BENEFICIAL INSECTS IN SOUTHEASTERN PEACHES: ELUCIDATING EFFECTS OF MANAGEMENT ON NATURAL ENEMIES AND POLLINATORS

by

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(Under the direction of Jason Schmidt and Brett Blaauw)

ABSTRACT

Peaches are a valuable crop in the southeastern USA. Despite the popularity of peaches, little is known about the communities of beneficial arthropods in orchards that may contribute ecosystem services such as biological control and pollination. Peaches also experience heavy pest pressure. To combat this pest pressure, commercial growers manage pests with broad spectrum pesticides. These broad spectrum pesticides may negatively affect communities of beneficial arthropods and their corollary ecosystem services. Some growers chose to manage peach pests with a greater IPM emphasis by using fewer, less intense, or organic pesticides in an effort to conserve beneficial arthropods. This study aims to catalog the abundance, diversity and spatiotemporal activity of communities of beneficial arthropods in peach orchards under different management regimens. Ultimately, this project will elucidate how management alters these communities, providing invaluable data for developing future IPM programs.

INDEX WORDS: Integrated pest and pollinator management (IPPM), ecosystem services, pollination, biological control, molecular gut-content analysis (MGCA)

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DEDICATION

To my parents, professors, and all other teachers I’ve had.

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Overview

Peaches (*Prunus persica)* are a perennial crop now grown around the world (Fideghelli et al. 1997; Hans, Shah, and Bansal 2020; Luo et al. 2022). Peach production requires certain climatic conditions (Parker and Abatzoglou 2019), and are normally grown in warmer temperate climates, between latitudes 30-45° North and South (Hancock, Scorza, and Lobos 2008; Hans, Shah, and Bansal 2020). In Georgia and South Carolina alone, there are more than 24,800 acres of orchards, producing over 122,000 tons of peaches, valued at over $170 million (USDA 2022). However, the humid conditions of the southeast also lead to heavy pest pressure, and key pests and pest control recommendations are well-documented. Bud to harvest is from roughly March through July in the Southeastern US, however peaches are managed year-round to combat pests and disease during dormancy (Blaauw et al. 2022). To combat pest pressure, growers rely on calendar sprays of chemical pesticides and fungicides (Johnson et al. 2002; Blaauw et al. 2022). However, these chemicals may negatively affect delivery of naturally occurring ecosystem services such as biological control and pollination (Tylianakis and Binzer 2014; Crowder and Jabbour 2014; Bengtsson 2015).

Despite the popularity and value of peaches in the southeast, little is known about the community of beneficial arthropods and how management may affect them. Increased biological diversity of natural enemies leads to greater pest suppression (Griffin, Byrnes, and Cardinale 2013). Identifying these communities of beneficial taxa and understanding their spatiotemporal activities is an important step toward a comprehensive integrated pest management (IPM) program (Crowder and Harwood 2014; Le Gal et al. 2020). Promoting and preserving beneficial taxa leads to increased ecosystem services and significant economic value of over $57 billion in the USA (Losey and Vaughan 2006). There are many considerations for how these estimations are reached and at what scale (Chee 2004; Hein et al. 2006), but conserving beneficial taxa remain a key aspect.

Because peaches are valuable and have low economic damage thresholds, growers currently rely on calendar chemical applications (Popp and Scherm 2001; Jones et al. 2009). Unfortunately, chemical sprays alter beneficial taxa communities and their corollary ecosystem services (Krauss, Gallenberger, and Steffan-Dewenter 2011; Crowder and Jabbour 2014). When spatiotemporal data are combined with diversity and abundance data, management of peaches could be amended to promote or preserve key taxa. The goal of this study is to elucidate the spatiotemporal activity of the key taxa in southeastern peaches that may contribute to pollination and biological control. To accomplish this task, pan traps and tree beats were used to capture and estimate the beneficial community. To quantify biological control contributions, molecular gut content analysis of canopy predators was used along with spatiotemporal activity.

Overview of pests

Pest pressure on peaches varies across the US and throughout the year. Like the southeast, California is also a massive producer of peaches with over 500,000 tons produced in 2021 (USDA 2022). One major difference in peach production is the difference in pest pressure stemming from climatic differences in the eastern US compared to the west. The arid conditions in the west reduces disease pressure and has less insect pest pressure compared to the more humid east (Jones et al. 2009). For example, plum curculio, *Conotrachelus nenuphar* (Curculionidae), pressure ranges throughout the US. Plum curculio is only present east of the Rocky Mountains. It has one generation in northern states, and is multivoltine in southern states (Chapman 1938; Zhang et al. 2008). Another example is the peach tree twig borer, *Anarisa* *lineatella* (Gelechiidae). While present throughout the US, peach tree twig borers are rarely cause for concern in eastern peach production, while they are considered a serious pest west of the Rocky Mountains (Horton et al. 2005; Horton, Fuest, and Cravedi 2008). Most of the difficulty in controlling pests and developing an IPM program comes from natural enemy compatibility with management. Historically, broad spectrum insecticides such as organophosphates have been used to control primary pests. Broad spectrum insecticide toxicity reduced natural enemy's capacity for control, giving rise to secondary pests (Jones et al. 2009; Agnello et al. 2009; Biddinger, Leslie, and Joshi 2014; Hill, Macfadyen, and Nash 2017). Chemical control is necessary, but finding the right management program that does not disrupt natural enemy contributions is one of the fundamental aspects of IPM (Stern et al. 1959). This highlights the importance of finding the balance of chemical and biological control. For this project, we focus on the four primary insect pests in the southeastern peach orchards (Ames 2012; Knutson, Ong, and Ree 2019; Blaauw et al. 2022). Here, we introduce the biology and management for these primary pests of southeast (SC and GA) peach systems.

Plum curculio

Plum curculio, *Conotrachelus nenuphar* Herbst (Curculionidae), are a considerable pest of stone and pome fruits such as apple, peach and plum in Eastern North America. Plum curculio have two generations in the southeast, first generation are present from petal fall through late April, second generation are present from late May into July (Chapman 1938; Zhang et al. 2008). Adults are small darkly colored black/brown with jagged bumps on their elytra. Larvae are small white/cream colored grubs with a brown or reddish head. Adults overwinter in leaf litter in and around the orchard, and move into the orchard and begin feeding and mating during bloom (Chapman 1938). Adults create "C" shaped injuries in the fruit and lay their eggs inside, and eggs hatch two to 12 days later (Ames 2012). From there the developing larvae eat toward the center of the fruit. This feeding causes the fruit to become gnarled, scarred, and bumpy, which impacts the marketability and yield for growers. Once larvae are fully grown in the fourth instar, larvae exit the fruit and drop to the ground where they will pupate (Akotsen-Mensah, Boozer, and Fadamiro 2012). The larvae can also release pectin enzymes that cause the fruit to drop prematurely, where they exit the fruit directly into the ground where they will pupate (Ames 2012).

The life cycle of the plum curculio makes them difficult to control with biological control alone, as many of their vulnerable life stages are out of sight. Chemical management for plum curculio begins by targeting overwintering generation around petal fall and shuck split. Multiple pyrethroid sprays allow for residual control of emerging plum curculio. Organophosphates can also be used in rotation to combat any pyrethroid resistance. Using a degree day calculator, one can estimate when the second generation will emerge, which is generally around June. This new emergence can be targeted with rotations of pyrethroid and organophosphate sprays (Horton et al. 2005; Blaauw et al. 2022).

There are also cultural management practices to mitigate damage from plum curculio, however, these practices alone are not enough to control their population. Akotsen-Mensa et al. (2012) also found that weed management/ substrate ground cover may influence emergence of PC after pupation, and found centipede grass to have the lowest emergence rate over natural weeds, pine bark, and bare ground. Another practice is removing fruit that drops to the ground, which may reduce the number of larvae that emerge and pupate. To target the pupae, the soil around the infested trees can be tilled or cultivated to mechanically destroy the pupae, however this practice may result in soil damage and erosion (Ames 2012).

There is evidence natural enemies of the plum curculio contribute to some biological control. Some of the most abundant predators known to prey on PC on the ground are ground beetles (*Carabidae*), ants (*Formicidae*), and spiders (Araneae) (Jenkins et al. 2006; Akotsen-Mensah, Boozer, and Fadamiro 2012). Schmidt et al. (2016) monitored biological control of plum curculio in apple orchards using MGCA and found that several taxa across different orders fed on the plum curculio. Screened taxa that tested positive for plum curculio DNA included Hemiptera, Coleoptera, and Araneae- which is particularly interesting as they all feed with different mouth part mechanisms. This highlights the importance of generalist predators contributing to biological control (Schmidt, Szendrei, and Grieshop 2016).

San Jose scale

San Jose scale, *Comstockaspis pernicious* Comstock (Diaspididae) (referred to as SJS in this document) are present throughout the US, and are a serious pest to tree fruits such as peaches, nectarines, plums, apples, cherries and more (Blaauw et al. 2022). SJS are small piercing/sucking insects that feed on the phloem sap of their host tree. SJS have a high reproductive potential, having approximately four generations a season, and females may produce 10 offspring per day for 2-3 weeks at a time (Blaauw 2021). SJS may anchor themselves anywhere on their host tree including the trunk, limbs, leaves and fruit (Gentile and Summers 1958). The adult females are immobile, and secrete a wax coating over themselves so they may feed undisturbed. Adult males are mobile, very small, two-winged flying insects. The mobile stage of the immature SJS is known as a ‘crawler’. SJS crawlers are yellow/green and very small. Immature crawler abundance within the orchard can be monitored with sticky tapes on tree limbs (Rychla 2014). Once a set threshold is reached, reactionary in-season sprays can be applied to combat this vulnerable stage of the scale (Blaauw 2021).

Although they were considered a secondary pest in the southeast, their prevalence and their potential damage are cause for consideration as a primary pest requiring intensive management (Blaauw 2021).The most common management of SJS is two dormant horticultural oil sprays (Blaauw et al. 2022; Pless, Deyton, and Sams 1995).These oils are applied in the off season when peach trees are dormant, so as not to damage fruits and leaves of the trees. These oils coat the trees and scales, smothering them. As mentioned above, the immature crawler stage of the scale can be combated with a contact insecticide or an insect growth regulator (Blaauw 2021). Cultural management of scales is also necessary in cases of infestation. When entire limbs of the tree are covered in scales, these limbs can be pruned and burned. Additionally, proper pruning can open up the canopy for more complete spray coverage (Blaauw et al. 2022; Pless, Deyton, and Sams 1995).

There have been several success stories of the biological control of various scale species. Michaud et al. (2012) detail examples of coccinellid specialists and generalists on several varieties of scales and mealybugs, including introductions to the California citrus industry, in various crops in tropical and subtropical regions, and in the Middle East and Africa (Michaud 2012). One study from apple trees in India found that a hymenopteran parasitoid, *Encarsia perniciosi* (Aphelinidae), and a beetle, *Coccinella infernalis* (Coccinellidae), had some success in reducing damage from SJS (Gupta 2005). There is also evidence that generalist predators can contribute to biocontrol of the scale *Aonidiella aurantii* Maskell (Diaspididae) biocontrol. These generalists include Araneae, Coleoptera, Neuroptera, and Dermaptera (Bouvet et al. 2019). There are fewer studies on the biological control of the SJS, as it is widely accepted that biological control alone is not enough to suppress their population. However, this study will reveal novel information about effective predators for scales in southeastern peaches.

Lepidoptera

The most common lepidopteran pests for southeastern peaches are the oriental fruit moth (Tortricidae: *Grapholita molesta*), peach tree borer (Sesiidae: *Synanthedon exitiosa*), and lesser peach tree borer (Sesiidae: *Synanthedon pictipes*) (Agnello et al. 2009; Blaauw et al. 2022). Different lepidopteran pests feed on different parts of the tree, for example, oriental fruit moth larvae feeding directly on the buds, leaves, or fruit, and peach tree borer and lesser peach tree borer larvae feeding on the inner layers of bark and on the roots of the tree (Myers, Hull, and Krawczyk 2014; Cottrell, Fuest, and Horton 2008; Shapiro-Ilan et al. 2015). Oriental fruit moth will often attack the shoots of the peach tree (Allen 1958). Shoots are the new growth of limbs, leaves, flower stems and buds. The young shoots are more vulnerable to attack as this new growth has not developed secondary cell walls or any tough fiber, making the new growth easier to digest for the pest (Ames 2012). Peach borers will also exploit mechanical damage to the tree, and oviposit their eggs into the cracks in the trunk, where the larvae will feed on the inner layers of bark and roots (Cottrell, Fuest, and Horton 2008; Shapiro-Ilan et al. 2015).

