 207.01 ml/ha (Carbine©, 857.3 ml a.i/liter, FMC Corporation, Philadelphia, PA, USA), sulfoxaflor 203.3 ml/ha (Closer©, 3,429.2 g a.i./liter, Corteva Agriscience, Wilmington, DE), low rate afidopyropen 221.8 ml/ha (Sefina©, 720.12 g a.i./liter, BASF Ag Products, Research Triangle Park, NC), high rate afidopyropen 443.6 ml/ha or a non-treated control. These rates are based on the recommended labelled rates. Treated trees were buffered from other treatments by being located in every other row and allowing at least two trees between each treated pair within the same row.

The average high temperature during the 2019 sampling period was 34.75 °C and the average low temperature was 19.9 °C. The hottest day recorded during the sampling period was 38.5 °C on September 27th, while the coolest night was 16.1°C on September 21st. Average rainfall during the sampling period was 0.06 mm per week. For 2020, the average high temperature was 32.78 °C while the average low temperature was 22.4 °C. The hottest day during the sample period was 35.4 °C on August 18th and the coolest night was 20.72 °C on September 7th. The average rainfall during the sampling period was 3 mm per week.

Post-spray assessments were done on 17 September (7-days), 25 September (14-days), and 2 October (21-days) during 2019 and 23 August (7-days), 31 August (14-days), and 7 September (21-days) during 2020. These days after treatment were selected based on the slow mortality rate of the mode of action of the selected insecticides (Sparks and Nauen 2015).

For the assessment, five compound leaves were randomly selected from the upper (~6.1 - 9.1 m) and lower canopy (~1.5 - 1.8 m) of each treated and control tree. Because the number of leaflets can vary, only the middle three pairs of leaflets in each leaf were sampled and taken to the lab. Leaflets were examined using a microscope (Luxeo 6z, Labomed®, Fremont, CA) for 'yellow aphid' nymphs and adults, black pecan aphid nymphs and adults and the mummies of parasitized

aphids. In addition, one yellow sticky card (7.6 X 12.7 cm, Olson Products Inc.; Medina, OH) was placed in the upper and lower pecan canopy (40 cards total) of one tree in each replicate pair on 17 (7-14 days) and 25 (14-21 days) September during 2019 and 23 (7-14 days) and 31 (14-21 days) August during 2020 for one week to assess populations of the aphid parasitoid *A. perpallidus*, in response to treatments.

During both years of the study, black pecan aphid was the dominant species averaging 94.15% and 60.70% across the sampling period in 2019 and 2020 respectively. In contrast to other multi-aphid species systems, previous studies of insecticidal effects on pecan aphid species were shown to be non-species specific (Dutcher 2005, Dutcher et al. 2010). Thus, all subsequent analyses of mean aphid populations included all aphid species. Data were examined for normality and homogeneity of variance and subjected to transformations (log +1) when needed prior to analyses (Ott and Longnecker 2015). A two-way analysis of variance (ANOVA) with product and canopy location as fixed effects and block as a random effect was used to evaluate canopy location interactions, overall product effects, and canopy location effects. A one-way ANOVA was used to evaluate effects of aphicides in the upper and lower canopy separately. Tukey's HSD was used for post hoc analysis at $\alpha = 0.05$. All statistical analyses were performed using JMP® Pro 14.1.0 (SAS Version 14.1.0, Cary, NC).

RESULTS

2019 Results

Effects of Insecticidal Treatment on Pecan Aphids. At 7 days post-application, there was no significant interaction between product and canopy location (Table 4.1). Aphid populations did not differ significantly between the upper (4.75 ± 2.05/leaf) and lower canopy (3.81 ± 6.98/leaf)

(Table 4.1). An overall product effect was observed (Table 4.1). Aphid numbers were significantly different across the products in both canopies (Table 4.1). In the upper canopy, the non-treated control trees had a significantly greater number of aphids compared to the trees treated with aphicides (Table 4.1). In the lower canopy, non-treated control trees had a significantly greater number of aphids compared to the treated trees (Table 4.1). Trees sprayed with low-rate afidopyropen had significantly greater aphid populations compared to trees treated with sulfoxaflor (Table 4.1).

At 14 days post-application, there was no significant interaction detected between product and location (Table 4.1). No significant difference was detected between canopy locations (Upper: 1.93 ± 0.33/leaf, lower: 2.28 ± 0.40/leaf Table 4.1). A significant difference in aphid numbers among the treatment groups was observed overall (Table 4.1). As well, significant differences among products were detected in the upper and lower canopies. In the upper canopy, flonicamid had significantly greater numbers of aphids than trees treated with sulfoxaflor or high-rate afidopyropen (Table 4.1). In the lower canopy, control trees and trees treated with low-rate afidopyropen had significantly more aphids than trees treated with sulfoxaflor (Table 4.1).

At 21 days post-application, no significant interaction was found between product and location (Table 4.1). Significantly, more aphids were found in the lower canopy (4.35 ± 0.67/leaf) than in the upper canopy (1.65 ± 0.23/leaf) (Table 4.1). Overall, there was a significant difference in aphid numbers among treatment groups (Table 4.1). A significant difference in aphids among product treatments was found in both the upper and lower canopy (Table 4.1). In the upper canopy, significantly more aphids were found in the control than in sulfoxaflor (Table 4.1). In the lower canopy, significantly more aphids were found in the control trees than all other treated trees except for flonicamid (Table 4.1)

Effects of Insecticidal Treatment on Aphid Mummies. There was no significant interaction between product and canopy location on aphid mummies across any sampling period (Table 4.2). Significantly more aphid mummies were found in the lower canopy than in the upper canopy location 14-days (Upper: 0.045 ± 0.02//leaf, Lower: 0.355 ± 0.13/leaf) and 21-days (Upper: 0 ± 0/leaf, Lower: 0.24 ± 0.04/leaf) post-treatment (Table 4.2). The aphicidal effects on the number of aphid mummies were not significantly different across any of the assessment periods (Table 4.2). No significant differences in aphicidal effects were found in either the upper or lower canopy (Table 4.2)

Effects of Insecticidal Treatment on Adult Parasitoids. No interaction was found between product treatment and canopy location on the adult parasitoid numbers (Table 4.3). Significantly more adult parasitoids were found in the upper canopy (7.5 ± 2.66/card) than in the lower canopy (1.85 ± 0.67/card) at 14-21 days post treatment (Table 4.3). No significant difference in *A. perpallidus* populations among the insecticidal treatments was detected on either the 7-14 or 14-21 post application intervals (Table 4.3). No significant difference in aphicidal effects on aphid numbers was found in either the upper canopy or lower canopy during either sampling period (Table 4.3).

2020 Results

2020 Pre-Sample. While the pre-sample was only done in the lower canopy in 2019, the 2020 pre-sample was done in the both canopy locations. The pre-sample found significantly more aphids in the lower canopy (2.33 ± 0.17) than in the upper canopy (0.51 ± 0.17) ($P<.0001$). The same is true for mummies as well, who were significantly more abundant in the lower canopy (0.93 ± 0.11) than in the upper canopy (0.035 ± 0.03) ($P<0001$).

Effects of Insecticidal Treatment on Pecan Aphids. At 7-days post assessment, no interaction was found between product treatment and canopy location (Table 4.4). Significantly more aphids were found in the lower canopy (0.665 ± 0.26/leaf) than in the upper canopy (0.125 ± 0.04/leaf). Overall aphid numbers were significantly different among treatments (Table 4). No significant differences in aphid numbers were found in the upper canopy among any of the treatment groups (Table 4.4). In the lower canopy, significantly more aphids were found in the control than in any of the treated trees except for sulfoxaflor (Table 4.4). Trees treated with sulfoxaflor had significantly more aphids than any of the other treated trees (Table 4.4).

During the 14-day post assessment, no interaction was found between product treatment and canopy location (Table 4.4). Significantly more aphids were found in the lower canopy (0.73 ± 0.20/leaf) than in the upper canopy (0.17 ± 0.04/leaf). A significant difference among the treatments was found (Table 4.4). Aphid numbers across treatments in the upper canopy were similar (Table 4.4). In the lower canopy, significantly more aphids were found in the control than in sulfoxaflor (Table 4.4).

At 21 days post assessment, no interaction was found between product treatment and canopy location (Table 4.4). Aphids were significantly more abundant in the lower canopy (2.18 ± 0.45/leaf) than in the upper canopy (0.50 ± 0.09/leaf) (Table 4.4). No significant overall production effect was found (Table 4.4). No significant effect was found in the 21-day post assessment among any of the treatment groups (Table 4.4). No significant effect of the aphicides on aphid numbers was found in either the upper or lower canopy (Table 4.4).

Effects of Insecticidal Treatment on Aphid Mummies. No significant interaction was detected between the product and canopy location on aphid mummies across any of the sampling days (Table 4.5). Mummies were significantly more abundant in the lower canopy compared to the

upper canopy during the 7-day (Upper: 0.03 ± 0.01/leaf, Lower: 0.975 ± 0.10/leaf), 14-day (Upper: 0.025 ± 0.08/leaf, Lower: 0.71 ± 0.08/leaf), and 21-day (Upper: 0 ± 0/leaf, Lower: 0.73 ± 0.14/leaf) sampling periods (Table 4.5). There was no significant difference in mummy abundance among any of the treatment groups across any of the sampling days (Table 4.5). There was no significant difference in aphicide effects on mummy number in either the upper or lower canopy during any of the sampling periods (Table 4.5).

Effects of Insecticidal Treatment on Adult Parasitoids. No significant interaction was detected between the product and canopy location on adult parasitoids across any of the sampling periods (Table 4.6). There were no significant differences in parasitoid numbers between the upper (183.3 ± 31.7/card) and lower canopy (156.1 ± 35.3/card) on the 7-14 day or the upper (21.6 ± 4.6/card) and lower canopy (21.5 ± 6.09/card) on 14-21 day (Table 4.6). A significant overall product effect was detected during the 14-21 day sampling period (Table 4.6). However, no significant effect of aphicides on parasitoids was found in either the upper or lower canopy (Table 4.6.)

DISCUSSION

Insecticide use is often necessary to manage aphids feeding on pecan foliage because aphid predators often arrive later than their prey or leave before the aphids begin to overwinter (Kunkel and Cottrell 2007). Predators such as lacewings may have lifecycles that lag behind their potential prey by as much as one week (Kunkel and Cottrell 2007). We demonstrated through this study that pecan growers have insecticidal options for aphid management. In 2019, trees sprayed with aphicides had significantly lower aphid populations than the non-treated control 7 days post-treatment application. Though aphid reduction varied among the aphicides, our analysis suggests that each aphicide can be used to successfully manage pecan aphids. The difference in aphid

numbers between the control and treatments diminishes at 14 and 21-days post application. This diminished effect could also be attributed to seasonal changes in pecan aphid populations, which usually peak in July and August before decreasing in late September and October when they begin to overwinter (Wells and Conner 2007). This is evident by the decrease in mean aphid numbers in the non-treated control as well as the treated trees. The 2020 spray trial was done earlier in the year to better understand the effects of the aphicides on the yellow aphid complex whose populations were dropping naturally during the 2019 study. However, aphid numbers were low during the growing season and thus any diminishing effects were not apparent.

The aphicides used in this study are formulated to inhibit the feeding of insects with piercing-sucking mouthparts such as aphids, whiteflies, and psyllids (Sparks and Nauen 2015, Joseph 2020). This not only makes them effective for managing the target insect but also helps prevent damage to natural enemy populations. Non-target effects are common in the pecan orchard setting and can result in a secondary outbreak of additional pests due to natural enemy suppression (Shapiro–Ilan et al. 2013). An example of this in the pecan system is the use of carbaryl for pecan weevil management causing a surge in pecan aphid populations as a result of reduced natural enemies (Dutcher et al. 1985). Laboratory studies on effects of insecticides on various aphid predators including lacewings, lady beetles, and the mummies of *A. perpallidus* indicated that no insecticide may be safe for all species in an orchard. Individual insecticides tested have reported differing effects among the predators and parasitoids (Mizell III and Schiffhauer 1990). In Mexico, application of tebufenozide and chlorpyrifos for management of hickory shuckworm had adverse effects on lacewings and ladybeetles (Quiñones-Pando et al. 2009).

Our findings in this spray trial found no significant difference in adult parasitoids or mummies in treated trees compared to the non-treated control across both years of the study. The

exception to this was overall product on adult parasitoids from 14-21 day post-treatment application in 2020. However, we did not see these significant differences in the 7-14 day. Therefore, it seems likely that these aphicides can be used to manage aphids while not adversely affecting *A. perpallidus*. In previous studies, no major non-target effects on beneficial insects such as *Apis mellifera* L. (Hymenoptera: Apidae), and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) were found for flonicamid (Hanson et al. 2017, Nauen et al. 2019). Sulfoxaflor has low toxicity to terrestrial invertebrates, beneficial insects, and earthworms (Sparks and Nauen 2015). However, a study done on *Trichogramma* (Hymenoptera: Trichogrammatidae) found that sulfoxaflor exposure had lethal effects and impaired their parasitism ability (Jiang et al. 2019). The non-target effects of afidopyropen are poorly understood, due to it being a new insecticide. A study done on green peach aphid and its predator, the two spotted ladybeetle (*Adalia bipunctata* L.) (Coleoptera: Coccinellidae) found a significant difference in green peach aphid numbers between pre- and post-treatment but found no significant difference in two spotted ladybeetle larvae under the same param (Joseph 2020). A laboratory study with soybean aphid found low toxicity to convergent ladybeetle (*Hippodamia convergens* Guerin-Meneville) (Coleoptera:Coccinellidae) and moderate toxicity to *Aphelinus certus* Yasnosh (Hymenoptera: Aphelinidae) (Koch et al. 2020). The results of our study are similar with these results. However, previous literature also highlights the importance of species-to-species assessments of non-target effects. Our study is potentially one of the first to analyze the effects of afidopyropen on natural enemies in a field setting. In addition, this is one of the few recent studies to assess the effects of aphicides on natural enemies in an orchard production system.