There are different recommended treatments depending on what time in the season one is managing for lepidopteran pests. Pesticides are applied after the tree's flower and petal drop to avoid exposing pollinators to these chemicals (Blaauw et al. 2022). However, it is important to treat the trees when the various lepidopteran pests are vulnerable (Trimble, Pree, and Carter 2001; Blaauw et al. 2022). Pyrethroids or organophosphates are applied when petals start to drop to control oriental fruit moth attacking new growth (Atanassov, Shearer, and Hamilton 2003; Blaauw et al. 2022). Diamides are the most effective spray management against most immature lepidopteran pests. Larvae that come in contact with the spray are paralyzed and will die within 1-3 days. Immature caterpillars will also die if they consume treated plant material (Blaauw et al.

2022). The life cycle of the peach tree borer and lesser peach tree borer make them difficult to kill via direct exposure once they are already under the bark. Physical barriers such as plastic wrap or paint on the trunk may be an option for peach tree borers, inhibiting larvae from entering the trunk of the tree, but there is only anecdotal evidence supporting this (Ames 2012). For adults, mating disruption pheromones work well, but are not fully reliable if used without supplemental insecticide sprays (Atanassov, Shearer, and Hamilton 2003; Blaauw et al. 2022).

Biological control of lepidopteran peach pests can be difficult due to how the larvae feed. The oriental fruit moth larvae burrow inside the new shoots and work their way inside the fruit, feeding near the pit. And peach tree borers oviposit inside cracks in the trunk, where larvae feed on inner layers of bark and roots. There has been success in controlling both pear tree borers using nematodes applied to the soil surrounding trees, as these microscopic organisms are able to attack larva "out of sight" (Cottrell, Fuest, and Horton 2008; Shapiro-Ilan et al. 2015). Oriental fruit moth can be attacked by ground predators such as spiders and carabids when pupating or emerging as an adult (de Roince et al. 2012). Other natural enemies such as parasitoids (Braconidae, Ichneumonidae, other Chalcidoidea), coccinellids, anthocorids and chrysopids will attack eggs (Atanassov, Shearer, and Hamilton 2003; Zhang et al. 2021).

Catfacing Insects

A catfacing insect is a hemipteran insect with piercing/sucking mouthparts. Plant bugs and stink bugs, as well as other Heteroptera, make up the majority of the catfacing insect complex (Blaauw et al. 2022). Catfacing insects feed on the phloem sap, leaves, or directly on the fruit of peaches, which results in deformed, scarred, or dimply peaches that reduces marketability of the fruit (Blaauw, Polk, and Nielsen 2015). The Brown Marmorated Stink Bug

(BMSB) *Halyomorpha halys* (Stål) (Pentatomidae), is the primary catfacing insect pest for peaches.

BMSB are a widespread pest in the US since their introduction from Asia, first discovered in the US in 2001 (Hoebeke and Carter 2003). BMSB has over 170 host plants including many species of tree fruit, resulting in serious efforts by researchers and extension agents to combat the invasion (Ludwick et al. 2020). BMSB have 1-2 generations in the US, depending on photoperiod and temperature, and are likely multivoltine in the warmer southeastern US (Nielsen, Hamilton, and Matadha 2008). Females lay their eggs on the underside of leaves and have five instars before reaching their adult stage (Nielsen, Hamilton, and Matadha 2008; Rice et al. 2014). BMSB emerge in May and are present throughout the season through harvest. Mobile stages are capable of feeding on the host plant, and they are able to complete their entire development in peaches (Blaauw, Polk, and Nielsen 2015). These attributes make them a particularly devastating pest in peaches.

Insecticides are the primary way growers combat BMSB and other catfacing insects. Calendar-based sprays of broad spectrum insecticides (primarily pyrethroid and neonicotinoid based) have been effective against BMSB (Nielsen, Hamilton, and Matadha 2008; (Blaauw, Polk, and Nielsen 2015). However, these blanket sprays may be negatively affecting natural enemies that may help mitigate pest populations via biological control. This reduction in natural enemies may give rise to secondary pests such as SJS.

An integral part of reducing the presence of catfacing insects with cultural management in a peach orchard is reducing vegetation on the orchard floor, specifically flowering broadleaf weeds. Reducing overwintering habitat and alternative hosts for the pests ultimately reduces their presence in the orchard, and reduces damage to the peaches. Once pest presence is low in the early season, most of the remaining pest pressure can be mitigated with insecticide sprays at petal fall (Blaauw et al. 2022). Using perimeter focused insecticide applications (IPM-CPR), growers can reduce the amount of catfacing insects overwintering in the surrounding habitat from entering the orchard, while reducing cost and amount of insecticide applied (Blaauw, Polk, and Nielsen 2015).

Biological control of the BMSB is difficult in their adult stage, as the insect is highly sclerotized (Pote and Nielsen 2017). However, there are several taxa known to attack their eggs in the southeast. There are 18 known parasitoids, seven chewing predators, and seven piercing/sucking predators that are documented preying on BMSB eggs (Tillman et al. 2020). This again highlights the importance of biological diversity and generalist predators (Pote and Nielsen 2017).

Overview of predatory arthropod communities in peaches

Biological diversity is a good metric for assessing ecosystem health and closely correlated with ecosystem services (Crowder and Harwood 2014; Crowder and Jabbour 2014; Wan et al. 2019). While biological diversity is indeed important, it is only the first step to determining predator contributions to biological control. A diverse ecosystem supports the "enemies hypothesis" that increased diversity will fill more niches and achieve a more comprehensive suppression of pests (Russell 1989; Wan, Ji, and Jiang 2014). In the context of this study, increased biodiversity in peach orchards may lead to increased ecosystem services such as biological control and pollination.

Presently, the natural enemy community in southeastern peaches is largely unknown, especially in regard to predator roles in biocontrol. Predicting and quantifying biological control contributions can be difficult. Biotic and abiotic variables such as habitat diversity and how that habitat is managed may influence predator behaviors and feeding habits (Symondson, Sunderland, and Greenstone 2002; Nguyen and Nansen 2018). Predator feeding habits can also be influenced by other predators and alternate prey (Koss and Snyder 2005). Biological control delivery can be imprecise in a complex multitrophic system without understanding the population dynamics of the key predators and their interactions with prey (Symondson, Sunderland, and Greenstone 2002). By capturing known natural enemies with spatiotemporal data, we begin to map population dynamics of predators in peaches.

Historically, growers had to rely on direct observations of feeding or parasitism to identify natural enemies (Jones et al. 2009). But now, we can use molecular gut content analysis (MGCA) to determine predator functions in agricultural systems. Using MGCA allows researchers to detect pest DNA in the guts of a screened predator, and estimate biological control contributions and reveal trophic interactions (King et al. 2008; Pompanon et al. 2012; Schmidt, Acebes-Doria, et al. 2021). By detecting pest DNA in the guts of a screened predator, we know that this predator is a potential biological control agent of the pest. When we screen thousands of predators from multiple taxa, from multiple sampling sites, and over multiple years, we start to understand who the key beneficials in the system are. Understanding trophic interactions and knowing the key taxa in a system is an important step towards a better IPM program. Then, management practices could be amended to accommodate or promote some of the more effective natural enemies.

A major component of MGCA is primer design. Species specific primers are great for detecting individual taxa for a more targeted study, however, to reveal trophic interactions on a wider scale for generalist predators, broader primers are required. Sint et al. (2014) details the methodology associated with creating a primer for higher taxonomic groups, such as Lepidoptera or Hymenoptera as a whole (Sint et al. 2014). Some of the advantages of multiplex primers are screening for multiple prey species in a single PCR reaction, which saves time and money.

MGCA is a useful tool in estimating biocontrol contributions and trophic interactions, but when paired with spatiotemporal data of the predator community, a more comprehensive estimation of the ecosystem can be attained. Diversity and abundance of predators alone is a useful metric to estimate potential biological control contributions (Crowder and Harwood 2014; Wan et al. 2019). However, when predator community information is paired with MGCA and seasonality data, it will reveal how active natural enemies are in feeding on pests during different stages of the crop (Schmidt et al. 2014). Peaches are a perennial crop, meaning they do not need to be replanted every year. While perennial systems may remain fairly constant, there is an expected flux of habitat availability throughout the year at the different growth stages. Availability of habitat can influence predator abundance and diversity in the system, which in-turn corresponds to biological control contributions (Staab and Schuldt 2020; Bowers, Toews, and Schmidt 2021).

Current knowledge of key predators in peach systems

Coccinellidae

Lady beetles (Coleoptera: Coccinellidae) are effective generalist predators in their adult and larval stages. Coccinellidae are holometabolous and have four larval instars before they pupate and become adults (Honěk and Hodek 1996; Hodek, Honek, and Van Emden 2012). Their rate of development is dependent on temperature and amount of prey consumed. They also vary in their voracity. In some cases, high voracity is beneficial to killing as many pests as possible. However, in some cases a lower voracity coccinellid species can be beneficial, as their population can persist even when prey populations are low (Michaud 2012).

Coccinellids breed and hibernate dense brush, forests, and orchards (Honěk and Hodek 1996; Hodek, Honek, and Van Emden 2012). Supporting overlap of pests and predators is a good way to encourage biological control. Many biological control programs have been implemented, but the role of naturally occurring coccinellids also shows promise to potentially suppress some pest populations (Obrycki and Kring 1998; Michaud 2012). This makes coccinellids a great option for both augmentative or conservation biological control.

Formicidae

Ants (Hymenoptera: Formicidae) have an interesting relationship with biological control, and community structure within peaches. Peach trees with extrafloral nectaries had a higher abundance of ants but lower arthropod community diversity and richness, compared with trees that did not have extrafloral nectaries. However, the trees with extrafloral nectaries and ants experienced less herbivory and increased productivity (trunk growth, bud production), suggesting ants are effective biological control agents (Mathews, Brown, and Bottrell 2007; Mathews, Bottrell, and Brown 2009). However, when ants are present, oriental fruit moth (*Grapholita molesta*) eggs are preyed upon less often, suggesting ants disrupt natural enemies that would otherwise prey on the egg (*Trichogramma minutum, Harmonia axyridis*). This competition may be influenced by the EFN resource, but it still impacts the efficiency of biological control of the egg stage (Mathews, Brown, and Bottrell 2007).

In GA and FL peach orchards, three ant species (*S. invicta, D. bureni, Paratrechina* sp.) were responsible for over 60% predation of plum curculio (PC) larva attempting to bury themselves in the soil to pupate (Jenkins et al. 2006). However, it should be noted that these lab-reared larvae were placed on the ground, which is not natural and may have increased the predation rate (Jenkins et al. 2006).

Chrysopidae

Green lacewings (Neuroptera: Chrysopidae) are effective generalist predators in their larval stage, and can be predatory, nectar and honey dew feeders in their adult stage. They are also known biological control agents with the capability to be mass reared (New 1975; Saminathan, Baskaran, and AN 1999; Mahzoum et al. 2020). Additionally, multiple studies have demonstrated the ability of chrysopids to tolerate some pesticides, further demonstrating their promise as a biological control agent in agricultural settings (Giolo et al. 2009; Castilhos et al. 2014).

Castilhos et al. (2014) found that most of the 14 chemicals tested (insecticides, fungicides, herbicides) were harmless, but mineral oil reduced egg hatch and pupal eclosion of *Chrysoperla externa*. Similarly, Silva de Armas et al. (2019) performed a bioassay of nine common pesticides to assess their effect on larvae and pupae of *Chrysoperla externa*, and 5/9 were classified as "harmless" to larvae and pupae. Silva de Armas et al. suggests that Azadirachtin (1%), chlorantraniliprole (14%), deltamethrin (40%) should be prioritized for IPM in peaches (De Armas et al. 2019). Another laboratory bioassay found that eggs and pupae of *Chrysoperla carnea* were largely resilient to pesticides, and found that abamectin, deltamethrin and methoxyfenozide should be considered for IPM in peaches, and Phosmet should be avoided (Giolo et al. 2009).

Reduviidae

Assassin bugs (Hemiptera: Reduviidae) are an effective generalist predator and will kill more insects than they need to survive (Readio 1927; Hopwood et al. 2016). Predatory Heteroptera have several attributes which make them a good biological control agent. Predatory Heteroptera are generalists, and have a wide range of host pests, and they can be long-lived under the right conditions (Hopwood et al. 2016). Their populations can also persist and grow on non-crop plants that act as a refuge, allowing a continuous source of predators to colonize the cropland (Hopwood et al. 2016). Some species of Reduviidae are shown to be tolerant of pesticides, which could lead to an addition to an IPM program. However, with the high cost of commercial rearing for inundative release, they may not be an economical option for every crop system (Grundy 2007).

Araneae

Spiders are an often-overlooked predator in regard to biological control, as there is more research required to determine their role (Michalko, Pekár, and Entling 2019). However, they are an effective generalist predator, accepting most insect taxa encountered as prey as well as having longer generation times than most of their prey (Riechert and Lockley 1984; Riechert and Bishop 1990). A meta-analysis was done to elucidate how landscape management will affect spider abundance and species richness (Prieto-Benítez and Méndez 2011). Spiders were shown to have more success in forest plantations, compared to rangeland or other agroecosystems. This suggests spiders may have increased abundance and species richness in peach orchards.

Just because spiders are present in peach orchards, does not necessarily mean they are a net positive on pest suppression. Abrams & Cortez (2015) found that generalist predators that share the same prey may negatively influence each other, disrupting predator-prey dynamics (Abrams and Cortez 2015). Schmidt-Entling & Siegenthaler (2009) suggest that the presence of spiders can negatively influence other predators via intraguild predation or altering behavior, ultimately increasing presence of herbivores (Schmidt-Entling and Siegenthaler 2009). Spiders are also known to reduce control of aphids by intraguild predation on parasitoids (Traugott et al.

2012). Because this study did not include PCR screenings for intraguild predation, spiders were not included in the results.

Overview of pollination and pollinators in peaches

Pollination is another key ecosystem service in agroecosystems and holds a significant amount of economic importance (Gallai et al. 2009; Barfield et al. 2015). Peaches are considered to be self-fertile, meaning they do not need pollen from a different plant to germinate and begin setting fruit (Szabó and Nyéki 1999; McCraw 2007; Herrera et al. 2021). Some studies have found that insect pollinators have no effect on the fruit set of peaches (Robertson, Joshi, and Dowling 2020), while others have found that insect pollinators significantly increase proportions of fruit set (Saran, Mall, and Garhwal 2010; Tanda 2021). Also, in the event of a freeze where less flowers survive, pollinators may be important in supplementing pollination (Szabó and Nyéki 1999). For this reason, it is important to understand the flower-visiting insect community in peach orchards.

Regardless of the impact on yield, monitoring the flower-visiting insect biodiversity in an ecosystem is important to understand how chemical treatment may alter the community (Brittain, Bommarco, et al. 2010; Krauss, Gallenberger, and Steffan-Dewenter 2011; Biddinger and Rajotte 2015). Growers are recommended to make an effort to reduce pollinator exposure to pesticides by spraying after petal fall (Blaauw et al. 2022).

Insect pest management in the peach systems

In the southeast, peaches are managed with variations of IPM programs. Integrated pest management has several definitions or defining principles, but in simplistic terms, it is combining multiple pest management practices into a cohesive program in an effort to increase pest suppression and reduce chemical applications (Kogan 1998; Barzman et al. 2015; Dara

2019). There are many benefits to reducing the amount of chemicals applied into an agricultural system including reduced cost of chemicals, reduced chemical leaching or build up into the surrounding landscape, and reducing non-target effects on other organisms. There are a range of chemical managements considered in this study; conventional (high input), reduced-risk (low input), and organic.

Recommended conventional management for peaches in the southeast is explained in full detail in Blaauw et al. (2022) The primary pests in the southeast (Plum curculio, San Jose scale, Brown Marmorated Stink Bug, Lepidoptera) are generally combated with broad spectrum insecticides such as pyrethroids, neonicotinoids and organophosphates. These chemicals are applied on a calendar basis, or as certain thresholds of pests are reached. Chemical sprays are an integral part of pest management, but not all chemicals should be considered for an IPM program. For example, when controlling for scale in peaches, some chemicals (buprofezin and fenoxycarb with summer oil) controlled scale populations with minimal impacts on predators (Coccinellidae and Cybocephalidae), whereas methidathion harmed the predators to the point where it should not be considered in an IPM program (Erkılıç and Uygun 1997). However, as mentioned in the Introduction, due to how valuable peaches are, and how low economic thresholds of damage are, growers won’t risk relying on biological control for pest suppression, and will use calendar chemical sprays (Popp and Scherm 2001; Jones et al. 2009). It should be noted that in conventional management growers are recommended not to spray until after petal drop, to avoid exposing pollinators to the pesticides (Blaauw et al. 2022).

Non-target beneficial species may be exposed to these chemicals via direct contact or from the residual activity of the pesticide. Direct exposure to some chemicals may kill beneficials, but sub-lethal effects must also be considered. Sub-lethal effects may alter a beneficials behavior, mating, fecundity, or impact less studied life stages such as eggs (Biddinger, Leslie, and Joshi 2014; Biddinger and Hull 1995). He et al. (2012) evaluated imidacloprid (neonicotinoid) applied at the recommended rate to combat white fly *Bemisia tabaci* (Alyrodidae), and the non-target effects on the biological control agent, *Serangium japonicum* (Coccinellidae). The direct exposure to treated leaves and eggs did not kill *S. japonicum*, however they experienced increased handling time for *B. tabaci* eggs, and a reduction in the number of eggs consumed. So, while the management may not directly kill the beneficials, the sub-lethal effects may impact their reproduction rates, population growth and functional response (He et al. 2012).

Reducing natural enemy presence in a system may give rise to secondary pests that would have otherwise been controlled by these beneficial species (Horton et al. 2000; Dutcher 2007; Hill, Macfadyen, and Nash 2017). In this study, the San Jose scale is an example of a “secondary pest” in peaches that would normally be controlled by specialist and generalist predators. But SJS has since become a primary pest in peaches in the southeast (Blaauw 2021). In an effort to reduce non-target effects, some growers choose to implement a reduced risk program. A reduced risk program is a loose definition and varies case by case. It may mean there are fewer calendar sprays of conventional chemicals, lower concentrations of conventional chemicals, alternating conventional and organic, or any combination. In the context of this study, reduced risk is fungicide only-no insecticide.

In a comparison of conventional vs. reduced-risk chemical management in peaches almost all beneficial taxa (predators and parasitoids) were positively associated with the reduced-risk program (Atanassov, Shearer, and Hamilton 2003; Krauss, Gallenberger, and Steffan-Dewenter 2011; Biddinger, Leslie, and Joshi 2014). Roubos et al. (2014) also examined different chemical effects on natural enemies. They used 14 different pesticides including conventional/commercial, “reduced-risk”, and certified organic in bioassays to study acute effects on lady beetles, *Orius*, green lacewings, and parasitoids in blueberries. They found that the broad-spectrum insecticides were the most toxic, followed by the reduced-risk, then the organic; however, there was considerable variation among the different insecticides and predators. Some “reduced-risk” products were more toxic to some predators than the broad-spectrum products. And while some organic products aren’t as acutely toxic to predators, their low residual levels require more frequent sprays than other products, ultimately increasing overall chemical input into the ecosystem (Roubos et al. 2014).

To compare how management may affect the arthropod community in peach production, we surveyed farms under several different management regimens. In Georgia in 2020 we surveyed both commercially managed farms (insecticide and fungicide) and reduced-risk farms (fungicide only). In 2021, we surveyed the same commercially managed farms in Georgia, but also surveyed organic farms (organic insecticide and fungicide) in South Carolina. Organic farms have a strict legal definition. To be certified organic, a farm must be free of synthetic chemicals and commercial fertilizers for three years (Ames 2012). This survey pattern over two years may introduce some confounding variables that may impact the local arthropod community such as geographic or climatic differences. However, to make claims about southeastern peach production as a whole, it is valuable to consider a wider range of management across different locations in the southeastern US.

Objectives

The overarching long-term goals are to develop cost-effective, ecologically-based IPM strategies in peach orchards in the southeastern US. To understand how to build ecologically-based practices into IPM programs, it is imperative to understand the spatiotemporal activity of beneficial arthropods peach production systems. In this study, we monitor the abundance, diversity, and spatiotemporal activity of canopy predators (natural enemies) and flower-visiting insects (pollinators). Additionally, we use molecular gut content analysis (MGCA) to reveal trophic interactions and potential biological control contributions of canopy predators. With an understanding of where and when these beneficial arthropods are active, we may synergize their ecosystem services with standard chemical management with less harmful overlap. Reducing frequency and reliance of chemical applications will lower costs for growers and mitigate damage to the environment.

Objective 1: Determine the spatiotemporal activity and diversity patterns of predatory arthropods in the peach system.

Hypotheses: There will be greater diversity and abundance of natural enemies in the reduced-risk/ organic sites than in the conventional orchards (Atanassov, Shearer, and Hamilton 2003; Krauss, Gallenberger, and Steffan-Dewenter 2011; Biddinger, Leslie, and Joshi 2014; Roubos et al. 2014). We also predict predator distribution will be more concentrated on the edges of orchards in all treatments, either due to habitat heterogeneity, chemical avoidance, or distribution of prey species (Blaauw, Polk, and Nielsen 2015; Nguyen and Nansen 2018). Finally, we may see greater presence of natural enemies later in the season, as pesticide applications are reduced (Zivan, Bohbot-Raviv, and Dubowski 2017; Blaauw et al. 2022).

Objective 2: Identify trophic interactions of predatory insects on key peach pests using molecular gut content analysis.

Hypotheses: Based on the abundance, we predict the most important biological control contributors to be Coccinellidae, Formicidae, Chrysopidae and Reduviidae. These taxa are all generalist predators (Saminathan, Baskaran, and AN 1999; Jenkins et al. 2006; Michaud 2012; Hopwood et al. 2016). Coccinellidae in particular we predict will have a higher percentage of SJS DNA, as it is a known predator of scales (Michaud 2012; Amouroux et al. 2019). We also predict a higher percentage of pest DNA on the edges, where there is likely more predator-prey interaction (Nguyen and Nansen 2018).

Objective 3: Evaluating effects of management on flower-visiting insect functional groups in peach orchards

Hypotheses: Similar to hypotheses of objective 1, we predict a greater diversity and abundance of flower-visiting insects in the reduced-risk/ organic orchards compared to the conventional (Brittain, Bommarco, et al. 2010; Krauss, Gallenberger, and Steffan-Dewenter 2011; Biddinger and Rajotte 2015). In contrast to objective 1, we predict little-to-no edge effect for flower-visiting insects at our scale, as most of our insects collected were solitary bees and flies (Woodcock et al. 2016). Also in contrast to objective 1, we predict fewer flower-visiting insects later in the season, as peaches bloom in March and there are fewer floral resources later in the season (Blaauw and Isaacs 2014b, 2014a; Blaauw et al. 2022).

Chapter 1 figures:

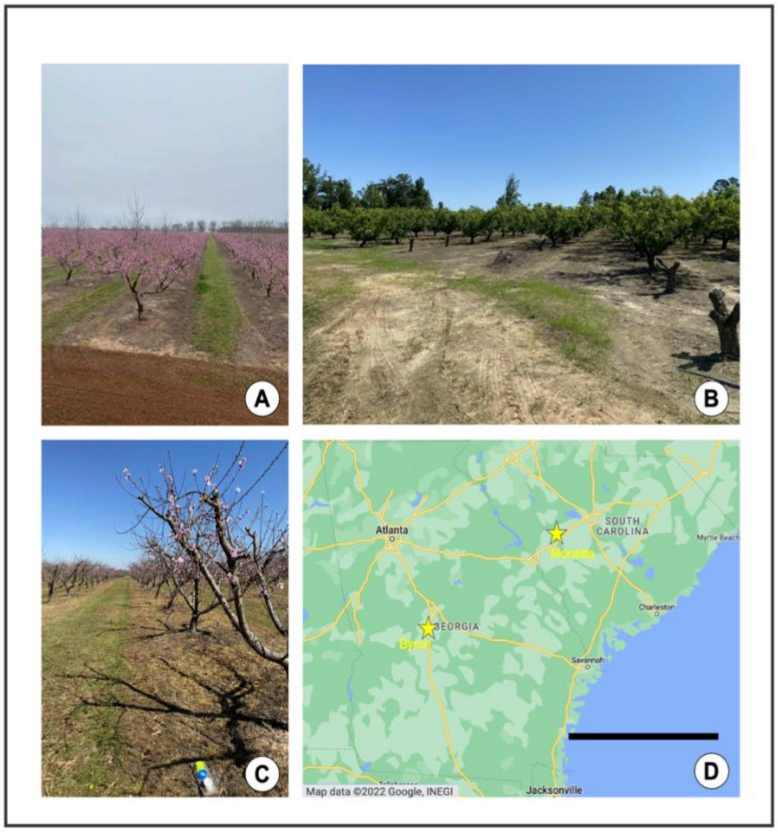

Figure 1.1. Field sites. (**A**) Commercial peach farm (high input), Byron, GA, 3/9/2021. (**B**) Organic peach farm (low input), Monetta, SC, 5/13/2021. (**C**) Commercial peach farm, Byron, GA, 3/16/2021. (**D**) Locations of field sites in Southeastern U.S. Map data © 2022 Google, INEGI (Google Maps) (Google 2022) Scale bar = 240 km.