Numbers of parasitized aphids were not significantly different among treatment groups throughout most of the study. Given that adult parasitoids were not affected significantly by the

treatments, it is likely they were able to continue to parasitize aphids. It has also been documented that the mummified aphid may offer some degree of protection to the developing parasitoid. However, this depends largely on a number of factors such as the penetrability of the insecticide as well as how soon the adult parasitoid will emerge (Longley 1999). Laboratory studies on aphids parasitized by *A. perpallidus* found that only methomyl and carbaryl caused significant mortality, 57 and 51%, respectively. All other insecticides tested were not toxic (Mizell III and Schiffhauer 1990). Lethal and sub-lethal effects of many modern insecticides on parasitoids in the pupal stage could be a basis of future studies.

Rainfall may have also been a factor in our study. A previous study by Kaakeh and Dutcher found a significant reduction in aphid numbers collected post-rainfall compared to pre-rainfall (Kaakeh and Dutcher 1993). This is in accordance with other studies in different systems (Molnár 2003, Javed et al. 2014). This may account for the lack of a significant difference in the upper canopy in 2020 compared to 2019 as the rainfall was greater in 2020 (3 mm) compared to 2019 (0.06 mm). The study mentioned above was done in a single field season and may not accurately depict long term trends. A multi-season study could help paint a bigger picture of the effects of rainfall on pecan aphid populations.

For this study, we analyzed the differences in population of aphids, mummies, and parasitoids between the upper canopy and lower canopy. In addition, we also assessed the interaction between the insecticides and canopy location. This was done to assess potential differences that canopy height had on treatment effects. Canopy height likely impacts spray coverage because the sprayer may be unable to supply adequate coverage to the upper canopy. Spray coverage area on spray cards placed at different heights in mature pecan trees decreases as height increases (Bock et al. 2015). In our study, no significant interactions were found between

insecticidal treatments and canopy location; however, aphid, mummy and parasitoid numbers differed significantly between upper and lower canopies. Aphids were more abundant in the lower canopies in 2019 at 21 days, and during all three sampling periods in 2020. Mummies were more abundant in the lower canopy during all sampling days during both years of the study except 7 days post application in 2019. Adult parasitoid distribution varied between upper and lower canopy only during the 14-21 day sampling period in 2019, when adult parasitoids were more abundant in the upper canopy. The significantly higher numbers of aphids in the lower canopy is interesting given that spray coverage should have been greater in the lower canopy. This could have been due to higher populations of aphids in the lower canopy to begin with. This was evident based off our pre-sample, where aphids and mummies were more abundant lower canopy than in the upper canopy. Even though we found little effect of canopy location on *A. perpallidus* adults, considering canopy height is also important for understanding differences in natural enemy populations. For instance, the number of lady beetle species inhabiting the pecan canopy is affected by canopy height; lady beetles respond negatively, positively, or neutrally to changes in canopy height (Cottrell 2017). Canopy height is a poorly studied factor that may have significant effects on aphicide efficacy as well as varying effects on natural enemy populations. Future studies are needed address the potential effects of canopy height on aphicide application.

LITERATURE CITED

Acebes-Doria, A. L., and W. G. Hudson. 2019. University of Georgia commercial pecan spray guide. University of Georgia

Bock, C. H., M. W. Hotchkiss, T. E. Cottrell, and B. W. Wood. 2015. The effect of sample height on spray coverage in mature pecan trees. Plant Dis. 99: 916-925.

Bueno Jr, R., and J. Stone. 1983. Phenology of a parasite of the blackmargined aphid in West Texas [*Aphelinus Perpallidus, Monellia Caryella*]. Southwest. Entomol.

Bueno Jr, R., and J. D. Stone. 1985. *Aphelinus Perpallidus* parasitism of *Monellia Caryella* populations in far West Texas. J. Entomol. Sci. 20: 325-330.

Bueno Jr, R., and J. Stone. 1987. Reproductive response of *Aphelinus perpallidus* (Hymenoptera: Aphelinidae) to age of its parent and density of its host, *Monellia Caryella* (Homoptera: Aphidae). Environ. Entomol. 16: 877-880.

Bueno Jr, R., and H. Van Cleve. 1997a. The effect of cold storage on the emergence of *Aphelinus Perpallidus*, a parasitoid of *Monellia Caryella*. Southwest. Entomol.

Bueno Jr, R., and H. Van Cleve. 1997b. The effect of temperature and host density on the reproduction of *Aphelinus Perpallidus*. Southwest. Entomol.

Byford, R. 2005. Growth and development of pecan nuts. New Mexico State University.

Cottrell, T. 2017. Trap height affects capture of lady beetles (Coleoptera: Coccinellidae) in pecan orchards. Environ. Entomol. 46: 343-352.

Dutcher, J., J. Payne, and W. Neel. 1985. The impact of pecan weevil control strategies on non-target arthropods, pp. 39-50, Pecan Weevil: Research Perspective. Quail Ridge Press, Brandon, MS.

Dutcher, J. D. 2005. Chemical control of aphids on pecan foliage. Arthropod Manag. Tests 30.

Dutcher, J. D., H. Karar, and M. G. Abbas. 2010. Effectiveness of two insecticides for control of foliage feeding pecan aphids. Arthropod Manag. Tests 35.

Hanson, A. A., J. Menger-Anderson, C. Silverstein, B. D. Potter, I. V. MacRae, E. W. Hodgson, and R. L. Koch. 2017. Evidence for soybean aphid (Hemiptera: Aphididae) resistance to pyrethroid insecticides in the upper midwestern United States. J Econ Entomol 110: 2235-2246.

Javed, H., J. Iqbal, and Z. Mateen. 2014. Response of different cultivars of groundnut, *Aarachis hypogaea* L.(Fabaceae: Fabales) to aphids, *Aphis craccivora* K.(Aphididae: Homoptera) in interaction with local weather factors. Pak. J. Zool. 46.

Jiang, J., X. Liu, Z. Zhang, F. Liu, and W. Mu. 2019. Lethal and sublethal impact of sulfoxaflor on three species of *Trichogramma* parasitoid wasps (Hymenoptera: Trichogrammatidae). Biol. Control 134: 32-37.

Joseph, S. V. 2020. Effect of afidopyropen against *Myzus persicae* (Hemiptera: Aphididae) and Its Predator, *Adalia bipunctata* (L.) in a greenhouse. J. Entomol. Sci. 55: 584-587.

Kaakeh, W., and J. D. Dutcher. 1993. Effect of rainfall on population abundance of aphids (Homoptera, Aphididae) on pecan. J. Entomol. Sci. 28: 283-286.

Kandil, M. A., I. S. Abdallah, H. M. Abou-Yousef, N. A. Abdallah, and E. A. Fouad. 2017. Mechanism of resistance to pirimicarb in the cowpea aphid *Aphis craccivora*. Crop Prot. 94: 173-177.

Koch, R. L., E. W. Hodgson, J. J. Knodel, A. J. Varenhorst, and B. D. Potter. 2018. Management of insecticide-resistant soybean aphids in the upper midwest of the United States. J. Integr. Pest Manag. 9: 23.

Koch, R. L., O. da Silva Queiroz, R. C. Aita, E. W. Hodgson, B. D. Potter, T. Nyoike, and C. D. Ellers-Kirk. 2020. Efficacy of afidopyropen against soybean aphid (Hemiptera: Aphididae) and toxicity to natural enemies. Pest Manag. Sci. 76: 375-383.

Koo, H.-N., J.-J. An, S.-E. Park, J.-I. Kim, and G.-H. Kim. 2014. Regional susceptibilities to 12 insecticides of melon and cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae) and a point mutation associated with imidacloprid resistance. Crop Prot. 55: 91-97.

Kunkel, B. A., and T. E. Cottrell. 2007. Oviposition response of green lacewings (Neuroptera: Chrysopidae) to aphids (Hemiptera: Aphididae) and potential attractants on pecan. Environ. Entomol. 36: 577-583.

Longley, M. 1999. A Review of Pesticide Effects Upon Immature Aphid Parasitoids within Mummified Hosts. Int. J. Pest Manag. 45: 139-145.

Mingeot, D., L. Hautier, and J. P. Jansen. 2020. Structuration of multilocus genotypes associated with insecticide resistance of the peach potato aphid, *Myzus persicae* (Sulzer), in potato fields in Southern Belgium. Pest Manag. Sci.

Mizell III, R. F., and D. E. Schiffhauer. 1990. Effects of pesticides on pecan aphid predators *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), *Hippodamia convergens*, *Cycloneda sanguinea* (L.), *Olla v-nigrum* (Coleoptera: Coccinellidae), and *Aphelinus perpallidus* (Hymenoptera: Encyrtidae). J Econ Entomol 83: 1806-1812.

Molnár, N. 2003. Population dynamics features of willow-feeding aphids. Acta Phytopathol. Entomol. Hung. 38: 125-135.

NASS, U. 2020. United States Department of Agriculture National Agricultural Statistics Service. 2017. Quick stats database.

Nauen, R., R. Slater, T. C. Sparks, A. Elbert, and A. Mccaffery. 2019. IRAC: Insecticide resistance and mode-of-action classification of insecticides, pp. 995-1012, Modern Crop Protection Compounds, vol. 3. Wiley-VCH.

Ott, R. L., and M. T. Longnecker. 2015. An introduction to statistical methods and data analysis, Nelson Education.

Quiñones-Pando, F. J., S. H. Tarango-Rivero, and C. A. Blanco. 2009. Effect of two insecticides on hickory shuckworm (Lepidoptera: Tortricidae) and predators of pecan pests. Southwest. Entomol. 34: 227-238.

Shapiro-Ilan, D. I., T. E. Cottrell, C. Bock, K. Mai, D. Boykin, L. Wells, W. G. Hudson, and R. F. Mizell III. 2017. Control of pecan weevil with microbial biopesticides. Environ. Entomol. 46: 1299-1304.

Sparks, D. 2005. Adaptability of pecan as a species. Hortscience 40: 1175-1189.

Sparks, T. C., and R. Nauen. 2015. IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Phys. 121: 122-128.

Tang, Q.-L., K.-S. Ma, Y.-M. Hou, and X.-W. Gao. 2017. Monitoring insecticide resistance and diagnostics of resistance mechanisms in the green peach aphid, *Myzus persicae* (Sulzer)(Hemiptera: Aphididae) in China. Pestic. Biochem. Phys. 143: 39-47.

Van Lenteren, J., Y. Drost, H. Van Roermund, and C. Posthuma-Doodeman. 1997. Aphelinid parasitoids as sustainable biological control agents in greenhouses. J. Appl. Entomol. 121: 473-485.

Wells, L. 2017. Pecan: America's Native Nut Tree, University of Alabama Press.

Wells, L., and P. Conner. 2007. Southeastern Pecan Growers' Handbook.

Winder, L., C. J. Alexander, C. Woolley, J. N. Perry, and J. M. Holland. 2013. The spatial distribution of canopy-resident and ground-resident cereal aphids (*Sitobion avenae* and *Metopolophium dirhodum)* in winter wheat. Arthropod-Plant Interact. 7: 21-32.

Wolfe, K., and K. Stubbs. 2019. Georgia Farm Gate Value Report 2018. University Georgia Cooperative Extension Service 17.

TABLES AND FIGURES

Table 4.1. Mean ± SEM of total pecan aphids per leaf across treatments and sample days in 2019. Differing letters in columns indicate a significant difference between treatments ($p < 0.05$ Tukey's honestly significant difference (HSD). Canopy-specific product effects and *p*-values for product*canopy interaction, overall product effect, and canopy location across sampling days are also shown. Application rates for each aphicide are based on the standard label rate.

Product	**7-Day Post-Treatment**		**14-Day Post-Treatment**		**21-Day Post-Treatment**	
	Upper	Lower	Upper	Lower	Upper	Lower
Control	18.93 ± 8.85a	13.30 ± 3.77a	2.90 ± 1.11ab	3.03 ± 0.79a	2.53 ± 0.59a	9.25 ± 2.11a
Flonicamid	0.95 ± 0.40b	1.28 ± 0.72bc	2.90 ± 0.79a	2.00 ± 0.66ab	2.08 ± 0.54ab	4.18 ± 1.11ab
Sulfoxaflor	0.48 ± 0.17b	0.28 ± 0.14c	0.75 ± 0.10b	0.73 ± 0.20b	0.70 ± 0.30b	1.73 ± 0.46b
Low-Rate Afidopyropen	2.40 ± 1.21b	3.58 ± 1.45b	2.30 ± 0.59ab	3.95 ± 1.42a	2.08 ± 0.43ab	3.10 ± 0.77b
High-Rate Afidopyropen	0.98 ± 0.65b	0.60 ± 0.22bc	0.80 ± 0.25b	1.70 ± 0.48ab	0.85 ± 0.36ab	3.48 ± 1.17b
Canopy-specific Product Effects	$F_{df} = 10.4_4$ $P = <.0001$	$F_{df} = 12.2_4$ $P = <.0001$	$F_{df} = 4.20_4$ $P = 0.0076$	$F_{df} = 3.67_4$ $P = 0.0144$	$F_{df} = 4.00_4$ $P = 0.0097$	$F_{df} = 6.37_4$ $P = 0.0007$
Product*Canopy Location (*P*-Value)	0.9293		0.3111		0.2829	
Overall Product Effect (*P*-Value)	<.0001		0.0002		<.0001	
Canopy Location Effect (*P*-Value)	0.9928		0.4548		<.0001	

Table 4.2. Mean ± SEM of mummified aphids per leaf across treatments and sample days in 2019. Differing letters in columns indicate a significant difference between treatments ($p < 0.05$ Tukey's HSD). Canopy-specific product effects and *P*-values for product*canopy interaction, overall product effect, and canopy location across sampling days are also shown. Application rates for each aphicide are based on the standard label rate.