Chapter 2: Diversity patterns and spatiotemporal activity of beneficial insects

Introduction

Promoting biodiversity in agroecosystems can increase ecosystem services such as biological control and pollination (Tscharntke et al. 2005; Mace, Norris, and Fitter 2012; Lefcheck et al. 2015). This concept provides the foundation for integrated pest management (IPM), which is a combination of pest management techniques that conserve beneficial arthropods to promote naturally occurring ecosystem services (Ehler 2006). Integrated pest and pollinator management (IPPM) is an ongoing shift in agricultural production to include pollinators into an established IPM paradigm (Biddinger and Rajotte 2015; Egan et al. 2020). Due to severe pest pressure and low economic damage thresholds, peaches are heavily managed with chemicals (Popp and Scherm 2001; Jones et al. 2009). It is important to consider how an agroecosystem is managed, especially in tree fruit and other perennial crops. Unlike annual cropping systems, populations of beneficial arthropods may be present year-round, carrying over ecosystem service benefits year-to-year (Tscharntke et al. 2005; Wan, Ji, and Jiang 2014; Gontijo 2019; Tooker, O'Neal, and Rodriguez-Saona 2020). There are some considerations in peach management for beneficial taxa, however the effects of management on communities of beneficial taxa are largely unexplored (Blaauw et al. 2022). In order to build an IPPM program, it is imperative to understand the key taxa that may provide ecosystem services in the agroecosystem. This study provides the foundation for this research by measuring abundance and diversity of key taxa, exploring their spatiotemporal activity, and considering how management alters their activity.

Pollination is a valuable ecosystem service that can be influenced by agricultural intensification (Tscharntke et al. 2005; Klein et al. 2007; Barfield et al. 2015; Porto et al. 2020). Greater intensity of management can reduce pollinator functional diversity and pollination services (Martins, Gonzalez, and Lechowicz 2015; Roquer-Beni et al. 2021). The relationship between pollinator biodiversity and pollination ecosystem services appears to be context-dependent (Radzevičiūtė et al. 2021), however there is evidence that increased biodiversity bolsters ecosystem resilience (Blüthgen and Klein 2011; Blaauw and Isaacs 2014a; Vasiliev and Greenwood 2020). For this reason, IPPM is a growing consideration to conserve pollinators by reducing their exposure to pesticides (Biddinger and Rajotte 2015; Egan et al. 2020). Pollinator activity also varies by location in the agroecosystem and throughout the season. Naturally, floral resource abundance drives much of the spatiotemporal activity of flower-visiting insects (Blüthgen and Klein 2011; Burkle and Alarcón 2011). Therefore, we would expect increased pollinator activity during bloom and less activity the rest of the season. To receive ecosystem services provided by pollinators in the orchard during this key time, recruiting flower-visiting insects from the surrounding landscape can be accomplished under the right management conditions (Kremen, Williams, and Thorp 2002; Lowe, Groves, and Gratton 2021). In some orchards flower-visiting insect diversity and activity decreased toward the interior of the orchard from the edge, which may result in inconsistent pollination (Chacoff and Aizen 2006). Monitoring flower-visiting insect spatiotemporal activity and management effect on this activity are important considerations for developing an IPPM program. Pollinators have an interesting relationship with self-pollinated crops such as peaches. Peaches are mostly wind pollinated, and do not need pollen from a different plant to germinate and begin setting fruit (Szabó and Nyéki 1999; McCraw 2007; Herrera et al. 2021). Peaches do need certain climatic conditions to grow, which include a required chill period (Parker and Abatzoglou 2019) and in cases where a freeze may damage or kill flowers, insect pollination becomes important (Szabó and Nyéki 1999). Growers are recommended not to spray pesticides until after bloom (Blaauw et al. 2022) even if some studies suggest pollinators have no effect on the fruit set of peaches (Robertson, Joshi, and Dowling 2020). However, other studies have found that pollinators do increase proportions of fruit set (Saran, Mall, and Garhwal 2010; Tanda 2021).

Biological control is an important ecosystem service to mitigate damage from pests (Van Driesche and Bellows Jr 2012; Naranjo, Ellsworth, and Frisvold 2015). In addition to spraying chemicals to control pests, natural enemies may provide additional pest suppression (Fravel 2005). For conservation biological control, it is important to consider factors such as habitat management, functional diversity, and spatiotemporal activity of key taxa (Tscharntke et al. 2008; Tylianakis and Binzer 2014; Shields et al. 2019). Recruiting and maintaining populations of predators requires a suitable habitat for them to thrive (Fiedler, Landis, and Wratten 2008; Shields et al. 2019). Maintaining a suitable habitat is not just related to structure and connectivity, but also how chemical management affects arthropod communities (Landis, Wratten, and Gurr 2000). Direct exposure to chemicals may kill some predators, while others may experience sub-lethal effects that alter behavior, mating or fecundity (He et al. 2012; Biddinger, Leslie, and Joshi 2014). Another aspect of conservation biological control correlated with maintaining suitable habitat is the functional diversity of predators (Tscharntke et al. 2005; Gagic et al. 2015; Jonsson, Kaartinen, and Straub 2017). Functional diversity is a consideration for all the variations and values of different species traits and abilities in an ecosystem, which ultimately influence how the ecosystem functions(Tilman 2001; Laureto, Cianciaruso, and Samia 2015). Habitat management intensity can alter functional diversity (Krauss, Gallenberger, and

Steffan-Dewenter 2011; Gallé et al. 2019). Increased biodiversity does not always increase biological control, however there is evidence that creates an ecosystem more resilient to disturbances (Tscharntke et al. 2012; Griffin, Byrnes, and Cardinale 2013; Jonsson, Kaartinen, and Straub 2017). Predator abundance and diversity also varies by distribution within the agroecosystem (space) and throughout the season (time) (Gallé et al. 2019; Le Gal et al. 2020). One of the significant drivers of this spatiotemporal variance is resource continuity (Iuliano and Gratton 2020). Resources such as primary or alternative prey or diet supplements (pollen or nectar) will vary throughout the season, which may influence predator communities. Edge effects are common in agroecosystems, which are likely influenced by habitat heterogeneity, distribution of prey species, and chemical avoidance (Blaauw, Polk, and Nielsen 2015; Gallé et al. 2019; Nguyen and Nansen 2018). Overall, it is important to consider the diversity, the spatiotemporal patterns, and the habitat of predators in an agroecosystem to build an IPPM program.

Peaches are subject to heavy pest pressure in the Southeastern USA. Currently in commercial peach orchards, these pests are controlled with calendar sprays of broad spectrum pesticides such as pyrethroids, neonicotinoids, organophosphates, and horticultural oils are applied when the orchard is dormant (Pless, Deyton, and Sams 1995; Knutson, Ong, and Ree 2019; Blaauw et al. 2022). These sprays are applied on a calendar basis, or as certain thresholds of pests are reached (Blaauw 2021; Blaauw et al. 2022). Rotations of these chemicals will reduce pest populations, but may also increase cost of production and damage to the surrounding ecosystem (Mariane de Souza et al. 2020; Rani et al. 2021). This year-round input of chemicals may alter communities of beneficial arthropods (Brittain, Vighi, et al. 2010; Crowder and Jabbour 2014). Additionally, these broad-spectrum pesticides may increase non-target effects on beneficial natural enemies, and reduce their capacity for biological control via direct exposure or sub-lethal effects (He et al. 2012; Biddinger, Leslie, and Joshi 2014). Some peach producers in the Southeastern USA manage pests with a “reduced-risk” approach in an effort to conserve natural enemies and pollinators (Krauss, Gallenberger, and Steffan-Dewenter 2011; Biddinger, Leslie, and Joshi 2014). “Reduced-risk” does not have a precise definition, but in the context of this study, it refers to orchards that spray only fungicides- no pesticides in 2020, and organic pesticides and fungicides in 2021. “Reduced-risk” programs in peaches have shown increased abundance and diversity of predators and pollinators compared to commercial management (Atanassov, Shearer, and Hamilton 2003; Krauss, Gallenberger, and Steffan-Dewenter 2011; Biddinger, Leslie, and Joshi 2014; Kovács-Hostyánszki et al. 2017). Other peach producers manage with organic pesticides. While organic pesticides are less toxic to natural enemies, their low residuals may require more frequent sprays, ultimately increasing overall chemical input into the ecosystem (Roubos et al. 2014). Understanding how different management alters natural enemy and pollinator communities is a key consideration for agriculturalists and researchers interested in ecosystem services. As mentioned above, the low economic threshold for damage to peaches may make growers hesitant to adopt IPPM strategies (Popp and Scherm 2001; Maas et al. 2021). However, the literature supporting IPPM success in conserving beneficial taxa is promising.

In the Southeastern USA the four major peach pests are the plum curculio *Conotrachelus nenuphar* Herbst (Curculionidae), San Jose scale *Comstockaspis pernicious* Comstock (Diaspididae, brown marmorated stink bug *Halyomorpha halys* (Stål) (Pentatomidae), and lepidopteran pests (e.g., oriental fruit moth, *Grapholita molesta* (Tortricidae)). The biology and life cycles of the individual pests make them difficult to control, as their more vulnerable life stages are largely out of sight. The plum curculio lay their eggs inside the peach where the larvae feed until their fourth instar, then exit the fruit and drop to the ground where they will pupate (Jenkins et al. 2006; Akotsen-Mensah, Boozer, and Fadamiro 2012). The larvae can also release pectin enzymes that cause the fruit to drop prematurely, then they exit the fruit directly into the ground where they will pupate (Ames 2012). San Jose scale (SJS) can anchor themselves on the bark, trunk, limbs and fruit of a peach tree where they will feed on the phloem or fruit (Gentile and Summers 1958). SJS secrete a waxy protective layer over themselves, which prevents them from direct exposure to some pesticides (Blaauw 2021). The oriental fruit moth larva will attack new growth buds, and bore into the peach, and will also pupate out of sight in the bark of the tree (Allen 1958). While brown marmorated stink bug (BMSB) are not as cryptic as the other pests, they are bulky and highly sclarotized in their adult stage (Hoebeke and Carter 2003). This makes them difficult to attack with natural enemies, however their eggs have several known predators and parasitoids (Tillman et al. 2020). Because the main pest species are particularly difficult to control via direct exposure to pesticides, there is an opportunity to explore supplementary biological control services.

In this study, we used molecular gut-content analysis to quantify predation events of key peach pests by natural enemies in southeastern peach orchards under different management regimens. Molecular gut content analysis (MGCA) is a useful metric for assessing trophic interactions by detecting fragments of pest DNA in the guts of a predator (Symondson and Harwood 2014; González-Chang et al. 2016). By detecting pest DNA in the guts of a screened predator, researchers can estimate biological control contributions and trophic interactions (King et al. 2008; Pompanon et al. 2012; Schmidt, Acebes-Doria, et al. 2021). MGCA is especially useful when studying generalist predators in a dynamic agroecosystem (Schmidt et al. 2014;

Schmidt, Szendrei, and Grieshop 2016). Generalist predator feeding habits are not always species-specific, their feeding habits may change over time and in different environmental conditions (Symondson, Sunderland, and Greenstone 2002; Roubinet et al. 2018; Le Gal et al. 2020). Quantifying trophic interactions of generalist predators using MGCA allows us to more precisely assign weight to certain taxa and create food webs (Kaartinen et al. 2010). Food webs allow us to visualize an ecosystem, determine which taxa are the most effective predators, and visualize how generalist predator feeding habits change in response to variables such as space, time, and management (Roubinet et al. 2018; Schmidt et al. 2014; (Bowers, Toews, and Schmidt 2021). By sampling predators from multiple managements in Southeastern peaches, we can compare how chemical management alters arthropod communities and their interactions in the agroecosystem (Krauss, Gallenberger, and Steffan-Dewenter 2011; Crowder and Harwood 2014).