Product	**7-Day Post-Treatment**		**14-Day Post-Treatment**		**21-Day Post-Treatment**	
	Upper	Lower	Upper	Lower	Upper	Lower
Control	0.40 ± 0.26a	0.53 ± 0.39a	0.08 ± 0.04a	0.45 ± 0.27a	0.00 ± 0.00a	0.35 ± 0.11a
Flonicamid	0.10 ± 0.08a	0.33 ± 0.15a	0.05 ± 0.03a	0.98 ± 0.58a	0.00 ± 0.00a	0.28 ± 0.08a
Sulfoxaflor	0.05 ± 0.03a	0.10 ± 0.04a	0.10 ± 0.10a	0.13 ± 0.08a	0.00 ± 0.00a	0.23 ± 10a
Low-Rate Afidopyropen	0.20 ± 0.08a	0.13 ± 0.08a	0.00 ± 0.00a	0.10 ± 0.05a	0.00 ± 0.00a	0.13 ± 0.08a
High-Rate Afidopyropen	0.03 ± 0.03a	0.23 ± 0.12a	0.00 ± 0.00a	0.13 ± 0.08a	0.00 ± 0.00a	0.23 ± 0.08a
Canopy-specific Product Effects	$F_{df} = 1.75_4$ $P = 0.1631$	$F_{df} = 0.78_{4\ P}$ =0.5455	$F_{df} = 0.90_4$ $P = 0.4740$	$F_{df} = 1.92_4$ $P = 0.1315$	$F_{df} = 0_4$ $P = 1$	$F_{df} = 0.82_4$ $P = 0.5225$
Product*Canopy Location (*P*-Value)	0.7333		0.2145		0.4973	
Overall Product Effect (*P*-Value)	0.2797		0.0988		0.4973	
Canopy Location Effect (*P*-Value)	0.2714		0.0038		<.0001	

Table 4.3. Mean ± SEM of adult parasitoids per card across treatments and sample days in 2019. Differing letters in columns indicate a significant difference between treatments ($p < 0.05$ Tukey's HSD). Canopy-specific product effects and *P*-values for product*canopy interaction, overall product effect, and canopy location across sampling days are also shown. Application rates for each aphicide are based on the standard label rate.

Product	**7-14 Day Post-Treatment**		**14-21 Day Post-Treatment**	
	Upper	Lower	Upper	Lower
Control	38.00 ± 34.02a	43.75 ± 43.41a	8.25 ± 4.44a	0.75 ± 0.48a
Flonicamid	7.75 ± 2.53a	17.25 ± 9.56a	2.25 ± 1.11a	1.50 ± 0.65a
Sulfoxaflor	16.50 ± 8.09a	3.50 ± 2.18a	13.25 ± 9.39a	1.50 ± 0.96a
Low-Rate Afidopyropen	2.50 ± 1.66a	1.50 ± 1.19a	8.25 ± 5.98a	4.75 ± 2.87a
High-Rate Afidopyropen	9.75 ± 8.09a	7.75 ± 4.19a	5.5 ± 3.11a	0.75 ± 0.75a
Canopy-specific Product Effects	$F_{df} = 1.37_4$ $P = 0.3011$	$F_{df} = 0.91_4$ $P = 0.488$	$F_{df} = 0.35_4$ $P = 0.8421$	$F_{df} = 1.70_4$ $P = 0.2136$
Product*Canopy Location (*P*-Value)	0.7221		0.5586	
Overall Product Effect (*P*-Value)	0.1922		0.7975	
Canopy Location Effect (*P*-Value)	0.186		0.01	

Table 4.4. Mean ± SEM of total pecan aphids per leaf across treatments and sample days in 2020. Differing letters in columns indicate a significant difference between treatments ($p < 0.05$ Tukey's HSD). Canopy-specific product effects and *P*-values for product*canopy interaction, overall product effect, and canopy location across sampling days are also shown. Application rates for each aphicide are based on the standard label rate.

Product	**7-Day Post-Treatment**		**14-Day Post-Treatment**		**21-Day Post-Treatment**	
	Upper	Lower	Upper	Lower	Upper	Lower
Control	0.23 ± 0.10a	1.83 ± 1.13a	0.13 ± 0.07a	1.60 ± 0.74a	0.63 ± 0.17a	3.08 ± 1.10a
Flonicamid	0.03 ± 0.03a	0.15 ± 0.08c	0.15 ± 0.07a	0.55 ± 0.18ab	0.53 ± 0.27a	1.88 ± 0.75a
Sulfoxaflor	0.28 ± 0.14a	1.03 ± 0.47ab	0.08 ± 0.06a	0.13 ± 0.20b	0.33 ± 0.16a	1.98 ± 0.86a
Low-Rate Afidopyropen	0.05 ± 0.03a	0.15 ± 0.08c	0.23 ± 0.10a	0.45 ± 0.17ab	0.43 ± 0.14a	1.00 ± 0.29a
High-Rate Afidopyropen	0.05 ± 0.05a	0.18 ± 0.06bc	0.28 ± 0.08a	0.93 ± 0.26ab	0.58 ± 0.26a	2.95 ± 1.63a
Canopy-specific Product Effects	$F_{df} = 2.15_4$ $P =$ 0.0973	$F_{df} = 3.81_4$ $P =$ 0.0121	$F_{df} = 0.93_4$ $P =$ 0.4567	$F_{df} = 3.00_4$ $P =$ 0.0327	$F_{df} = 0.55_4$ $P =$ 0.6988	$F_{df} = 0.94_4$ $P =$ 0.4612
Product*Canopy Location (*P*-Value)	0.1755		0.1072		0.7611	
Overall Product Effect (*P*-Value)	0.0007		0.0295		0.2755	
Canopy Location Effect (*P*-Value)	0.0013		0.0001		<.0001	

Table 4.5. Mean ± SEM of mummified aphids per leaf across treatments and sample days in 2020. Differing letters in columns indicate a significant difference between treatments ($p < 0.05$ Tukey's HSD). Canopy-specific product effects and *P*-values for product*canopy interaction, overall product effect, and canopy location across sampling days are also shown. Application rates for each aphicide are based on the standard label rate.

Product	**7-Day Post-Treatment**		**14-Day Post-Treatment**		**21-Day Post-Treatment**	
	Upper	Lower	Upper	Lower	Upper	Lower
Control	0.00 ± 0.00a	0.90 ± 0.13a	0.00 ± 0.00a	0.75 ± 0.11a	0.00 ± 0.00a	1.00 ± 0.27a
Flonicamid	0.08 ± 0.05a	0.75 ± 0.32a	0.00 ± 0.00a	0.73 ± 0.18a	0.00 ± 0.00a	0.63 ± 0.29a
Sulfoxaflor	0.03 ± 0.03a	1.20 ± 0.21a	0.00 ± 0.00a	0.70 ± 0.16a	0.00 ± 0.00a	0.70 ± 0.24a
Low-Rate Afidopyropen	0.03 ± 0.03a	1.18 ± 0.31a	0.10 ± 0.08a	0.60 ± 0.23a	0.00 ± 0.00a	0.60 ± 0.15a
High-Rate Afidopyropen	0.03 ± 0.03a	0.85 ± 0.12a	0.03 ± 0.03a	0.78 ± 0.27a	0.00 ± 0.00a	0.73 ± 0.23a
Canopy-specific Product Effects	$F_{df} = 0.74_4$ $P = 0.5694$	$F_{df} = 0.99_4$ $P = 0.4225$	$F_{df} = 1.46_4$ $P = 0.2372$	$F_{df} = 0.21_4$ $P = 0.9316$	$F_{df} = 0_4$ $P = 1$	$F_{df} = 0.54_4$ $P = 0.7086$
Product*Canopy Location (*P*-Value)	0.3256		0.7088		0.7233	
Overall Product Effect (*P*-Value)	0.607		0.997		0.7233	
Canopy Location Effect (*P*-Value)	<.0001		<.0001		<.0001	

Table 4.6. Mean + SEM of adult parasitoids per card across treatments and sample days in 2020. Differing letters in columns indicate a significant difference between treatments ($p < 0.05$ Tukey's HSD). Canopy-specific product effects and *P*-values for product*canopy interaction, overall product effect, and canopy location across sampling days are also shown. Application rates for each aphicide are based on the standard label rate.

Product	**7-14 Day Post-Treatment**		**14-21 Day Post-Treatment**	
	Upper	Lower	Upper	Lower
Control	219.00 ± 54.64a	202.00 ± 68.87a	45.00 ± 8.38a	42.25 ± 18.40a
Flonicamid	186.75 ± 75.21a	92.75 ± 36.75a	9.75 ± 2.56a	14.25 ± 10.96a
Sulfoxaflor	127.25 ± 52.16a	144.25 ± 46.20a	21.00 ± 11.25a	38.25 ± 17.06a
Low-Rate Afidopyropen	103.67 ± 12.12a	200.50 ± 153.59a	6.75 ± 1.80a	3.75 ± 0.25a
High-Rate Afidopyropen	261.25 ± 110.40a	141 ± 69.23a	25.5 ± 12.5a	8.75 ± 3.47a
Canopy-specific Product Effects	$F_{df} = 0.30_4$ $P = 0.8731$	$F_{df} = 0.34_4$ $P = 0.8490$	$F_{df} = 1.89_4$ $P = 0.1763$	$F_{df} = 2.76_4$ $P = 0.0771$
Product*Canopy Location (*P*-Value)	0.9714		0.844	
Overall Product Effect (*P*-Value)	0.6915		0.0064	
Canopy Location Effect (*P*-Value)	0.4042		0.4718	

CHAPTER 5

EFFECTS OF VERTICAL STRATIFICATION ON PECAN APHID AND THEIR PARASITOIDS IN PECANS

* Slusher, E. K., T. Cottrell, and A. L. Acebes-Doria. 2021. Effects of Vertical Stratification on Pecan Aphid and Their Parasitoids in Pecans. To be submitted to Biological Control.

ABSTRACT

The effects of vertical stratification on aphids in orchard systems has been poorly represented in the literature. Vertical stratification of the canopy can affect aphid density as food quality, predator density, and climate can change at different heights. In addition, natural enemies may also be affected by changes in vertical strata as their food choices may affect their density in a certain region of the canopy. One tree crop that has yet to have the effects of vertical stratification on pecan aphids and their parasitoids thoroughly studied is the pecan system. In this study we evaluated the effects of difference in canopy height on pecan aphids, mummies, and primary parasitoid *Aphelinus perpallidus* in both shorter trees (~9 m) in a commercial orchard and taller trees (~15 m) in an experimental orchard. Leaf samples at both sites indicated that pecan aphids and mummies were found at higher densities in the lower canopy of the tree in the commercial orchard and the lower half of tree in the experimental orchard. *A. perpallidus* was found at higher abundance in the lower half of the trees in the experimental orchard and in the upper canopy of the commercial orchard trees. These results indicate that growers should focus their effects on scouting the lower canopy in order to gather the best assessment of aphid populations in their orchard. In addition, the ability for *A. perpallidus* to disperse into the upper canopy can be of benefit to growers as they can help with pest management in areas where sprayers may struggle to efficiently cover the canopy.

KEYWORDS: Aphid-parasitoid interactions, yellow pecan aphid, blackmargined aphid, black pecan aphid

INTRODUCTION

The distribution of organisms across a vertical plane has become a subject of interest in several different systems including forest, water bodies, and soil as it has been linked to greater species richness (Basset et al. 2003, Procházka et al. 2018, Oliveira and Scheffers 2019), more niche space (Basset et al. 2003, Rissanen et al. 2019, Chmel et al. 2021), and habitat partitioning (Rissanen et al. 2019, Chmel et al. 2021, Littlefair et al. 2021). Insects are one of the most heavily studied groups in vertical stratification studies especially those that are forestry pests or disease vectors (McGregor et al. 2018, Procházka et al. 2018, Šigut et al. 2018). Interestingly, the effects of vertical stratification on tree crops are poorly represented with most studies on crops focusing more on row crops or only the lower canopies of tree crops (Rice et al. 1998, Athanassiou et al. 2003, Brown 2003, Cottrell 2017). The effects of vertical stratification are particularly significant in orchard systems as tree crops are uniquely taller than other crops and thus contain niches and species richness not found in smaller crops (Platková et al. 2020). Pecans, in particular, vary in height with trees easily exceeding 30 m as they get older (Bock et al. 2015, Wells 2017).

Pecan, like many other agricultural commodities, is susceptible to damage by an assemblage of pest whose life histories and management tactics vary (Wells et al. 2007). Three species of aphid feed on pecan in the southeastern US including yellow pecan aphid, *Monelliopsis pecanis* Bissell, blackmargined aphid, *Monellia caryella* (Fitch), and black pecan aphid, *Melanocallis caryaefoliae* (Davis), (Hemiptera: Aphididae) (Wood et al. 1987, Mizell III and Schiffhauer 1990, Wells and Conner 2007, Shapiro–Ilan et al. 2013). The yellow pecan aphid and blackmargined aphid are collectively referred to as the 'yellow aphid' complex (Wood et al. 1987, Mizell III and Schiffhauer 1990, Wells and Conner 2007, Shapiro–Ilan et al. 2013). All three species of aphid feed on the leaves of the tree causing both direct and indirect damage. Feeding on

the leaf removes nutrients from the leaf which are valuable for vital functions. In addition, as pecan aphids feed they secrete honeydew as a byproduct. Honeydew coats the leaf and overtime promotes the growth of sooty mold which can hinder the photosynthetic capabilities of the plant (Tedders 1978, Cottrell et al. 2009, Paulsen et al. 2013).