Despite the prominence and popularity of peaches in the Southeastern USA, remarkably little is known about the communities of beneficial arthropods present in the orchards. This study is a first step in cataloging these key taxa. Over a two-year study, we collected predators and pollinators from peach orchards in the Southeastern USA under different management paradigms. One goal of the study is to estimate pollinator communities using pan traps, and reveal how management alters their and location and activity within the orchard. The other goal is to estimate communities of natural enemies and their biological control contributions using MGCA, and reveal how management and location within the orchard alters their community and predation. By monitoring the spatiotemporal activity of these beneficial communities, we can provide researchers and agriculturalists information on where and when pollination and biological control ecosystem services may be delivered. With a more complete understanding of the peach agroecosystem, future IPPM management may be altered to promote or preserve key taxa providing ecosystem services, and reduce reliance on pesticides. This study is an important foundation for future research related to beneficial arthropods in southeastern peaches.

Materials and Methods

Sites description and field design

In Georgia and South Carolina, USA, a total of ten sites under different management paradigms were sampled for this study over two years. The treatments for these sites were chosen to assess how different chemical management, intended to mitigate pest damage, may alter food webs or affect natural enemy communities. We sampled three commercially managed orchards, three “reduced-risk” orchards, and four organic orchards. The peach orchards in this study underwent different management regimes, but much of the site structure was the same. Peach trees were planted 5m apart in rows, with the space between the rows about 7m. The soil in the peach orchards was largely sandy, and grass was allowed to grow between the rows of trees until it was mowed once a month. The area below the tree canopy is sprayed with herbicide to reduce grass growth (Figure 1.1).

The commercial orchards located in Byron, GA, were sampled in both 2020 and 2021. These commercially managed sites are managed with the protocols in Blaauw et al. (2022), which are calendar sprays of broad spectrum pesticides such as pyrethroids, neonicotinoids and organophosphates. The “reduced-risk” orchards located at the USDA southeastern fruit and tree nut research station, Byron, GA, were sampled in 2020. The “reduced-risk” sites were managed with only fungicide, no insecticides. The organic farms located in Monetta, SC, were sampled in 2021. The organic orchards are managed with organic fungicides and pesticides. To be certified organic, a farm must be free of synthetic chemicals and commercial fertilizers for three years (Ames 2012). One of the original organic sites was bulldozed ~6/24/21, so it was replaced with another organic site at the same location.

To monitor the spatial activity of beneficial arthropods within the peach orchards we selected nine trees arranged in a transect at each site to sample. The transect is arranged with three trees along the edge, three trees at 25m from the edge, and three trees at 50m from the edge. In an effort to maintain consistency in the study, the "edge" was selected to be along a dirt road at each of the sites.

Sampling approach

Peach management varies according to the developmental stage and the time of year. Different peach varieties experience developmental stages (e.g., bloom, pre-harvest, post-harvest, dormancy, etc.) at slightly different times of year (Blaauw et al. 2022). In an effort to capture a representative analysis of southeastern peach production as a whole, we sampled several varieties of peaches during these key developmental stages, as well as the under-studied late season leading into dormancy. To monitor the temporal activity of beneficial arthropods within peach orchards, samples were taken biweekly from July-October in 2020 and from March-October in 2021. In 2020, we began sampling in July, when majority of peaches were in their summer or pre-harvest developmental stage. We had 14 total sample dates (seven pollinator collections, seven predator collections), with the last sample date on 10/2/2020. This allowed us to summarize how communities of arthropods changed when transitioning from summer until post-harvest, and dormancy. In 2021, we escalated our sampling efforts, beginning before bloom in March. We totaled 44 sample dates (17 pollinator collections, 27 predator collections), with the last sample date on 10/5/2021. This allowed us to capture all developmental stages of peaches, including bloom and dormancy, with significant overlap during the growing season-successfully estimating the seasonality of beneficial arthropod communities in the southeast USA.

To monitor the presence of pollinators in peach orchards, we used colored pan traps to attract and capture flower-visiting insects (Montgomery et al. 2021). These pan traps are small plastic bowls painted with fluorescent blue, yellow and white spray paint. Bowls are filled with a solution of water and scentless dish soap to break surface tension, so any arthropod that visits the trap would sink to the bottom. Pan traps were deployed on the ground below each tree in the transect in sets of three (blue, yellow, white) under the edge of the canopy. These traps were collected after 24 hours, and arthropods were brought back to the lab for identification.

The same trees in the transect pattern described above are sampled for predators in the canopy. To monitor the presence of predators in peach orchards, we used a tree beating technique to directly sample predators in the canopy (Montgomery et al. 2021). Limbs of the peach tree were struck with a foam bat to dislodge predators in the canopy onto a beat sheet, where they are collected with aspirators and paintbrushes wet with ethanol. Beats were repeated three times per tree. Predators were collected into individual microcentrifuge tubes containing chilled ethanol. Specimens were stored in a cooler on ice while in the field, and transferred to a -20°C freezer upon returning to the lab for identification and processing.

Insect identification and processing (flower-visiting insects)

Flower-visiting insect specimens were identified to family using morphologic keys in Triplehorn and Johnson's (2005) "Borror and DeLong's Introduction to the Study of Insects". (Triplehorn and Johnson 2005). We say flower-visiting insects instead of strictly "pollinators", as there are other beneficial taxa, such as predators, that utilize flowers as a resource for pollen and/or nectar (Hogg, Bugg, and Daane 2011; Wardhaugh 2015; Uhl and Brühl 2019). Due to many rare taxa, even at the family level, the 47 families were categorized into the following functional groups; Nectar/pollen feeding Hymenoptera, predatory/parasitic Hymenoptera, predaceous Diptera, and other Diptera. These broad functional groups are an estimation of insects that may use flowers as a resource, as they can accurately be monitored with pan traps (Campbell and Hanula 2007).

Predator identification and processing (Canopy predators)

Taxa from several orders and functional groups of arthropods feed on pest species, reducing their abundance. Some examples of the diversity of natural enemies in peaches can be found in Table 1 of Biddenger et al. (2014). Insect specimens were identified to family using morphologic keys in Triplehorn and Johnson's (2005) "Borror and DeLong's Introduction to the Study of Insects". Insects were identified to family, as specialized keys are needed for genus and species level identification. Also, management considerations are often applied at the family level as growers often do not have specialized identification training, with the exception of key pests (Blaauw et al. 2022). Further, we wanted to explore these families as generalist predators, rather than focusing on species-specific predator-prey pairings. All spiders were identified to Araneae, as the large majority of the spiders were immature. Without the key taxonomic descriptors such as labial palps, reproductive structures, or hair fully developed, spiders cannot be easily identified past family. As such, we focus on the insect natural enemy community for analysis and trophic links.

After canopy predators were captured, sorted, and identified, specimens were cleaned with 10% bleach, molecular grade water, and a final rinse of 100% ethanol to remove any external environmental contaminants. Whole body DNA was extracted following the manufacturer's protocol for animal tissue protocol using the QIAGEN DNeasy 96 Blood and Tissue Kit (Schmidt et al. 2014, Bowers et al. 2021). The extracted predator gut DNA was cleaned with 75 µL of AE buffer, and stored in a -20° C freezer until PCR.

Before the DNA gut contents of the predators are amplified to screen for the target prey species, primers of the prey species must be optimized or designed. Protocols for primer design of PC, BMSB, and Lepidoptera were available in the literature (Schmidt et al. 2014; Sint et al. 2014; Athey, Sitvarin, and Harwood 2017). We designed a primer for San Jose scale (*Diaspidiotus perniciosus*) following common practices (King et al. 2008; Chapman et al. 2013). Primers for SJS were designed using sequences available in GenBank (KY085371.1, HM474184.1) using PRIMER 3 and primer blast (Chapman et al. 2013; Schmidt, Szendrei, and Grieshop 2016). Three sets of primers were tested for specificity (detailed in Table 2), and sensitivity in the laboratory, and the annealing temperature as optimized. The best of the three SJS primers for satisfying conditions, and with the ability to be run with primers for plum curculio (Schmidt, Szendrei, and Grieshop 2016) was selected. The best primer was DpForward-5'-CAGGATGAACATTATACCCTCCATT-3', and DpReverse – 5'-ACCATTTCCTCTTGGATTGAAAAA-3' with PCR conditions detailed below, produced a ~293 bp amplicon. No cross-reactivity on non-*D. perniciosus* DNA samples was observed. DNA extractions were screened for the presence of pest DNA using Lepidoptera primers, BMSB, and a duplex PCR screening for PC and SJS (King et al. 2008; (King et al. 2008, Chapman et al. 2013, Schmidt et al. 2014; Sint et al. 2014, Schmidt et al. 2016, Athey, Sitvarin, and Harwood 2017).

PCRs were all conducted in a Bio-Rad C1000 Touch Thermal Cycler. The protocol for multiplex PCR screening follows general 12.5 µL multiplex conditions (King et al. 2008, Schmidt et al. 2014, Sint et al. 2014, Athey et al. 2017). The duplex PCR mix for plum curculio and San Jose scale includes 6.25 µL 2x Qiagen multiplex master mix, 0.3 µL BSA, 2.7 µL molecular grade water, 0.3125 µL each of the forward and reverse primer for San Jose scale (Dp304), 0.3125 µL each of the forward and reverse primer for plum curculio (PC), and 1.5 µL of extracted predator DNA. The PCR protocol for PCDp is 15 minutes at 95° C, then 35 repeated cycles of 30 seconds at 94° C, 30 seconds at 58° C, and 40 seconds at 72° C, followed by five minutes at 72° C, and then held indefinitely at 8° C. For the multiplex PCR protocol of Lepidoptera, the mix includes 6.25 µL 2x Qiagen multiplex master mix, 0.31 µL BSA, 3.69 µL molecular grade water, 0.3125 µL each of the forward and reverse primer for Lepidoptera (Lep), and 1.50 µL of extracted predator DNA. The PCR protocol of Lepidoptera is 15 minutes at 95° C, then 35 repeated cycles of 30 seconds at 94° C, 90 seconds at 66° C, and 60 seconds at 72° C, followed by five minutes at 72° C, and then held indefinitely at 12° C. For the multiplex PCR protocol of BMSB, the mix includes 6.25 µL 2x Qiagen multiplex master mix, 0.3 µL BSA, 2.7 µL molecular grade water, 0.3125 µL each of the forward and reverse primer for BMSB (BMSB), and 2.0 µL of extracted predator DNA. The PCR protocol of BMSB is 15 minutes at 95° C, then 39 repeated cycles of 45 seconds at 94° C, 45 seconds at 61° C, and 30 seconds at 72° C, followed by five minutes at 72° C, and then held indefinitely at 12°C.

Results of the PCR were read on a QIAxel Advanced System. To ensure there is no contamination between multiple PCR runs, a positive and negative control are included in every 96-well plate. In this study, PCR data is read in a binary, either the predator had the pest DNA or it did not. For this study, a positive result was determined if the pest DNA being screened for was above a concentration of >0.075 RFUs (Schmidt et al. 2014, Sint et al. 2014).

Data management and statistical analysis (flower-visiting insects)

For our initial exploration of the data we used MANOVA to test if abundances of flower-visiting insect functional groups were significantly influenced by management, location in the orchard, and sample date, as well as interactions between the variables. Once we had statistical support from MANOVA (test=Pillai), we proceeded with univariate models. To assess spatial, temporal, and management effects on the abundance and diversity of flower-visiting functional groups, we fit linear mixed effects models (LMMs) (Pinheiro et al. 2021) for each group as the response variable with field site nested within time as a random variable to account for repeated measures of fields. We pooled catch data by combining blue, white, and yellow traps for an individual tree, and combined trees in the sample transect on an individual sample date. Natural logarithm transformed counts were used as the response variable, which helped normalize the distribution and improve model fit (i.e. spread of residuals). We also use the common metrics of measuring diversity in ecology, using species richness, Shannon index, and Simpson index. All analyses were conducted in R. (Pinheiro et al. 2021; Team 2021).

Data management and statistical analysis (Canopy predators and trophic interactions)

The composition of predators and their interactions (i.e. frequency of predation determined by MGCA) with pests were analyzed using PERMANVOA models (adonis2, package: vegan) using time, spatial location, and management as fixed effects and site location as a blocking factor (i.e., strata = site) to account for repeated sampling of the same plot across each season using 999 permutations and Bray-Curtis similarity measures (Legendre and Legendre 2012). Bi-partite consumer webs were produced using the package “bipartite” (Dormann, Gruber, and Fründ 2008) to visualize the communities feeding links to the four common pests by management practice and transect location. For univariate initial exploration of the data, we calculated the total predator abundance and Shannon Diversity index. All analyses were conducted in R (R CoreTeam 2021).

Results

Flower-visiting insects

Over two years, we deployed 2,252 pan traps and caught 11,105 flower-visiting insects, which were identified to family. Due to the large data set with many rare taxa, we combine these flower-visiting insects into functional groups based on diet (Table 1). There were 45 unique taxa observed that were assigned to functional groups. The most abundant functional groups were 'Predaceous Diptera' (n=3468), then 'Other Diptera' (n=3289), followed by 'Nectar/Pollen Feeding Hymenoptera' (n=2923), and finally 'Predaceous/Parasitic Hymenoptera' (n=1425). Total community data for 2020 and 2021 are displayed in (Figure 2.1) and (Table 1).

For our initial exploration of the data we used MANOVA to test if abundances of flower-visiting insect functional groups were significantly influenced by management, location in the orchard, and sample date, as well as interactions between the variables. These initial explorations found that flower-visiting insect functional groups were influenced by management ($F_{4,105}$=14.61, P<0.0001), sample date ($F_{40,432}$=6.89, P<0.0001), and an interaction between management and sample date ($F_{20,432}$=2.02, P<0.0001). We then proceeded with univariate models to explore these significant variables from the MANOVA (Figure 2.1&2.2). For univariate analysis, we combined traps by transect, treatment, field, and date to form pooled samples. After pooling catch data, the sampling effort was fairly even by location with 50 at the edge, 49 at 25m, and 50 at 50m. Pooled data by management was slightly higher in the high-input with 81 pooled samples, and 68 in the low-input. Some sample dates did not overlap for high-input and low-input, so to balance the data for location analysis, all dates that didn't have measurements for both were dropped for 2021. We did the same for 2020.

In 2020, nectar and pollen feeder activity in peaches varied by date ($F_{6,107}$=2.74, p=0.0161), with an interaction with management indicating that abundance over time is related to management practices ($F_{6,107}$=2.74, p=0.0161) (Figure 2.2A). Predaceous and parasitic Hymenoptera were significantly influenced by management practice, with higher abundance in the high-input management ($F_{1,107}$= 14.25, p<0.0003) (Figure 2.2B). In 2020, variance in predaceous Diptera activity was significantly influenced by a combination of date ($F_{6,107}$=5.09, p<0.0001) and management ($F_{1,107}$= 8.38873, p<0.0046), with an interaction early in the season ($F_{6,107}$=4.49, p<0.0004) (Figure 2.2C). Other Diptera activity varied throughout the season ($F_{6,107}$=11.44, p<.0001) and in relation to field management, with higher abundance in the high-input management ($F_{1,107}$=6.88, p<.0001) (Figure 2.2D).

In 2021, nectar and pollen and pollen feeders varied by management, with a higher abundance in the high-input orchards ($F_{1,100}$=22.18, p<.0001) (Figure 2.3A). Predatory and parasitic Hymenoptera abundance varied significantly by sample date in both managements ($F_{1,100}$=4.43, p=0.0377) (Figure 2.3B). Predaceous Diptera did not appear to have a strong trend, however there was a significant interaction between management and sample date ($F_{1,99}$=5.92, p=0.0168) (Figure 2.3C). Predaceous Diptera abundance in the high-input increases in late April, and abundance in the low-input decreases in May, indicating that abundance over time may be related to management. Other Diptera abundance varied significantly over time ($F_{1,100}$=22.14, p<.0001), and had a higher abundance on the edge relative to the other areas of the orchard ($F_{2,100}$=7.92, p=0.0006) (Figure 2.3D).

Abundance and diversity of canopy predators

A total of 4440 predators were collected in peach canopies over the two-year study, with 51 unique predatory taxa identified to the family level (Table 3). Spiders were identified to order

(Araneae), due to mostly immatures in samples. Although many unique predator taxa were collected, only 13 taxa were present in greater than 4% of all samples, of which six taxa were the most common (Figure 2.4, Table 3). Predator taxa occurring in at minimum >4% of samples were included further analyses. The most abundant predators in the canopy predator community of peaches were Araneae, Formicidae, Coccinellidae, Chrysopidae, Anthocoridae, and Elateridae (Figure 2.4) (Table 3).

Overall, there were more predators collected from the high-input orchards (n=2412) than in the low-input orchards (n=2017). However, the sampling effort in the high-input orchards (n=511), were greater than the sampling effort in the low-input orchards (n=434). The sampling effort across the transects was even at the edge (n=322), at 25m (n=318), and at 50m (n=322).

To assess management intensity, transect, and date effects we fit generalized least squares (gls) using field nested within date to account for repeated measures of field. In this case there was obvious non-linearity in the date variable, so we fit a number of polynomial versions of date into the model, and instead opted for using data as a factor in the model, which greatly improved the fit of the model (residual plots and produced much lower AIC values). One interaction term was entered into the model to test for dependencies of spatial location and management treatment, and was tested for need to include in the model using AIC and BIC of <delta2. Overall, treatment (management) did not have a strong effect of field location effects on diversity ‘H’ or total abundance of natural enemies in the canopy ‘tot’ (Figure 5), with the exception of 2020 diversity.

Seasonal abundance and diversity

The seasonal patterns did have significant effect on diversity and abundance in both years. In 2020, the abundance of canopy predators varied by time of year, with fewer canopy predators caught later in the season although the result was not significant (Figure 2.5A). Also in 2020, there was a marginal interaction between transect location and management, with predator abundance slightly higher on the edges of the high-input compared to the low-input ($F_{2,94}$=3.02, p=0.0536). In 2021, the abundance of canopy predators varied by time of year ($F_{12,215}$=9.23, p<.0001), but there was no significant influence of management. However, there was an interaction of management similar to 2020, with predator abundance higher on the edges of the high-input compared to the low-input ($F_{2,215}$=3.87, p<.0001) (Figure 2.5B). In 2020, the diversity of canopy predators was higher in the high-input sites ($F_{1,96}$=15.33, p=0.0002) and varied by time of year ($F_{5,96}$=2.31, p=0.0497) (Figure 2.5C). In 2021, diversity of canopy predators also varied by time of year with diversity steadily growing until roughly June, and fell later in the season ($F_{12,217}$=9.49, p<.0001) (Figure 2.5D).

Management effects on canopy community over time

Management had different effects on individual taxa. Univariate analyses were followed with principle response curve. Principal response curves are used to assess treatment effects on community structure measured repeatedly over time (Auber et al. 2017). In the principle response curve (Figure 2.6), predator community structures in the low-input are compared to the control (commercial standard high-input). Taxa above the 0.0 on the y axis are more abundant than expected relative to the control, and taxa below the 0.0 are less abundant than expected relative to the control. In other words, taxa above the line performed better than expected in the low-input sites, and taxa below the line performed better than expected in the high-input sites. In 2020, we see an effect of management, where overall canopy community was generally more abundant in the low-input orchards relative to the high-input orchards over the season ($F_{1,93}$=13.49, p<.0001) (Figure 2.6A). In 2021, we see a less consistent effect of management, where specific communities fluctuated in the low-input orchards over the season ($F_{1,208}$=14.52, p<.0001) (Figure 2.6B).

Location effects on canopy predator community over time

For test for location effects on canopy predators, we again used principal response curves to assess effects on community structure measured repeatedly over time (Auber et al. 2017). We chose the control as the 25m mark, as it is the "average" of our sample locations. We assessed if community structure is subject to change based on the location within the orchard (Figure 2.7). Taxa above the 0.0 on the y axis are more abundant than expected relative to the control, and taxa below the 0.0 are less abundant than expected relative to the control. Overall, there were no significant changes in community structure in transects over time. Meaning regardless of the location (edge, 25m, 50m) in the orchard, the relative canopy community structure will be the same throughout the season. This is a positive result that indicates even distribution of potential biological control delivery.

Canopy predator trophic interactions (MGCA)

We screened 2444 individual canopy predators for the DNA of the four major peach pests in the Southeast (Table 4). Predation rates were overall very low, with a total number of positives: 241 SJS, 87 plum curculio, 41 Lepidoptera, and 3 BMSB. Of the 2444 predators we screened, the mostly common six taxa in the canopy communities (Figure 2.4) accounted for roughly 86.72% of SJS positives, 95.40% of plum curculio positives, 80.48% of lepidopteran positives, and 100% of BMSB positives (Figure 2.8). For screening ants (Formicidae), groups of ants were pooled together in the same PCR and treated as one sample. We tested if grouping ants together would influence predation frequency with logistic regression, presence/absence of prey in relation to number of ants in a sample. There was no correlation between number of ants included in a sample and proportion positive for prey (X^2=0.42, df=1, p=0.5147). The proportion positive figure estimated the proportion of predators that tested positive for pest DNA with a 95% CI via the R package “binconf” (Hmisc) (R CoreTeam 2021).

We collected canopy predators from different treatments (high-input and low-input) and locations (edge, 25m, 50m), and screened their gut content for the presence of the four target peach pests: brown marmorated stink bug (bmsb), San Jose scale (SJS), Lepidoptera (lept), and plum curculio (pc). The predation results were grouped by location and management, to ask questions about effects of management on overall predation and differences in predation frequencies on the four target peach pests. Using multivariate analysis to detect significant dissimilarity in communities based on Bray-Curtis distance metric, there were no significant dissimilarities in the predation on pests in relation to treatment or location within the orchard (Figure 2.9). While there were more predators screened from the high-input management, there was no evidence of differences in feeding frequency between the two systems or along our spatial gradient (Figure 2.9). Overall predation on the BMSB and Lepidoptera was very low, with four different taxa infrequently testing positive. Predation on plum curculio was rare, but a few taxa tested positive for the presence of PC (Figure 2.8). The SJS had all 13 of the abundant taxa choose them as prey. For the consumer webs (Figure 2.9), we see this rate of predation frequencies are consistent regardless of location or management. The strongest links are predators eating SJS, as this pest was frequently preyed upon. The smaller links are for the ‘lept’ and ‘bmsb’, as these pests were infrequently preyed upon. These results suggest a high potential for SJS biological control, as many predators tested positive for SJS DNA, and those predation frequencies were consistent in all treatments and locations.

Discussion

Flower-visiting insects

Pollination is a valuable ecosystem service that occurs naturally, but chemical management can reduce pollinator abundance and diversity (Tscharntke et al. 2005; Martins, Gonzalez, and Lechowicz 2015; Roquer-Beni et al. 2021). In order to build an IPPM program, it is valuable to consider how management may alter beneficial communities (Biddinger and Rajotte 2015; Egan et al. 2020). This study monitored flower-visiting insects under different management intensities. We use the term flower-visiting insect instead of strictly 'pollinator', because there are many beneficial arthropods that utilize flowers that may not be considered strictly pollinators. A wide breadth of taxa will visit flowers; including predators, parasites, decomposers, nectar and pollen feeders, and other arthropods that use flowers as refuge or breeding grounds (Koptur, Jones, and Pena 2015; Wardhaugh 2015; Ollerton 2017; Uhl and Brühl 2019). Wardhaugh et al. (2015) describes "the big four" orders that utilize flowers (Hymenoptera, Diptera, Coleoptera, and Lepidoptera). In this study, we used pan traps to estimate flower-visiting insects, which we organized into functional groups based on diet. However, pan traps do not provide a representative estimation of Coleoptera and Lepidoptera (Montgomery et al. 2021). So we organized our functional groups as nectar and pollen feeding Hymenoptera, predaceous and parasitic Hymenoptera, predaceous Diptera, and other Diptera. To restate, while all of these taxa may not be obligate pollinators, all of these groups provide pollination services (Orford, Vaughan, and Memmott 2015). We consider the effects of management on these potentially beneficial groups to inform on future IPPM decisions.

Nectar and pollen feeding Hymenoptera functional group was defined as Hymenoptera that are obligate feeders of nectar and pollen (Uhl and Brühl 2019). In 2020 nectar and pollen feeder activity in peaches varied suggest abundance is related to both time and management (Figure 2.1). Halictidae were among the most abundant taxa in the system, making up 83.13% of the 'Nectar/Pollen feeding Hymenoptera' functional group. Other studies in peaches have found Halictidae to be the dominant pollinator in the system (Dar et al. 2020). Peaches have extrafloral nectaries (Marazzi, Bronstein, and Koptur 2013) and Halictidae are known to frequent extrafloral nectaries (GALETTO and BERNARDELLO 2004; Koptur, Jones, and Pena 2015; Sandoval-Molina et al. 2018). It is possible that the drop in population at the end of 2020 is driven by resource depletion as peaches become dormant. The interaction with management in 2020 may also be driven by the transition to the dormant stage, as growers in the high-input begin to apply horticultural oils to combat SJS (Blaauw et al. 2022). The large drop in abundance in September for the high-input population relative to the low-input population could be explained by this difference in treatment. An unexpected result was the higher abundance in the high-input relative to the low input in 2021. Brittain et al. (2010) found no difference in flower-visiting insect diversity and abundance between organic farms and conventional farms. Brittain et al. suggest that this finding could be related to landscape context rather than management. Many other studies describe the importance of landscape context for species abundance and diversity (Kremen, Williams, and Thorp 2002; Lowe, Groves, and Gratton 2021; Tscharntke et al. 2012). In our case, the surrounding landscape for the high-input orchards may be much richer in resources or refuge for the nectar and pollen feeding Hymenoptera. We did not measure flower-visiting insects in the surrounding landscape, so this is a good area of study for future directions.