Aphids may distribute themselves throughout the canopy for several reasons including food quality, climate, and predator avoidance (Dixon 2005, Costamagna and Landis 2011, Platková et al. 2020). However, this varies according to study. For example, predation avoidance patterns vary based on the predator (Dixon 1969, Clements and Yeargan 1997, Costamagna and Landis 2011, Kirstová et al. 2017, Platková et al. 2020). Previous studies on the distribution of aphids across the vertical strata have shown that aphids preferred the lower canopy of non-woody crops such as cotton or soybean (Costamagna and Landis 2011, Fernandes et al. 2012) while distribution on cereal crops differed between species (Dean 1973, Winder et al. 2013). Thus, it is apparent that aphid distribution differs according to species and canopy highlighting the importance of assessing the effects of vertical strata on different aphid species in multiple systems.

In addition, this variation in height can affect the distribution and abundance of natural enemies in pecan trees. A study of Coccinellidae populations on pecans found that individual species can have a positive, negative, or neutral association with elevation (Cottrell 2017). Unpublished data by the authors has found that overall, significantly more natural enemies (*e.g.* lady beetles, lacewings, parasitoids, spiders, syrphid flies, ants, ground beetles, minute pirate bugs, assassin bugs, and predatory stinkbugs) were present in the upper canopy of pecan trees compared to the lower canopy. Thus, a thorough assessment of the effects of canopy location on key pecan aphid natural enemies is necessary.

Aphelinus perpallidus Gahan (Hymenoptera: Aphelinidae) is a primary parasitoid of pecan aphids (Tedders 1978, Bueno Jr and Stone 1983, Bueno Jr and Stone 1985, Bueno Jr and Stone 1987). While several studies have investigated the effects of seasonality, agricultural inputs, and intraguild competition on *A. perpallidus* (Tedders 1978, Bueno Jr and Stone 1983, Bueno Jr and Stone 1985, Mizell III and Schiffhauer 1990, Bueno Jr and Van Cleve 1997b, Mizell 2007), the effects of elevation on adult and mummy numbers have not been fully assessed. Previous studies have examined the relationship between parasitoids and vertical strata with most of these studies being in the forestry sector. These studies have documented a variety of reactions of parasitoids to changes in vertical strata indicating that generalist parasitoids usually lack a canopy preference while specialists tended to prefer the canopy location where their host was present (Di Giovanni et al. 2015, Šigut et al. 2018). Given that *A. perpallidus* is a specialist on pecan aphid (Tedders 1978), it is likely it will congregate where host distribution is highest. In addition, application of insecticides may potentially alter the distribution of natural enemies in canopies as it often does in row crop fields (De Jiu and Waage 1990, Holland et al. 2000, Hill et al. 2017). Therefore, it appears as though the relationship between vertical stratification and parasitoid abundance and success is not uniform across both plant and insect taxa. This highlights the importance of assessing individual relationships between parastioids and their habitat.

The objective of this study was to assess the effects of vertical stratification in mature pecan trees on pecan aphid and *A. perpallidus* populations at a commercially managed orchard and at an experimental orchard. Two different canopy locations were assessed in 6-9 m tall trees in a commercial orchard and four different canopy locations were assessed in ≥ 15 m tall trees in an experimental orchard. Assessments were performed in orchards with different tree sizes in order to assess how pecan aphids and *A. perpallidus* distribute in pecan trees of with varying canopy

regions. In addition, we also wanted to look at potential differences in aphid and parasitoid distribution in a commercial orchard where insecticides were frequently applied versus an experimental orchard where insecticide application was kept to minimum. Effects of vertical stratification on aphids, aphid mummies, and adult *A. perpallidus* numbers were assessed at each location. In addition, the relationship between parasitoids and aphid populations in the experimental orchard were examined.

MATERIAL AND METHODS

Commercial Orchard Study

This study was conducted over the course of one week from 27 July to 3 August 2020 and 29 July to 4 August 2021 in Lowndes County, Georgia on 'Morrill' pecan trees measuring ~9 m in height. The orchard was divided into four blocks with five replicates each represented by eight mature pecan trees. A lift (Nelson Tree Squirrel, Nelson Manufacturing, Yuba City, CA) was used to place two yellow sticky cards (7.6 x 12.7cm, Olson Products Inc.; Medina, OH) in the upper (~9 m) and lower (~6 m) canopy of the third and sixth tree in order to sample *A. perpallidus* adults. Sticky cards were left out for one week and then retrieved. In addition, leaf sampling was done to quantify pecan aphid species *M. pecanis*, *M. caryella*, and *M. caryaefoliae* and aphids parasitized by *A. perpallidus* (from here on referred to as mummies). For aphid and mummy sampling, four whole leaves were collected from the upper and lower canopy of the third and sixth tree in each replicate. These leaves were stored in labeled 3.79 L Ziploc® bags upon collection which were then kept in coolers during transport to the laboratory. Leaf samples were stored in a refrigerator and examined within 48 hours of collection. The interior of all bags was examined to account for

aphids/mummies that moved or fell off leaf's pre-examination. Sticky cards were covered in plastic and brought back to the lab where they were stored in a freezer until assessment.

Experimental Orchard Study

This study was conducted from June – October 2020 and 2021 in Peach County, Georgia, at the United States Department of Agriculture (USDA) Southeastern Fruit and Tree Nut Research Laboratory on mature 'Stuart' and 'Schley' pecan trees. All trees were ~15.2 m or greater in height. A lift (JLG, McConnellsburg, PA) was used to perform all measuring and sampling. Prior to sampling, the tree canopies were measured and marked at 6 m, 9 m, 12 m, and 15 m in six trees in the orchard. To help increase aphid population in the experimental orchard, in 2020 carbaryl + pyrethroid (Baythroid XL©, 119.8 g a.i./liter, Bayer Cropscience) at 146.1 ml/h was applied on 20 July and 3 August 2020. In 2021, Diflubenzuron (Dimilin©, 238.7 g a.i./liter, Bayer Cropscience) at 1,168 ml/h and carbaryl + pyrethroid (Baythroid XL©, 119.8 g a.i./liter, Bayer Cropscience) at 146.1 ml/h was applied on 22 July 2021 and Zeta-cypermethrin (Mustang®, 179.0 g a.i./liter, FMC Corporation) were applied for the same reason. Five compound leaves were taken from each height in each tree once a month to assess for aphid and mummy numbers. In addition, a yellow sticky card was placed at each height in each tree to quantify the adult parasitoid populations at each height. Each year, leaves and cards were taken to the laboratory to quantify the number of live aphids, mummies (both emerged and unemerged), and adult parasitoids. Since whole leaves were taken, the number of leaflets on each leaf was quantified.

Statistical Analysis

All commercial orchard data were transformed using Log+1 in order to meet assumption of normality (Ott and Longnecker 2015). Effects of height on aphid and aphid parasitoids at the commercial orchard were analyzed using a two-tailed t-test. For the experimental site, aphid and

mummy data were transformed using Log+1 in order to meet the assumption of normality (Ott and Longnecker 2015). Adult *A. perpallidus* data were square root transformed (Ott and Longnecker 2015). Effects of height on aphids and aphid parasitoids at the experimental orchard were analyzed with a One-way Analysis of Variance. Tukey's HSD was used to separate means among sampling height at $\alpha = 0.05$. Spearman's correlation was used to analyze the correlation between aphids, mummies and adult parasitoid populations at $\alpha = 0.05$. All analyses were conducted in JMP® Pro 14.1.0 (SAS Version 14.1.0, Cary, NC).

RESULTS

Commercial Orchard

Aphids and Mummies on Leaf Samples. In 2020, yellow pecan aphid numbers were statistically greater in the lower canopy than in the upper canopy (DF= 28.4, *P*= 0.0029; Fig. 5.1). Black pecan aphid numbers were equal between the two canopy locations (DF= 24.985, *P*= 0.06; Fig. 5.1). The numbers of mummified aphids in the lower canopy were also greater than in the upper canopy (DF= 25.5, *P*< .0001; Fig. 5.1). In 2021, there was no significant difference in yellow pecan aphid numbers between the upper and lower canopy (DF= 43, *P*= 0.9324; Fig. 5.2). Black pecan aphids were also similar between the two canopy locations (DF= 28.5, *P*= 0.1472; Fig. 5.2). Aphid mummies were significantly more abundant in the lower canopy than in the upper canopy (DF= 26.9, *P*= 0.0002; Fig. 5.2).

Adult Parasitoids on Sticky Cards. In 2020, adult *A. perpallidus* numbers were comparable between the two canopy locations (DF= 38.0, *P*= 0.4376; Fig. 5.1). However, in 2021, significantly more *A. perpallidus* adults were found in the upper canopy than in the lower canopy (DF= 43, P< .0001; Fig. 5.2)

Experimental Orchard

Aphids and Mummies on Leaf Samples. In 2020, there was minimal effect of canopy height on pecan aphid numbers throughout the season. Total seasonal numbers of yellow pecan aphids were statistically greater at 6 m than at 15 m (F= 7.5, DF= 3, 116, P= 0.0001; Fig. 5.3). Yellow pecan aphids varied statistically by height only for the 5 August sample, where aphid numbers at 6 m were greater than aphid numbers at 12 and 15 m; aphids at 9 m were greater than 15 m (F= 9.28, DF= 3,20, P= 0.0005; Fig. 5.3). Black pecan aphid numbers differed only for the 14 October sample date, when there were more black pecan aphids at 6 m than at 15 m (F= 4.23, DF= 3, 20, P= 0.0181; 5.3). Season-long mummy numbers were higher at 6 m than at 15 m (F= 4.77, DF= 3,116, P= 0.0036; Fig. 5.3). The numbers of mummified aphids were significantly different for the 5 August sample when mummies were more abundant at 6 m than at 15 m (F= 3.42, DF= 3, 20, P= 0.0370; 5.3).

In 2021, leaf samples again revealed effects of canopy height on aphid numbers throughout the season. In June, yellow pecan aphids were detected in higher numbers at 9 m than at 12 or 15 m. Total seasonal numbers of yellow aphids were statistically greater in at 9 m than at 12 or 15 m (F= 5.62, DF= 3, 92, P= 0.0014; Fig. 5.4). In addition, yellow pecan aphids were present in higher numbers at 6 m than at 15 m (F= 3.6, DF= 3, 20, P= 0.0312; Fig. 5.4. In July, significantly more yellow pecan aphids were found at 6 m than at any other heights sampled (F= 3.4, DF= 3, 20, P= 0.0391; Fig. 5.4). In August, yellow pecan aphids were significantly more abundant at 6 m in height than at 12 or 15 m (F= 4.97, DF= 3, 20, P= 0.0097; Fig. 5.4). Later during September, significantly more yellow pecan aphids were found at 6 m than 12 or 15 m (F= 2.95, DF= 3, 20, P= 0.0574; Fig. 5.4). Black pecan aphids only differed in vertical stratification during September, when black pecan aphids were less abundant at 15 m than at 6 or

9 m (F= 6.9, DF= 3, 20, P = 0.0022; Fig. 5.4). Overall, season-long mummy abundance was higher at 6 m than any other canopy height (F= 5.1, DF= 3, 92, P= 0.0026; Fig. 5.4). Monthly mummy assessments only detected differences in August, during which mummies were significantly more abundant at 6 m than at any other height sampled (F= 1.4, DF= 3,20, P= 0.0014; Fig. 5.4).

Aphelinus perpallidus. In 2020, adult *A. perpallidu*s populations followed a similar trend with yellow pecan aphids. Analysis of season-long numbers revealed that adult *A. perpallidus* numbers were greater at 9 m in the canopy than at 12 or 15 m in the canopy (F= 4.87, DF = 3, 116, P= 0.0031; Fig. 5.5). The only statistical difference in height found was during the 5 August sampling period where adult *A. perpallidus* numbers were greater at 6 and 9 m than at 12 or 15 m (F= 3.72, DF = 3, 20, P= 0.0283; Fig. 5.5). There was no statistical difference in *A. perpallidus* abundance at other heights throughout the season. In 2021, there was no significant difference in *A. perpallidus* abundance across any of the heights in the study (Fig. 5.6).

Correlation between Aphids and Parasitoids. In 2020, Spearman's analyses suggest a relationship between yellow aphids, aphid mummies, and *A. perpallidus*. At 6 m, aphid mummies and yellow aphids were positively associated with each other. *A. perpallidus* numbers were positively correlated with both yellow aphid and aphid mummies at 9 m in the canopy, yellow aphid populations were positively correlated with both aphid mummy (Spearman ρ= 0.3852, P= 0.0356; Table 5.1) and *A. perpallidus* (Spearman ρ= 0.5923, P= 0.0006; Table 5.1). At 12 m, the only positive correlation was found between *A. perpallidus* and yellow aphid numbers (Spearman ρ= 0.3739, P= 0.0418, Table 5.1). No correlations among the live aphid and parasitized aphid counts were found in the canopy at 15 m.

In 2021, at 6 m in the canopy, yellow pecan aphid populations were positively correlated with *A. perpallidus* (Spearman ρ= 0.4046, P= 0.0499; Table 5.2). In addition, mummies were positively correlated with black pecan aphids (Spearman ρ= 8746, P= <.0001; Table 5.2). At 9 m in the canopy, mummies were again positively correlated with black pecan aphids (Spearman ρ= 0.4694, P= 0.0207; Table 5.2).