Predaceous and parasitic Hymenoptera functional group was defined by their diet and behavior, differing from the nectar and pollen feeding Hymenoptera group because parasitoids and predatory wasps often feed on nectar in their adult stage, while immatures are insectivores or consume hosts (Wardhaugh 2015; Uhl and Brühl 2019). Predaceous and parasitic Hymenoptera abundance were influenced by management practices in 2020, with higher abundance in the high-input management (Figure 2.2B), and varied by sample date in 2021 (Figure 2.3B). 2021 Seasonal variations did not have a distinct pattern, but there was a significant drop in abundance after bloom when pesticide sprays begin for both managements. But in 2020, we again see the unexpected higher abundance of this group in the high-input management. There is some evidence that predaceous and parasitic Hymenoptera can be successful in high-intensity management. Cutler et al. (2015) found parasitic wasps had high rates of parasitism in conventionally managed fields (Cutler et al. 2015). Schmidt et al. (2021) surveyed parasitoid activity in multiple management practices in blueberry systems in the Southeast. While they did not find significantly greater abundance in the conventional management, they did find unique parasitoid community structure depending on management and location in the field. While this phenomenon is likely landscape dependent (Tylianakis and Binzer 2014; Schmidt, Whitehouse, et al. 2021), it does provide some evidence this group could contribute to biological control in conventional management and fold neatly into future IPPM management.

Predaceous Diptera were mostly composed of predaceous families, but parasitic groups were counted in this grouping as well. In 2020, variance in predaceous Diptera activity was significantly influenced by a combination of date and management (Figure 2.2C). Dolichopodidae made up 72.76% of the predaceous Diptera functional group. Cicero et al. (2017) found that Dolichopodidae abundance peaked from June until August in citrus orchards in Florida, which we also see in our 2020 results. There was also an interaction between date and management in both 2020 (Figure 2.2C) and 2021 (Figure 2.3C). Unraveling how the individual taxa are individually responding to management and time are difficult. Pfister et al. (2017)

monitored predatory fly activity in response to management, and also found that families responded differently to management (Pfister et al. 2017). They found that surrounding landscape was a better predictor of predatory fly activity. Another study by Kautz & Gardiner (2019) found that Dolichopodidae were actually more abundant in the managed cropland than in the surrounding fields (Kautz and Gardiner 2019). This finding suggest that prey resources may be driving their activity rather than response to management. Although we did not monitor prey activity, several of the known prey of Dolichopodidae were present in our bycatch and in rotting peaches on the ground. Predaceous Diptera were the most abundant functional group in the pan traps over two years, so despite their inconsistent results, they are an important group to consider in peach management.

The Other Diptera functional group are other flies that are not considered predators. This functional group may be among the most important pollinators in the system based on their abundance. Other studies have found that 'non-syrphid' Diptera are among the most important pollinators based on their abundance and pollen carrying ability (Orford et al. 2015, Uhl and Brühl 2019). Other Diptera abundance fluctuated throughout both seasons (Figure 2.2D,2.3D). This is a diverse functional group, but many of the abundant families such as Drosophilidae utilize resources like rotting fruit. This temporal variation is likely in response to resource availability such as rotting fruit, nectar, and other resources fly mouthparts are adapted for (Krenn, Plant, and Szucsich 2005). This may explain the large drop in abundance after bloom in 2021, and account for the peaks and valleys from fruit thinning or culling in both years (Blaauw et al. 2022). 2020, other Diptera had a higher abundance in the high-input management ($F_{1,107}$=6.88, p<.0001) (Figure 2.2D), and had a higher abundance on the edge in 2021 ($F_{2,100}$=7.92, p=0.0006) (Figure 2.3D). These responses to management are more consistent with previous literature suggesting edge-biased distribution may be related to increased habitat heterogeneity or chemical avoidance (Chacoff and Aizen 2006). Due to their sheer abundance and potential pollination services (Orford et al. 2015), this functional group may be beneficial in Southeastern peaches.

Canopy predator communities

We successfully captured 4440 peach canopy predators over two years. By collecting throughout the season, from different management regimens, and in a spatial gradient within the orchards, we can describe how canopy communities change in response to space, time, and management. We screened 2444 individual canopy predators for the DNA of the four major peach pests in the Southeast. Using this data, we can describe predation patterns and highlight specific taxa that may be effective biological control agents. This information will inform researchers and growers about the spatiotemporal activity of beneficial arthropods communities, and their response to different management in these agroecosystems.

Management effects on predator abundance and diversity varied with no significant differences in predator community structure by location within the orchard in 2020 or 2021 (Figure 2.7). This result suggests the time of year did not play a role in where predators were located, however management may have played a role. An interesting pattern in both years was the interaction of management and location on predator community abundance and diversity. Raw catch data suggests predators in the high-input were most abundant on the edge (n=966), compared to the 25m (n=749), and center (n=697) of the orchard. While abundance of predators in the low-input were fairly evenly dispersed across the edge (n=656), 25m (n=654), and center (n=707). The interaction observed between management and field location suggests that predator communities had an edge-biased distribution along the edges of the high-input orchards. There are several studies and reviews that have explored driving factors for predator edge-effects (Tscharntke et al. 2002; (Whitehouse, Sial, and Schmidt 2017) Gallé et al. 2018; Nguyen and Nansen 2018). Emerging narratives for why edges may have higher species richness and abundance vary. Some studies attribute natural enemy distribution between the interior and edge to increased habitat heterogeneity and prey resources at the edge (Tscharntke et al. 2002, Tylianakis et al. 2004, Rand et al. 2006, Gallé et al. 2018, Nguyen & Nansen 2018). Other studies show evidence that management (chemical or mechanical) reduces predator numbers in agroecosystem interiors, and drives natural enemies to the edges (Thorbek & Bilde 2006, Whitehouse et al. 2017). As is often the case in science, it is likely a combination of the factors, and more research is needed. In this study, only the high-input orchards had evidence for edge-biased distribution of natural enemies, providing evidence of management may play a role.

Management did not affect the diversity of predators in 2021, however in 2020, the high-input orchards had greater diversity than the low-input orchards. This is a surprising result, as historically, predator diversity and abundance has been greater in “reduced-risk” compared to commercial management (Atanassov et al. 2003, Tscharntke et al. 2005, Krauss et al. 2011, Biddinger et al. 2014, Tylianakis & Binzer 2014, Crowder & Harwood 2014). There are a few considerations that may explain this result. One is the size of the orchard. In an effort to maintain consistency, we set our transects along the edge, at 25m and at 50m, so the sample area remained the same, however these orchards ranged in size. We were limited by site selection in this study, as there are no large-scale peach agroecosystems that manage with only fungicides. We were fortunate to use the USDA fruit and tree nut station for our low-input sites in 2020. The high-input orchards were large commercial size roughly 3 ha, where the reduced-risk orchards in 2020 were only about 1 ha. The concept of species richness decreasing with decreasing area is well documented (Debinski et al. 2001, Zimmerer 2010, Tscharntke et al. 2012, MacArthur & Wilson 2016, Magurran 2021). The sample area was the same for each orchard, but when the sample area is taken from a larger "patch", we may see this increased diversity. Another possible explanation for this diversity result could be the management differences between 2020 and 2021. The high-input orchards were the same in both years, however the low-input in 2020 was fungicide-only (no pesticides), and the low-input for 2021 was organic pesticides and fungicides. There was greater diversity of natural enemies in the no-insecticide low-input compared to the high-input, where the organic pesticides had similar diversity to the high-input (Figure 2.5C,2.5D). Organic pesticides are often considered "safer" for natural enemies, but in practice there is considerable variation in their effect on natural enemies (Bahlai et al. 2010, Puech et al. 2014, Roubos et al. 2014, Orpet et al. 2020). This is not to say that there are no benefits to organic management, however these studies emphasize the need for an integrated pest management approach.

There was significant variation throughout the season for canopy predator diversity in 2020, and for diversity and abundance in 2021 (Figure 2.5). Interestingly, predator populations fluxed throughout the season regardless of management regime (Figure 2.5, 2.6). This trend is especially visible in 2021, as diversity and abundance of predators continues to rise even though more intensive management begins after bloom (Blaauw et al. 2021). So, if management was not the driving factor, perhaps it was prey availability. In any ecosystem, there is an expected amount of flux driven by seasonality and resource continuity (Tscharntke et al. 2008, Iuliano & Gratton 2020). We did not monitor counts of pests (natural enemy resource), but we are inferring that pest presence will be higher when their host crop (peaches) are present and fruiting. The abundance of natural enemies in 2020 dropped around September after harvest was complete, and there are fewer resources in the orchards (Figure 2.5). In 2021, we see a more complete trend of diversity and abundance rising and falling accordingly with resource availability (the peach growing season). A key aspect of conservation biological control is maintaining refuges for natural enemies (Symondson et al. 2002, Shields et al. 2019). Meaning if there are habitat and/or resources for these natural enemies when peach orchards are dormant, they may carry over biological control services year-to-year. Greater diversity and abundance of natural enemies leads to a more buffered ecosystem (Yachi & Loreau 1999, Tscharntke et al. 2008). So, in the event of intensive management, or limited resources, growers may maintain viable populations of natural enemies for biocontrol services. The important consideration for peach growers is keeping a viable population of natural enemies that can deliver biological control services when they are needed.

Trophic interactions of canopy predators on key pests.

The gut content results were slightly unexpected, as we did not see a difference in predation activity in relation to management or by location (Figure 2.9). Previous literature predicts that predation activity may be higher along the edges (Blaauw et al. 2014, Nguyen & Nansen 2018) or reduced in higher intensity management (He et al. 2012, Biddinger et al. 2014). Because overall predation rates were low, it is possible our sample size was not high enough to reveal any interactions with space or management. Habitat management can alter the functional diversity of arthropod communities (Krauss et al. 2011, Tsharntke et al. 2012, Gallé et al. 2018). We did see the predator community structure change according to management, location, and sample date (Roubinet et al. 2018), however these variables did not alter the rate of predation by any individual taxa in this study. This may be promising for peach agriculture, as it suggests that communities of canopy predators may be resilient to management conditions.

In the rate of predation or proportion positive result (Figure 2.8), we see that three of the four major pests were infrequently attacked. As mentioned in the introduction, these species life cycles have few vulnerable life stages that are “visible”, which may result in less interactions with predators. The ecological success of these pests are part of the reason they are so heavily managed, and difficult to control in peaches (Knutson 2019, Blaauw et al. 2022). We do see a promising result for biological control of the SJS, as every common taxon that was screened tested positive for SJS DNA. These common taxa were naturally occurring insect predators in the peach orchards from five different orders. These diverse predators had different feeding mechanisms with chewing (Coleoptera, Hymenoptera), sucking (Hemiptera, Neuroptera), and rasping/sucking (Diptera), and all successfully fed on SJS. This finding reinforces the importance of functional diversity of predators (Griffin et al. 2013, Crowder & Jabbour 2014, Wan et al. 2019). Any reformation of future management in peaches should consider maintaining this diversity, and promote the most effective biocontrol agents.

In this study, two of the most abundant and effective predator families in peach orchards are Coccinellidae and Chrysopidae. Historically, there has been many studies that explored conservation biological control of Coccinellidae (Obrycki et al. 2009, Michaud et al. 2012, Amouroux et al. 2019) and Chrysopidae (Saminathan et al. 1999, Bouvet et al. 2019, Mahzoum et al. 2020). Coccinellidae and Chrysopidae share some behavioral and biological traits that may begin to explain their shared success against these pests in the peach system, most notably, their high rate of predation on SJS. Of the 460 Coccinellidae screened for SJS DNA, 12.82% were positive. Of the 374 Chrysopidae screened for SJS DNA, 24.86% were positive. Coccinellidae and Chrysopidae are both known predators of several species of scales (Michaud et al. 2012, Amouroux et al. 2019, Mahzoum et al. 2020). Both Coccinellidae and Chrysopidae are predaceous in their immature stages, and even have some morphological and behavioral similarities. The larvae forage by crawling along the surface of the plant scanning for prey until they encounter the prey and begin feeding (Clark & Messina 1997, Michaud et al. 2012). This foraging technique may give them an advantage for hunting the sessile SJS over predators that rely on visual ques.