DISCUSSION

The results of this study provide evidence that pecan aphid abundance differs based on canopy height. While significant differences in pecan aphid abundance were not detected during every month of the study, when aphid numbers were significantly different, it was apparent that pecan aphids were more abundant in the lower parts of the canopy in both the experimental orchard and commercial orchard. These results are similar with previous studies showing that aphids prefer the lower canopies of woody plants (McClure 1982, Dahlsten et al. 1999, Dixon 2005, Platková et al. 2020). This higher abundance in the lower canopy may be due to a more suitable climate and higher-quality food in the lower canopy compared to the upper canopy (Dixon 2005, Platková et al. 2020). Previous studies have shown that leaf nutritional quality is poorer in the upper canopies of other tree species (Dixon 2005, Platková et al. 2020). In addition, aphids are more vulnerable to wind, rain, and ultraviolet light in the upper canopy (Dixon 2005, Platková et al. 2020) . Higher temperature can also be a factor with aphids choosing to avoid the upper canopy where temperatures can differ by as much as 10°C between the upper and lower canopies of some trees (Dixon 2005). The highest abundance of pecan aphids in the lower canopy was most apparent during peak times of the year for each aphid species (yellow aphid complex: June-September; black pecan aphid: September-October) (Tedders 1978). This may indicate that pecan aphids

congregate and build up populations in the lower canopy where conditions are better. Previous research on canopy distribution of pecan aphids has shown that canopy preference decreases as populations increase (Edelson and Estes 1987). We did not observe this in our study due to lack of steady population increase in aphid populations preventing aphids from reaching population saturation. Future studies with heavier pecan aphid populations may be useful in order to see if the distribution of pecan aphids in the canopy changes as populations increase.

Aphelinus perpallidus numbers only differed significantly according to canopy height in the experimental orchard during August of 2020 when they were more abundant in the lower canopy. This trend was also observed overall during the 2020 season. However, in the commercial orchard in 2021, *A. perpallidus* were found in greater abundance in the upper canopy than the lower canopy. This contrasts with previous studies on parasitoids in forests where specialists often were found in one part of the canopy in contrast to more generalist parasitoids which were more evenly distributed throughout the canopy (Šigut et al. 2018). This may have been due to low aphid numbers throughout the season, which may have forced *A. perpallidus* to distribute more throughout the canopy in order to find hosts. Such long distance dispersal has previously been reported in parasitoids due to host scarcity, so it is possible *A. perpallidus* may be displaying this behavior here (Cameron et al. 1981). Our multivariate analysis found a positive correlation between the number of yellow pecan aphids and *A. perpallidus*, especially in the lower canopy. Interestingly, the positive correlation of this relationship often decreased in significance as canopy height increased indicating that increases in canopy height may weaken some ecological relationships.

Mummies were low throughout the season which coincided with low aphid numbers. Mummies typically were found in the lower canopy in both the experimental orchard and commercial orchard often following the population trends of yellow pecan aphids. This makes sense because *A. perpallidus* often prefer yellow pecan aphids over black pecan aphids (Tedders 1978, Paulsen et al. 2013). Interestingly, we saw a positive correlation between mummies and black pecan aphids in 2021 despite evidence that black pecan aphids are rarely, if ever, parasitized by *A. perpallidus* (Tedders 1978, Paulsen et al. 2013). This may have been due to decrease in yellow aphid populations via parasitism allowing black pecan aphid populations to increase. However, we did not see this trend in 2020 so further study is needed to assess whether *A. perpallidus* parasitism of yellow pecan aphid positively benefits black pecan aphid.

While *A. perpallidu*s were detected more often in the lower canopy, it is still impressive to see that both prey and parasitoid can colonize trees up to 15 m despite potential dispersal limits due to size and mobility. This can be useful as it means parasitoids can possibly be relied upon to manage pest populations in the upper canopy. This is useful as previous research has shown that insecticide coverage decreases significantly a pecan canopy height increases (Bock et al. 2015). A study looking at spray coverage at different heights in mature pecan trees has shown that spray coverage decreases significantly as canopy height increases (Bock et. al, 2015).

The effects of vertical stratification on natural enemies are poorly understood and analysis of a single family or even species should not be used as a blanket statement for all beneficial insects. A study on lady beetle (Coleoptera: Coccinellidae) populations at different heights revealed that lady beetles may respond negatively, neutrally, or positively to height depending on the species (Cottrell, 2017). A study on Ichneumonid wasps found that the

community differed between the lower canopy and the upper canopy. Certain groups of Ichneumonid were captured in one location over the other while the other groups were captured equally in both locations (Di Giovanni et al. 2015). In addition, studies assessing parasitism rates of leaf-chewing pest found that parasitism rates increased from the first level (area closest to the ground floor) to the third level (uppermost area of a given tree) in smaller tree species and decreased from first level to the third level in taller tree species (Šigut et al. 2018). This phenomenon in smaller trees was believed to be due to spatial avoidance of predators by parasitoids. Since predators were more abundant in the first level, which was close to the forest floor, parasitoids may have moved higher in the tree to avoid predation. For the taller tree species, it was argued that parasitoids avoid the harsh abiotic conditions of the third level and thus are more prevalent in the lower two levels. In addition, due to the first level being much farther from the forest floor there was less risk of predation (Šigut et al. 2018). This suggests that all-natural enemies are not affected by elevation equally even on a species to species level. Future studies should look at the effects of elevation on numerous species of natural enemies in order to fully understand canopy height effects.

Trends between the commercial and experimental orchard were similar with pecan aphids and mummies being found in higher abundance in the lower parts of the tree. This information could be beneficial for growers as it means they can continue to scout the lower canopy and acquire valuable information on aphid numbers regardless of how tall their trees get. By only having to spend time on the lower part of the canopy regardless of whether a tree is 9 m tall or 15 m tall growers may be able to save time and manpower on scouting. In addition, this information can be useful for implementing pest management plans by informing growers on which management tactics (i.e. chemical control to vs biological control) could be most effective.

LITERATURE CITED

Athanassiou, C. G., N. G. Kavallieratos, V. S. Ragkou, and C. T. Buchelos. 2003. Seasonal abundance and spatial distribution of the predator *Macrolophus costalis* and its prey *Myzus persicae* on tobacco. Phytoparasitica 31: 8-18.

Basset, Y., P. M. Hammond, H. Barrios, J. D. Holloway, and S. E. Miller. 2003. Vertical stratification of arthropod assemblages. *In, Arthropods of Tropical Forests*: 17-27.

Bock, C. H., M. W. Hotchkiss, T. E. Cottrell, and B. W. Wood. 2015. The effect of sample height on spray coverage in mature pecan trees. Plant Dis. 99: 916-925.

Brown, M. 2003. Intraguild responses of aphid predators on apple to the invasion of an exotic species, *Harmonia axyridis*. BioControl 48: 141-153.

Bueno Jr, R., and J. Stone. 1983. Phenology of a parasite of the blackmargined aphid in West Texas [*Aphelinus Perpallidus, Monellia Caryella*]. Southwest. Entomol.

Bueno Jr, R., and J. D. Stone. 1985. *Aphelinus Perpallidus* parasitism of *Monellia Caryella* populations in far West Texas. J. Entomol. Sci. 20: 325-330.

Bueno Jr, R., and J. Stone. 1987. Reproductive response of *Aphelinus perpallidus* (Hymenoptera: Aphelinidae) to age of its parent and density of its host, *Monellia Caryella* (Homoptera: Aphidae). Environ. Entomol. 16: 877-880.

Bueno Jr, R., and H. Van Cleve. 1997a. The effect of cold storage on the emergence of *Aphelinus Perpallidus*, a parasitoid of *Monellia Caryella*. Southwest. Entomol.

Bueno Jr, R., and H. Van Cleve. 1997b. The effect of temperature and host density on the reproduction of *Aphelinus Perpallidus*. Southwest. Entomol.

Cameron, P., G. Walker, and D. Allan. 1981. Establishment and dispersal of the introduced parasite *Aphidius eadyi* (Hymenoptera: Aphidiidae) in the North Island of New Zealand, and its initial effect on pea aphid. N. Z. J. Zool. 8: 105-112.

Chmel, K., S. M. Kamga, T. Awa, F. L. Ewome, G. Uceda-Gómez, D. Hořák, J. Mlíkovský, L. L. Molua, J. Riegert, and Š. Janeček. 2021. Vertical stratification and seasonal changes of the avian community in Mount Cameroon lowland rainforest. Afr. J. Ecol.

Cingolani, M. F., and N. Greco. 2018. Spatio-temporal variation of strawberry aphid populations and their parasitoids. Appl. Entomol. Zool. 53: 205-214.

Costamagna, A. C., and D. A. Landis. 2011. Lack of strong refuges allows top-down control of soybean aphid by generalist natural enemies. Biol. Control 57: 184-192.

Cottrell, T. 2017. Trap Height Affects Capture of Lady Beetles (Coleoptera: Coccinellidae) in Pecan Orchards. Environ. Entomol. 46: 343-352.

Cottrell, T. E., B. W. Wood, and X. Ni. 2009. Chlorotic Feeding Injury by the Black Pecan Aphid (Hemiptera: Aphididae) to Pecan Foliage Promotes Aphid Settling and Nymphal Development. Environ. Entomol. 38: 411-416.

Dahlsten, D., R. Zuparko, A. Hajek, D. Rowney, and S. Dreistadt. 1999. Long-term sampling of *Eucallipterus tiliae* (Homoptera: Drepanosiphidae) and associated natural enemies in a northern California site. Environ. Entomol. 28: 845-850.

De Jiu, G., and J. Waage. 1990. The effect of insecticides on the distribution of foracing parasitoids, *Diaeretiella rapae* [Hym.: Braconidae] on plants. Entomophaga 35: 49-56.

Dean, G. 1973. Distribution of aphids in spring cereals. J. Appl. Ecol.: 447-462.

Di Giovanni, F., P. Cerretti, F. Mason, E. Minari, and L. Marini. 2015. Vertical stratification of ichneumonid wasp communities: the effects of forest structure and life-history traits. Insect Sci. 22: 688-699.

Dixon, A. 1969. Population dynamics of the sycamore aphid *Drepanosiphum platanoides* (Schr.)(Hemiptera: Aphididae): migratory and trivial flight activity. J. Anim. Ecol.: 585-606.

Dixon, A. F. G. 2005. Insect herbivore-host dynamics: tree-dwelling aphids. Cambridge University Press.

Edelson, J., and P. Estes. 1987. Seasonal abundance and distribution of predators and parasites associated with *Monelliopsis pecanis* Bissell and *Monellia caryella* (Fitch)(Homoptera: Aphidae). J. Entomol. Sci. 22: 336-347.

Fernandes, F. S., F. S. Ramalho, J. B. Malaquias, J. L. Nascimento Junior, E. T. Correia, and J. C. Zanuncio. 2012. Within-plant distribution of cotton aphid (Hemiptera: Aphididae) in cotton cultivars with colored fibers. An. Acad. Bras. Ciênc. 84: 707-721.

Hill, M. P., S. Macfadyen, and M. A. Nash. 2017. Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks. PeerJ 5: e4179.

Holland, J., L. Winder, and J. Perry. 2000. The impact of dimethoate on the spatial distribution of beneficial arthropods in winter wheat. Ann. Appl. Biol. 136: 93-105.

Kirstová, M., P. Pyszko, J. Šipoš, P. Drozd, and P. Kočárek. 2017. Vertical distribution of earwigs (Dermaptera: Forficulidae) in a temperate lowland forest, based on sampling with a mobile aerial lift platform. Entomol. Sci. 20: 57-64.

Littlefair, J. E., L. E. Hrenchuk, P. J. Blanchfield, M. D. Rennie, and M. E. Cristescu. 2021. Thermal stratification and fish thermal preference explain vertical eDNA distributions in lakes. Mol. Ecol. 30: 3083-3096.

McClure, M. S. 1982. Distribution and damage of two Pineus species (Homoptera: Adelgidae) on red pine in New England. Ann. Entomol. Soc. Am. 75: 150-157.

McGregor, B. L., A. E. Runkel, S. M. Wisely, and N. D. Burkett-Cadena. 2018. Vertical stratification of Culicoides biting midges at a Florida big game preserve. Parasites & vectors 11: 1-11.

Mizell III, R. F., and D. E. Schiffhauer. 1990. Effects of pesticides on pecan aphid predators *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), *Hippodamia convergens*, *Cycloneda sanguinea* (L.), *Olla v-nigrum* (Coleoptera: Coccinellidae), and *Aphelinus perpallidus* (Hymenoptera: Encyrtidae). J Econ Entomol 83: 1806-1812.

Mizell, R. F. 2007. Impact of *Harmonia axyridis* (Coleoptera: Coccinellidae) on native arthropod predators in pecan and crape myrtle. Fla. Entomol. 90: 524-536.

Oliveira, B. F., and B. R. Scheffers. 2019. Vertical stratification influences global patterns of biodiversity. Ecography 42: 249-249.

Ott, R. L., and M. T. Longnecker. 2015. An introduction to statistical methods and data analysis, Nelson Education.

Paulsen, C., T. Cottrell, and J. Ruberson. 2013. Distribution of the black pecan aphid, *Melanocallis caryaefoliae*, on the upper and lower surface of pecan foliage. Entomol. Exp. Appl. 146: 252-260.

Paulsen, C. M. 2011. The distribution of the black pecan aphid *Melanocallis caryaefoliae* (Homoptera: Aphididae) between the upper and lower surfaces of pecan foliage. University of Georgia.

Platková, H., P. Pyszko, A. Coeur d´ Acier, E. Jousselin, and P. Drozd. 2020. Spatial distribution of aphids in the canopy of a temperate forest: where can they be found? Agric. For. Entomol. 22: 379-389.

Procházka, J., L. Cizek, and J. Schlaghamerský. 2018. Vertical stratification of scolytine beetles in temperate forests. Insect Conserv. Diver. 11: 534-544.

Rice, N. R., M. W. Smith, R. D. Eikenbary, D. Arnold, W. L. Tedders, B. Wood, B. S. Landgraf, G. G. Taylor, and G. E. Barlow. 1998. Assessment of legume and nonlegume ground covers on Coleoptera: Coccinellidae density for low-input pecan management. AJAA. 13: 111-123.

Rissanen, A. J., S. Peura, P. A. Mpamah, S. Taipale, M. Tiirola, C. Biasi, A. Mäki, and H. Nykänen. 2019. Vertical stratification of bacteria and archaea in sediments of a small boreal humic lake. FEMS microbiology letters 366: fnz044.

Shapiro-Ilan, D. I., T. E. Cottrell, C. Bock, K. Mai, D. Boykin, L. Wells, W. G. Hudson, and R. F. Mizell III. 2017. Control of pecan weevil with microbial biopesticides. Environ. Entomol. 46: 1299-1304.

Shapiro–Ilan, D. I., T. E. Cottrell, M. A. Jackson, and B. W. Wood. 2013. Control of Key Pecan Insect Pests Using Biorational Pesticides. J. Econ. Entomol. 106: 257-266.

Šigut, M., H. Šigutová, J. Šipoš, P. Pyszko, N. Kotásková, and P. Drozd. 2018. Vertical canopy gradient shaping the stratification of leaf-chewer–parasitoid interactions in a temperate forest. Eco. Evo. 8: 7297-7311.

Tedders, W. L. 1978. Important biological and morphological characteristics of the foliar-feeding aphids of pecan. Department of Agriculture, Science and Education Administration, 1978. 1578-1587.

Wells, L. 2007. Pecan Physiology pp. 1-7. In L. Wells (ed.), *In, Southeastern Pecan Growers' Handbook.* University of Georgia Extension, Athens, United States.

Wells, L. 2017. Pecan: America's Native Nut Tree, University of Alabama Press.

Wells, L., and P. Conner. 2007. Southeastern Pecan Growers' Handbook.

Wood, B. W., W. L. Tedders, and J. D. Dutcher. 1987. Energy drain by three pecan aphid species (Homoptera: Aphididae) and their influence on in-shell pecan production. Environ. Entomol. 16: 1045-1056.

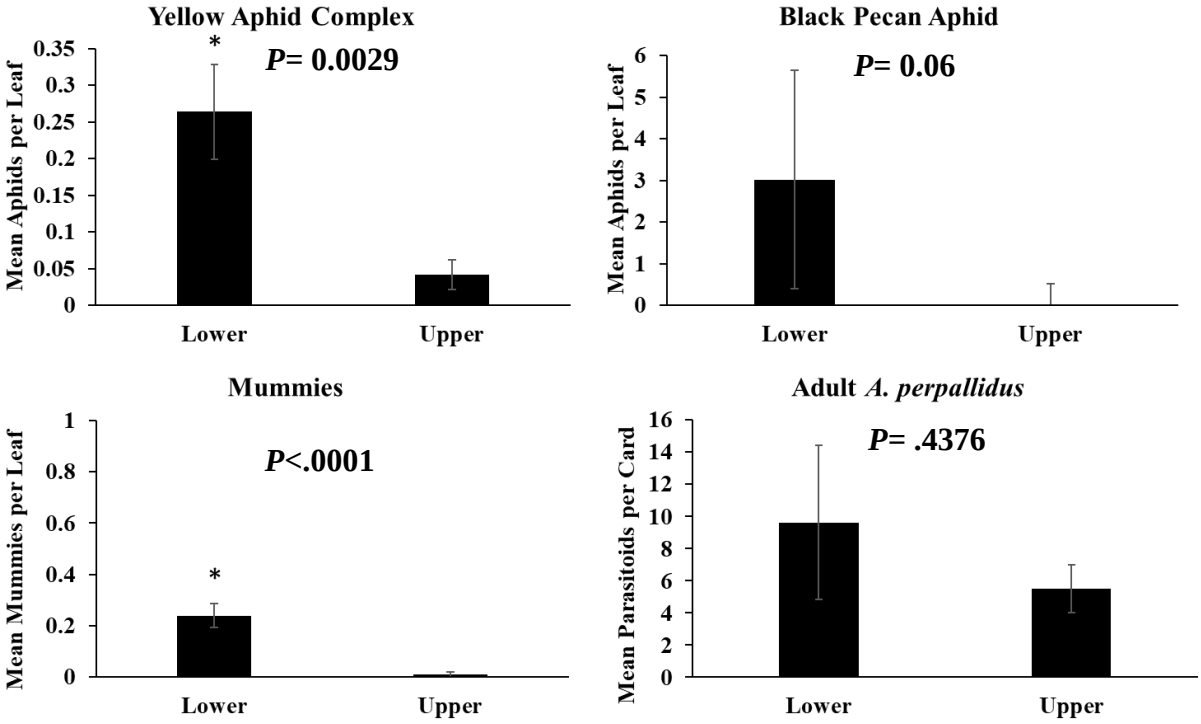

Figure 5.1. Mean number (±SEM) of aphids, aphid mummies and *A. perpallidus* adults surveyed in the lower (~6 m) and upper (~9 m) canopy of pecan trees at the commercial orchard in 2020. Presence of an asterisk designates a significant difference between the two canopy locations using Tukey's HSD at $\alpha = 0.05$.

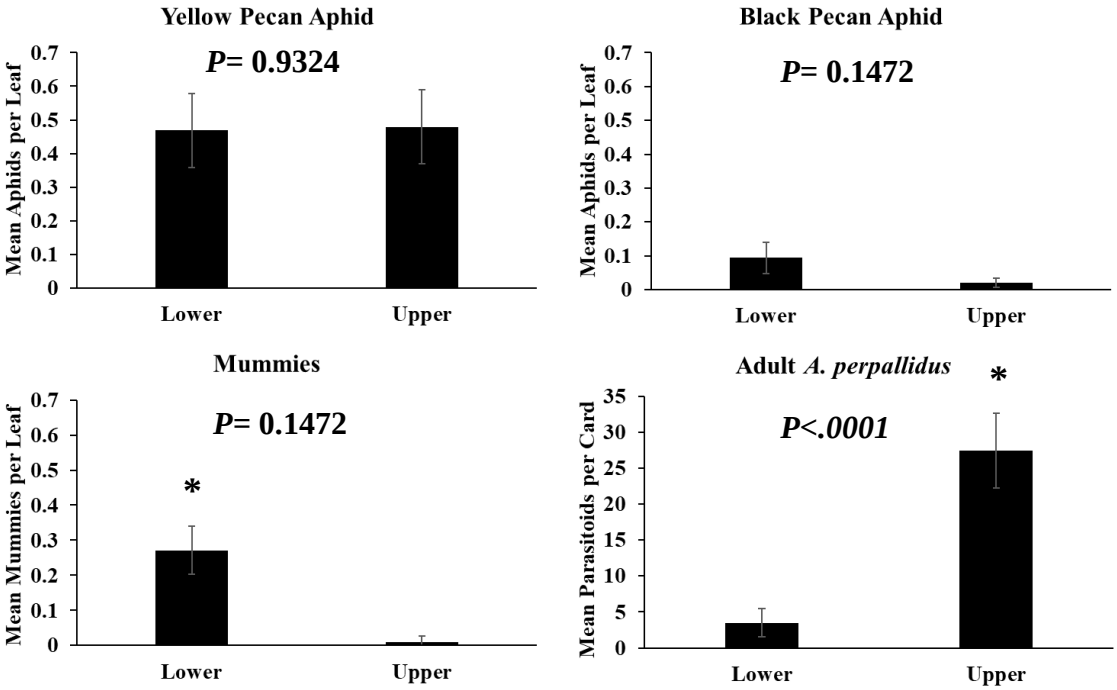

Figure 5.2. Mean number (±SEM) of aphids, aphid mummies and *A. perpallidus* adults surveyed in the lower (~6 m) and upper (~9 m) canopy of pecan trees at the commercial orchard in 2021. Presence of an asterisk designates a significant difference between the two canopy locations using Tukey's HSD at $\alpha = 0.05$.

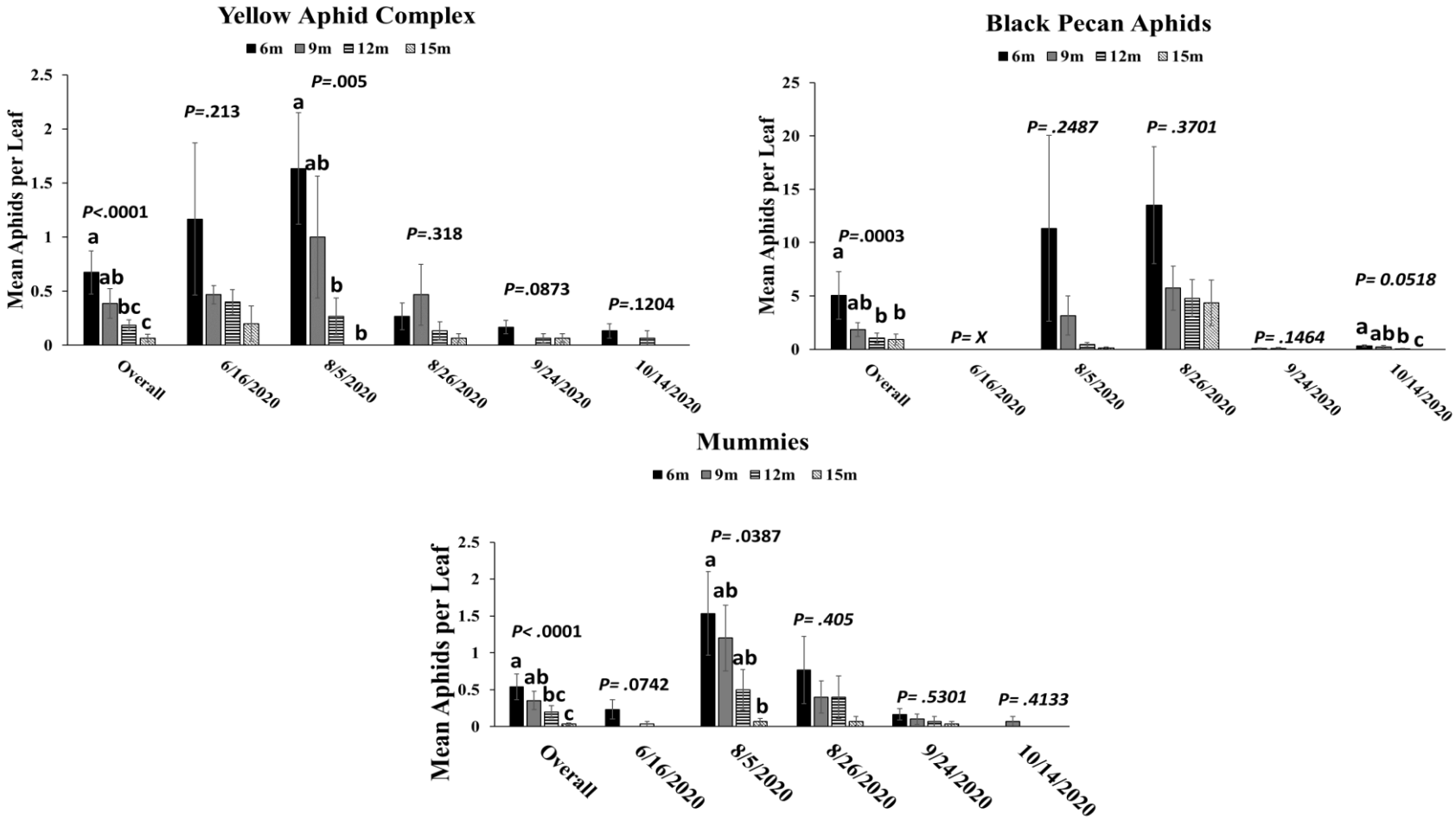

Figure 5.3. Mean number of aphids and mummies collected per leaf during each sampling date and throughout the whole season at the experimental orchard in 2020. Presence of differing letters designates a significant difference between the four canopy locations using Tukey's HSD at $\alpha = 0.05$.

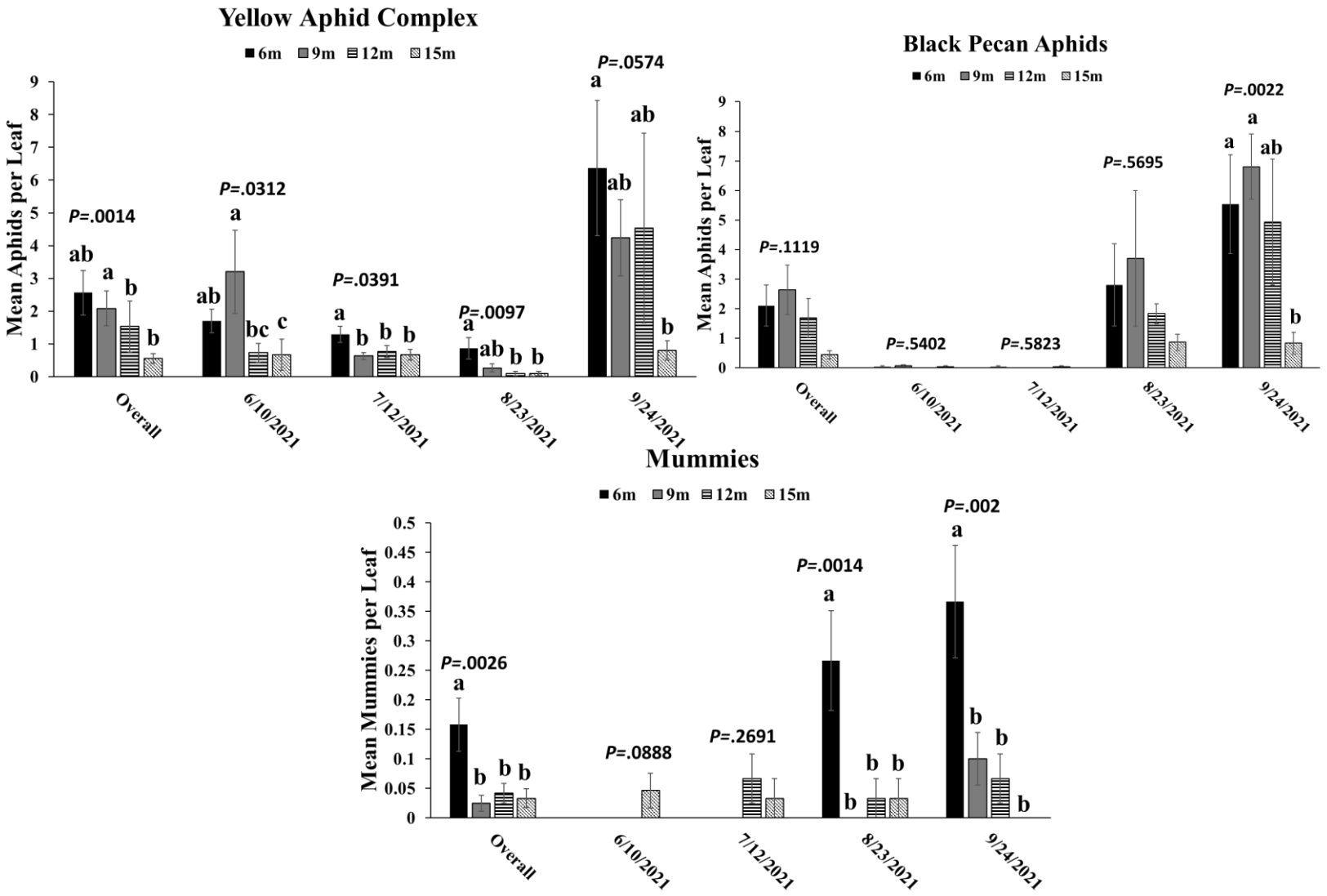

Figure 5.4. Mean number of aphids and mummies collected per leaf during each sampling date and throughout the whole season at the experimental orchard in 2021. Presence of differing letters designates a significant difference between the four canopy locations using Tukey's HSD at $\alpha = 0.05$.

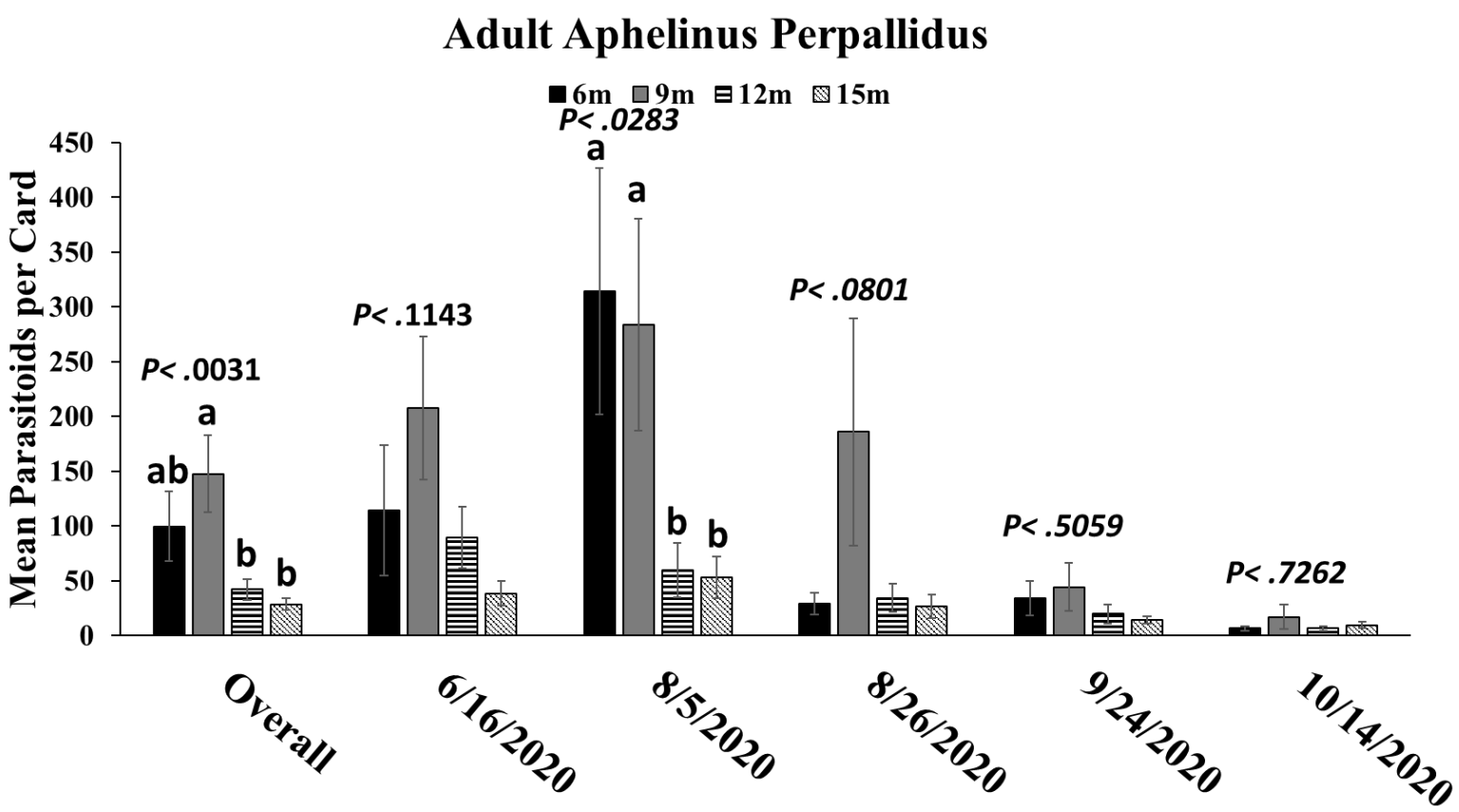

Figure 5.5. Mean number of *A. perpallidus* collected per card during each sampling date and throughout the whole season at the experimental orchard in 2020. Presence of differing letters designates a significant difference between the four canopy locations using Tukey's HSD at $\alpha = 0.05$.

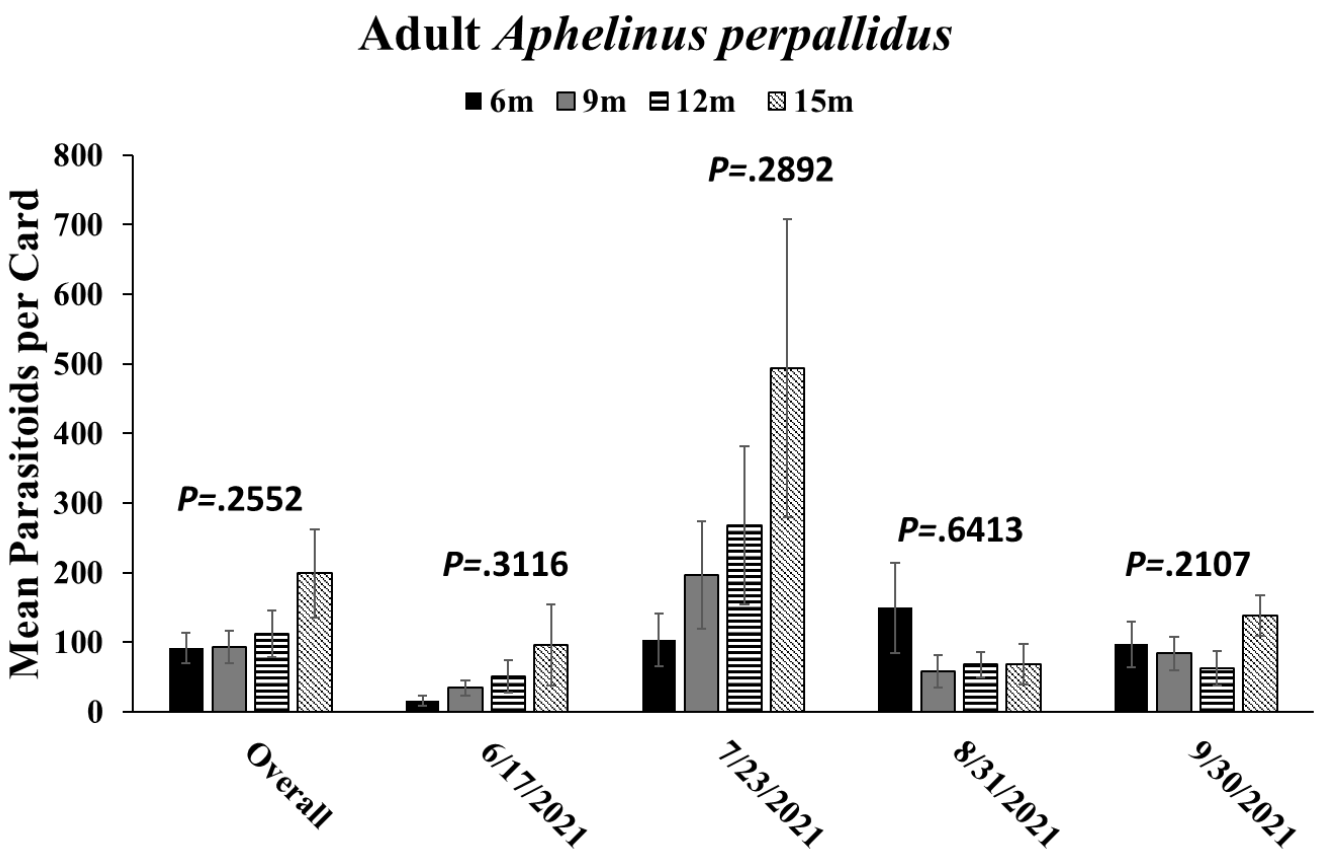

Figure 5.6. Mean number of *A. perpallidus* collected per card during each sampling date and throughout the whole season at the experimental orchard in 2021. Presence of differing letters designates a significant difference between the four canopy locations using Tukey's HSD at $\alpha = 0.05$.

Table 5.1. Spearman's multivariate analysis of aphid mummies versus aphids and *A. perpallidus* versus aphids according to height in the experimental orchard in 2020. Asterisks dictate significant difference based on $P = 0.05$

Comparisons	Canopy Height							
	6 m		9 m		12 m		15 m	
	Spearman's	*P*	Spearman's	*P*	Spearman's	*P*	Spearman's	*P*
Mummies x Yellow aphids	0.6179	0.0003*	0.3852	0.0356*	-0.0044	0.9818	0.0642	0.736
Mummies x Black aphids	-0.0427	0.8228	0.3595	0.051	0.2151	0.2537	0.2229	0.2364
A. perpallidus x Yellow aphids	0.596	0.0005*	0.5923	0.0006*	0.3739	0.0418*	-0.0359	0.8507
A. perpallidus x Black aphids	-0.2464	0.1893	0.1214	0.5229	0.0713	0.7082	0.0745	0.6956
A. perpallidus x Mummies	0.4256	0.019*	0.086	0.6515	0.1711	0.3661	-0.1435	0.4493

Table 5.2. Spearman's multivariate analysis of aphid mummies versus aphids and *A. perpallidus* versus aphids according to height in the experimental orchard in 2021. Asterisks dictate significant difference based on $P = 0.05$

Comparisons	Canopy Height							
	6 m		9 m		12 m		15 m	
	Spearman's	*P*	Spearman's	*P*	Spearman's	*P*	Spearman's	*P*
Mummies x Yellow aphids	0.2632	0.2139	0.2194	0.3029	0.3760	0.0702	0.3787	0.0680
Mummies x Black aphids	0.8746	<.0001*	0.4694	0.0207*	0.1902	0.3734	-0.1058	0.6228
A. perpallidus x Yellow aphids	0.4046	0.0499*	-0.2206	0.3003	0.1578	0.4615	0.2390	0.2608
A. perpallidus x Black aphids	0.0999	0.6422	-0.2581	0.2233	0.2195	0.3027	-0.1168	0.5869
A. perpallidus x Mummies	0.1028	0.6325	0.2093	0.3263	0.2372	0.2643	-0.2100	0.3246

CHAPTER 6

SUMMARY/EXTENSION

The work detailed in this dissertation was not only designed and implemented to provide information to the scientific community but was also done in order to benefit pecan growers and other interested stakeholders. In an uncertain world of insecticide resistance, climate change, economic uncertainty, and the constant movement of species across borders, scientist are often tasked with testing and supplying new tools for the grower's pest management toolbox. The hope is that the information gathered during the preparation of this dissertation will prove beneficial in pecan aphid management for growers. If not, at least it will establish a baseline for future research to build upon, and thus, develop future studies that will help the pecan growing community. In this chapter, we will highlight some of the finding of the previous studies and how these can be useful for growers in practical situations.

Multisite Seasonal Monitoring of Pecan Aphids and Their Parasitoid in Commercial Pecan Orchards

In this study, we quantified pecan aphids, mummies, and adult *A. perpallidus* at four Georgia commercial orchards with varying types of management tactics in 2019 and 2020. We took these samples from May – November which is the typical pecan season for Georgia growers (Wells and Conner 2007). The goal of this study was to examine some of the differences in seasonal aphid and parasitoid populations across orchards with different grower ideologies and philosophies on pest management.

Both the yellow pecan aphid complex and the black pecan aphid populations followed trends described in previous research (Tedders 1978; Dutcher 2010). Yellow pecan aphids

experienced populations peaks in May, June, July, September, and October while black pecan aphids peaked in late September and October. Thus, scouting efforts should focus on these particular aphid species during these times of the year.

Comparison of our sites revealed there were few consistent trends among the four sites examined in our study (Figure 2.2). For example, black pecan aphids were more abundant in Albany in 2019 while in 2020 they were more abundant in Marshallville (Figure 2.2). This highlights that growers may experience inconsistent pest populations from year to year. This means that growers should emphasize finding a good scout who can assess year to year and month to month in order to make sustainable choices on when to manage pest.

One trend that was consistent year to year was the low numbers of both yellow and black pecan aphids across sites. Despite these low numbers, some growers treated for aphids often throughout the growing season, while some growers only treated once (Table 2.2). The interesting outcome of this, was that the growers who treated less still had aphid number far below threshold (Acebes and Hudson 2019). This indicates that the growers spraying multiple times a year for aphids maybe applying unnecessary treatments, potentially wasting money and resources on aphid management. This was especially true for black aphids, which were being treated for at a couple sites, despite being present in numbers much lower than the threshold presented in the pecan aphid spray guide (Acebes-Doria and Hudson 2019).

A. perpallidus were most abundant at sites that applied insecticides less. This may provide evidence for non-target effects of insecticide/fungicides on *A. perpallidus*. However, further work would need to be done to provide support for non-target effects. It could be possible that *A. perpallidus* numbers may be low in these areas naturally.

Overall, this chapter provided a valuable insight into the aphid populations and parasitoid populations at just a few commercial pecan orchards in Georgia. It is important for growers to assess their fields and make management decisions on frequency of insecticide usage. Based off the results of this study, it appears that insecticide usage can be mitigated in most Georgia orchards. This can save growers time and money while preserving the surrounding environment.

Molecular Unraveling of Parasitoids and Hyperparasitoids Associated with Pecan Aphid

Use of genetic characterization to understand the members of pest-parasitoid food-webs can be a valuable tool for assessing potential issues with biological control agents. Relatively few cropping systems have had their pest-parasitoid systems characterized by use of molecular analysis despite the increased resolution that molecular analysis can provide compared to traditional rearing methods (Lefort et al. 2017; Sigut et al. 2017; Taberlet et al. 2018). We used molecular technology to define the pecan aphid-parasitoid-hyperparasitoid food web using both mummies and whole parasitoids collected from Georgia pecan orchards. Based on the results of this analysis we identified three species of aphid (yellow pecan aphid, blackmargined aphid, and black pecan aphid), one primary parasitoid (*A. perpallidus*), and five species of hyperparasitoids consisting of two pteromalid species, two figitid species, and one species of Signophorid (Table 3.1). This was the first time these interactions were characterized in the southeast. Analysis of the preliminary trophic interaction between these species reveal a potential issue for *A. perpallidus* as a biological control agent. The hyperparasitoids found in this study are all members of groups that are primarily generalist hyperparasitoids of aphid parasitoids. Due to the generalist life style of these hyperparasitoids, they are often associated with more than one cropping system. This means that hyperparasitoids maybe able to move in from other crops and reduce *A. perpallidus* populations as seen in previous work (Gomez-Marco et al. 2015; Lefort et

al. 2017). Growers who are interested in biocontrol and preserving their natural enemies may want to be aware of what crops are around their fields as they could potentially harbor hyperparasitoid populations that can move into the orchard. In addition, growers can monitor parasitoid populations in the field by either collecting mummies and hatching them out to look for hyperparasitoids or have them assessed genetically. This may be able to help growers assess the health of their primary parasitoids in the field. By understanding the biological diversity of their crop fields growers can potentially develop more sustainable agricultural techniques by working with the natural system in their orchard.

Effects of Aphicides on Pecan Aphids and Their Parasitoids in Pecan Orchards

Insecticide resistance is a major issue in pest management, especially in aphids where frequent insecticide resistance is documented (Tang et al. 2017, Ullah et al. 2020, Zu et al. 2019). This is often the result of over-reliance or incorrect usage of a few insecticides that leads to these resistant populations (Tang et al. 2017, Ullah et al. 2020, Zu et al. 2019). Therefore, it is critical for researchers to assess different insecticides for potential resistance. In addition, it is also important to assess non-target effects on natural enemies such as lacewings, ladybeetles, and parasitoids. Non-target effects can lead to issues such as secondary pest outbreaks or resurgence of the target pest in the absence of natural enemies (Shapiro-llan et al., 2017). In this study, we assessed the effects of three aphicidal products on the abundance of pecan aphids, mummies, and adult *A. perpallidus*: flonicamid 207.01 mL/ha (Carbine©, 857.3 mL a.i/liter, FMC Corporation, Philadelphia, PA, USA), sulfoxaflor 203.3 mL/ha (Closer©, 3429.2 g a.i./liter, Corteva Agriscience, Wilmington, DE, USA), low rate afidopyropen 221.8 mL/ha (Sefina©,720.12 g a.i./liter, BASF Ag Products, Research Triangle Park, NC, USA), and high rate afidopyropen

443.6 mL/ha. In addition, we also assessed the effects of these products on aphids and their parasitoids in the upper and lower canopy of mature pecan trees.

The results of this study provide evidence for the effectiveness of these products on pecan aphids. All three aphicides had significantly lower aphid populations than the non-treated control 7-days post-application in both years of the study (Table 4.1 and 4.4). This seems to indicate that growers have multiple products that they can use to manage aphid populations in pecans. In addition, all of these products have unique modes of action and are members of different Insecticide Resistance Action Committee (IRAC) classifications (Spark and Nauen 2015). This means that these insecticides can be used as part of a treatment rotation which can potentially mitigate insecticide resistance. However, the differences between the treated and control trees decreased 14 and 21-days post application in both years (Table 4.1 and Table 4.4). This means that growers may want to increase scouting during this time period in order to look for rebounds in aphid populations in order to determine if additional treatment needs to be applied.

In addition, neither *A. perpallidus* adults nor mummies were significantly different between the control and the treated trees in either year of the study (Table 4.3 and Table 4.6). This provides evidence for low to no non-target effects of these aphicides on *A. perpallidus* populations. This provides support to previous literature which report minimal to no non-target effects of these products on natural enemies (Joseph 2020; Koo et al. 2014; Nauen et al. 2019). This was one of the few studies to assess non-target effects on natural enemies in a field setting.

We also assessed canopy height on spray effects, as previous research has shown that spray coverage decreases as height increases (Bock et al. 2015). While we did not see any significant interaction between height and spray effects, we did see differences in the number of aphids, mummies, and adult *A. perpallidus* between canopies. Aphids were significantly more

abundant in the lower canopy at 21-days in 2019 and all three sample dates in 2020 (Table 4.4). Mummies were more abundant in the lower canopy during every assessment period except the 7-day in 2019 (Table 4.2). Adult parasitoids only differed significantly in the upper canopy during the 14-21-day sampling period in 2019 (Table 4.3). This information indicates that growers may find aphids in greater abundance in the lower canopy. This can be beneficial as spray coverage can be better in the lower canopy compared to the upper canopy (Bock et al., 2015). The presence of more adult *A. perpallidus* in the upper canopy is also good news. This means that these adult parasitoids are still present in the upper canopy where they can provide biological control in areas where insecticide might not provide adequate coverage.

Effects of Vertical Stratification on Aphids and Their Parasitoids

In this chapter, we assess the effects of vertical stratification on pecan aphids and their parasitoids in Georgia pecan orchards. Vertical stratification can be a major factor in where pecan aphids and their parasitoids maybe found as the quality of habitat, food, and climate can change significantly between the upper and lower canopies of mature pecan trees (Costamagna and Landis 2011; Dixon 2005; Platkova et al. 2020). In addition, pecan trees grow throughout their lives sometimes exceeding 30 m in height which can further alter these communities by providing more canopy structure (Wells, 2017). In this study, we assessed pecan aphid, mummy and parasitoid populations at different canopy heights in shorter trees (~9 m) at a commercial orchard and taller trees (~15 m) in an experimental orchard. We found that while aphids, mummies, and *A. perpallidus* were not consistently dense in one canopy over the others, they tended to be most dense in the lower canopy of the shorter trees in the commercial orchard and the lower half of the canopy (6-9 m) in the taller trees at the experimental orchards. The only exception to this was that *A. perpallidus* adults were more abundant in the upper canopy in the

commercial orchard in 2021 (Figure 5.2). The results of this study indicate that growers should focus scouting efforts on the lower canopy where pecan aphids are more likely to congregate due to ideal habitat conditions. In addition, *A. perpallidus* can still be found even at 15 m up the canopy meaning that they can still provide biological control in areas where insecticides may not be properly applied.

The results of this dissertation have added new knowledge to the understanding of pecan aphid and *A. perpallidus* ecology. Hopefully, growers and extension agents can find some valuable information in the preceding chapters and use it to improve the sustainability of their orchards. While this dissertation answered many questions, it raised just as many. Hopefully these chapters are just the starting point for an even greater wealth of knowledge waiting to be uncovered.

LITERATURE CITED

Acebes-Doria, A. L., and W. G. Hudson. 2019. 2020 Commercial Pecan Spray Guide. Univ. Georgia Coop. Ext. Pub. 841. Tifton, GA.

Bock, C. H., M. W. Hotchkiss, T. E. Cottrell, and B. W. Wood. 2015. The Effect of Sample Height on Spray Coverage in Mature Pecan Trees. Plant Dis. 99: 916-925.

Costamagna, A. C., and D. A. Landis. 2011. Lack of strong refuges allows top-down control of soybean aphid by generalist natural enemies. Biol. Control 57: 184-192.

Dixon, A. F. G. 2005. Insect herbivore-host dynamics: tree-dwelling aphids, Cambridge University Press.

Dutcher, J. D., H. Karar, and M. G. Abbas. 2010. Effectiveness of two insecticides for control of foliage feeding pecan aphids. Arthropod Manag. Tests. 35: 1-2.

Joseph, S. V. 2020. Effect of Afidopyropen against Myzus persicae (Hemiptera: Aphididae) and Its Predator, Adalia bipunctata (L.) in a Greenhouse. J. Entomol. Sci. 55: 584-587.

Koo, H.-N., J.-J. An, S.-E. Park, J.-I. Kim, and G.-H. Kim. 2014. Regional Susceptibilities to 12 Insecticides of Melon and Cotton Aphid, Aphis gossypii (Hemiptera: Aphididae) and a Point Mutation Associated with Imidacloprid Resistance. Crop Prot. 55: 91-97.

Lefort, M. C., S. Wratten, A. Cusumano, Y.-D. Varennes, and S. Boyer. 2017. Disentangling higher trophic level interactions in the cabbage aphid food web using high-throughput dna sequencing. MBMG: e13709.

Nauen, R., R. Slater, T. C. Sparks, A. Elbert, and A. Mccaffery. 2019. IRAC: Insecticide Resistance and Mode-of-Action Classification of Insecticides, pp. 995-1012, Modern Crop Protection Compounds, vol. 3. Wiley-VCH.

Platková, H., P. Pyszko, A. Coeur d´ Acier, E. Jousselin, and P. Drozd. 2020. Spatial distribution of aphids in the canopy of a temperate forest: where can they be found? Agricultural and Forest Entomology 22: 379-389.

Šigut, M., M. Kostovčík, H. Šigutová, J. Hulcr, P. Drozd, and J. Hrček. 2017. Performance of DNA metabarcoding, standard barcoding, and morphological approach in the identification of host–parasitoid interactions. PLOS One 12: e0187803.

Shapiro-Ilan, D. I., T. E. Cottrell, C. Bock, K. Mai, D. Boykin, L. Wells, W. G. Hudson, and R. F. Mizell III. 2017. Control of pecan weevil with microbial biopesticides. Environ. Entomol. 46: 1299-1304.

Sparks, T. C., and R. Nauen. 2015. IRAC: Mode of Action Classification and Insecticide Resistance Management. Pestic. Biochem. Phys. 121: 122-128.

Taberlet, P., A. Bonin, L. Zinger, and E. Coissac. 2018. Environmental DNA: For biodiversity research and monitoring, Oxford University Press.

Tedders, W. 1978. Important biological and morphological characteristics of the foliar-feeding aphids of pecan. USDA Technical Bulletin. 1579. Hyattsville, MD.

Tang, Q.-L., K.-S. Ma, Y.-M. Hou, and X.-W. Gao. 2017. Monitoring Insecticide Resistance and Diagnostics of Resistance Mechanisms in the Green Peach Aphid, Myzus persicae (Sulzer)(Hemiptera: Aphididae) in China. Pestic. Biochem. Phys. 143: 39-47.

Ullah, F., H. Gul, K. Tariq, N. Desneux, X. Gao, and D. Song. 2020. Functional Analysis of Cytochrome P450 Genes Linked with Acetamiprid Resistance in Melon Aphid, Aphis gossypii. Pestic. Biochem. Phys. 170: 104687.

Wells, L., and P. Conner. 2007. Southeastern Pecan Growers' Handbook.

Zhu, Y.-L., F. Yang, Z.-W. Yao, Y.-K. Wu, B. Liu, H.-B. Yuan, and Y.-H. Lu. 2019. A Molecular Detection Approach for a Cotton Aphid-Parasitoid Complex in Northern China. Sci. Rep. 9: 1-8.