Another promising result for Chrysopidae and Coccinellidae conservation biological control is their apparent tolerance to pesticides. In the principle response curve (Figure 2.6), predator communities in the low-input are compared to the control (commercial standard high-input) (Auber et al. 2017). Both Chrysopidae and Coccinellidae are less abundant in non-control modalities than in the control, meaning they show evidence of tolerating the high-input orchards. Other studies provide evidence that Chrysopidae (Giolo et al. 2009, Castilhos et al. 2014, Silva de Armas et al. 2019) and Coccinellidae (Biddinger & Hull 1995, Torres et al. 2015, Holý & Stará 2020) are tolerant of some pesticides. Additionally, both Chrysopidae (Mahzoum et al. 2020) and Coccinellidae (Michaud et al. 2012) were able to hunt prey at low densities, and maintain their populations, which is an important trait for conservation biological control. Both the past literature and empirical evidence from this study reinforce the evidence these natural enemies are promising candidates for IPM.

Ants are an interesting group for biological control in peaches. In this study, of the 648 ants screened, only 3% tested positive for the four main pests. While they may not be feeding on the main pests, they still may be providing biocontrol services. Other studies have demonstrated ants' ability to attack key peach pests such as the plum curculio (Jenkins et al. 2006), the BMSB (Cornelius et al. 2016), oriental fruit moth (Matthews et al. 2007) and various scale species (Martinez-Ferrer et al. 2003). Ants are one of the more abundant groups in the peach system.

Peach trees have extrafloral nectaries they use to attract predators (mostly parasitoids and ants) that provide biological control services (Cuautle & Rico-Gray 2003). Peach trees with extrafloral nectaries had higher populations of ants, higher rates of growth, and lower rates of herbivory (Matthews et al. 2007, Matthews et al. 2009). However, these studies found that when ants were present, overall predator diversity was lower, and oriental fruit moth predation was lower too- leading researchers to the conclusion ants disrupted predators that may have otherwise controlled oriental fruit moth populations (Matthews et al. 2007, Matthews et al. 2009). So, ants are a biological control agent, however they may disrupt certain community structures, requiring more research to conclude their overall effect in peach orchards.

Limitations and future directions

A possible limitation to this study is the sample collection technique. Tree beatings are an excellent way to obtain a point estimate of predator presence in the canopy, however they may underrepresent flying insects (Montgomery et al. 2021). We captured many flying insects for pest PCR screening, but the sample is likely lower because insects can escape quickly from the air. Other sampling methods could be considered to estimate canopy predator presence such as sweep netting or sucking sampling. However, the precision of insect collection into individual vials with beat sheets is preferable for MGCA, as suction sampling and sweep netting may result in insects becoming damaged, destroyed, or contaminated with another predator's DNA. The other limitation regarding site size and landscape was mentioned in the '*Management and community'* section of the discussion. We wanted to sample predator activity in all variations of management in Southeastern peaches. For the fungicide-only management, we were limited to the smaller experimental orchards. The same sampling area was applied to all the orchards; however, the surrounding landscape was not consistent for every orchard which may influence colonization into the orchards (Tscharntke et al. 2002). Future studies should consider sampling in an increased gradient into the natural area (Tylianakis & Binzer 2014, Schmidt et al. 2021).

Spiders are another promising area for future directions, as they are the most abundant taxa in our samples. Spiders have complicated and sometimes inconsistent roles in biological control (Michalko et al. 2019). Spiders can disrupt predator-prey dynamics either directly via intraguild predation (Traugott et al. 2012) or indirectly via behavior alterations in other predators (Schmidt-Entling & Siegenthaler 2009, Abrams & Cortez 2015). Spiders that utilize flowers as a hunting site may also reduce pollination services either by direct predation or behavioral alteration of pollinators (Jones & Dornhaus 2011, Wardhaugh et al. 2015). This is not to say that spiders are not beneficial. However, we did not PCR screen for intraguild predation or pollinators in this study. Future studies could increase the number of taxa screened for a more complete food web of Southeastern peaches.

Conclusions

One of the cornerstones of IPPM is conserving or promoting beneficial species to increase naturally occurring ecosystem services (Biddinger & Rajotte 2015, Egan et al. 2020). This is especially important in a perennial cropping system such as peaches, where beneficial species may carry over ecosystem services to the next year (Tscharntke et al. 2005, Wan et al. 2014, Gontijo 2019, Tooker et al. 2020). We surveyed Southeastern peach orchards under different management regimens for two years, and estimated beneficial arthropod communities and their spatiotemporal activity. We hypothesized we would find distinctly less diversity and abundance of beneficial arthropods in our high-input sites, however, we instead find that these communities and their activity may not be greatly different among the various management regimes. In some cases, we found greater abundance and diversity of pollinators and predators in the high-intensity management. There were many interactions with management and sample date. Naturally, with the seasonality of perennial crops there are going to be fluxes in resources during the different stages of the crop. These different crop stages are often paired with changes in management according to the stage the crop is in (e.g., : bloom, full fruiting, dormant) (Blaauw et al. 2022). So, the nature of peach management may be amplifying some of these interactions.

Using functional groups based on diet to explore the communities of potential pollinators allowed us to explore how abundance varied by management, sample date, with interactions between management and sample date. All of the functional groups abundances varied seasonally. The two Hymenoptera groups were generally more abundant in the high-input sites. And the two Diptera groups had significant interactions with management and sample date. Broadly assigning many families into functional groups is difficult ecologically, but it allows us to estimate potential pollination services. We know that the presence of the nectar and pollen feeding Hymenoptera may be an asset to IPPM services, as their presence was still high in commercial management. And the sheer abundance of the Diptera groups should be cause for consideration for growers, as these flies can provide significant pollination services (Orford et al. 2015, Uhl & Brühl 2019). In particular, we found that Dolichopodidae and Halictidae are among the most abundant flower-visiting insects in Southeastern peach orchards. Building an IPPM program with these families in mind may increase pollination, and potential biocontrol services with Dolichopodidae. We did not investigate pollination or flower visitation during bloom, but we now have a more complete understanding of the potential pollinator community.

Analyzing the gut content of canopy predator communities, paired with their spatiotemporal activity, allowed us to estimate predation rates on the key pests in peach orchards under different management regimens. Overall, the frequency of predation on these pests was low in both management regimens. By monitoring pest populations and predator populations simultaneously, we would gain a better perspective on how effective these predators are in true biological control contributions. Because we found the rate of predation and did not monitor pest populations, we cannot conclude how effective these predators are. However, rate of predation is useful information for building an IPPM program, as we know that promoting the key taxa, Coccinellidae and Chrysopidae, may increase biological control. We only found anecdotal evidence of edge-biased distribution of predators, but their communities were not significantly affected by location over time. The rate of predation did not vary by management or by location within the orchard. The diversity and abundance of predator communities did vary by time and management, with higher diversity and abundance in the high-input orchards at times. One of the cornerstones of conservation biological control is maintaining a functionally diverse group of predators (Tscharntke 2005, Gagic et al. 2015, Jonsson et al. 2017). We see this in our results as every abundant taxon fed on the SJS. Because SJS are particularly difficult to control with pesticides, this high rate of predation is promising for peach management. For this reason, growers should be mindful to maintain a diverse community of predators to ensure this potential biological control of the SJS.

This two year study in Southeastern peaches found unexpected success of beneficial arthropods in commercial management. However, the context of our sampling effort is important to consider. Our goal was to monitor these communities under all types of management, which meant that our site selection was limited. Some of our low-input sites were smaller than the high-input orchards, and the surrounding landscape ranged in composition. Patch size and landscape context are very important as they may predict species colonization rates (Blaauw & Isaacs 2012, Tscharntke et al. 2012, MacArthur & Wilson 2016). Future research should consider sampling the landscape surrounding the orchards, in order to evaluate how the management promotes or prevents colonization from naturally occurring beneficial arthropods. For the context of this study, management did alter beneficial community composition and activity throughout the season. However, the activity of pollinators and predation rates from natural enemies appears to be fairly resilient in Southeastern peach orchards.

Chapter 2 Tables

Table 2.1. Total catch data of flower-visiting insects. The 'taxa' column is organized with the functional group in bold, with the taxa belonging to the functional group below. The other columns are the total sum of the taxa within management and transect. The *Predaceous Diptera* functional group also contained parasitic Diptera. For this study, Cecidomyiidae, Ephydridae, and Phoridae were categorized into *Other Diptera*.

Taxa	low			high		
	edge	25m	50m	edge	25m	50m
Nectar/Pollen Feeding Hymenoptera	**314**	**325**	**248**	**643**	**607**	**783**
Andrenidae	9	5	2	18	15	11
Apidae (*Apis*)	1	1	0	1	0	1
Apidae (*Bombus*)	1	1	2	2	1	0
Apidae (Eucerini)	39	46	28	75	83	88
Apidae (Nomadinae)	0	0	0	0	1	1
Apidae (Xylocopinae)	2	3	2	10	8	9
Colletidae	2	4	1	1	3	7
Halictidae	257	264	211	536	496	666
Megachilidae	1	1	0	0	0	0
Pompilidae	1	0	1	0	0	0
Tenthredinidae	1	0	1	0	0	0
Predatory/Parasitic Hymenoptera	**152**	**171**	**171**	**298**	**299**	**320**
Crabronidae	16	15	3	120	151	169
Parasitoids	133	151	166	175	145	150
Sphecidae	3	5	2	3	3	1
Tiphiidae	0	0	1	0	0	0
Predaceous Diptera	**572**	**632**	**672**	**589**	**560**	**401**
Asilidae	15	29	19	13	5	5
Dolichopodidae	507	535	600	388	250	212
Empididae	1	0	0	0	0	0
Muscidae (*Coenosia*)	36	50	36	164	273	158
Pipunculidae	0	0	1	1	1	1
Syrphidae	9	10	15	22	31	25
Tachninidae	4	8	1	1	0	0
Other Diptera	**512**	**376**	**309**	**784**	**692**	**602**
Agromyzidae	0	1	1	4	4	2
Anthomyiidae	2	2	5	16	28	27
Bibionidae	1	0	0	1	0	0
Calliphoridae	2	0	0	4	1	0
Cecidomyiidae	93	54	52	121	166	107
Ceratopogonidae	1	2	0	2	2	0
Chironomidae	12	4	4	4	0	5
Chloropidae	1	1	1	5	2	6
Conopidae	0	0	0	0	1	0
Culicidae	1	0	0	0	1	0
Drosophilidae	151	132	51	178	180	224
Ephydridae	11	15	10	29	22	21
Heleomyzidae	1	0	1	1	5	3
Muscidae (Excluding *Coenosia*)	51	45	50	218	103	95
Mycetophilidae	3	1	0	1	0	1
Phoridae	39	23	38	42	46	25
Psychodidae	3	2	0	1	0	0
Sarcophagidae	90	41	67	89	57	45
Sciaridae	44	35	40	59	68	37
Sciomyzidae	0	0	0	1	0	0
Stratiomyidae	0	0	0	0	1	0
Tephritidae	1	3	2	8	4	3
Tipulidae	5	0	2	0	1	1

Table 2.2. Non-target taxa screened in primer specificity test for SJS. The taxa in bold are orders of arthropods screened, and below the orders are the families that were screened.

Non-target Taxa Screened	
Araneae	**Collembola**
Araneidae	**Diptera**
Lycosidae	Bibionidae
Oxyopidae	Dolichopodidae
Salticidae	Syrphidae
Thomisidae	Tipulidae
Coleoptera	**Hemiptera**
Anthicidae	Aleyrodidae
Coccinellidae	Aphididae
Elateridae	Cydnidae
Lampyridae	Diaspididae
Meloidae	Membracidae
Scarabaeidae	Miridae
Hymenoptera	Nabidae
Apidae	Pentatomidae
Formicidae	Pseudococcidae
Pompilidae	Reduviidae
Neuroptera	
Chrysopidae	
Hemerobiidae	
Thysanoptera	

Table 2.3. Canopy arthropod totals and means by treatment and location over the two-year sample period. The 'taxa' column is organized with the arthropod order in bold, with the taxa belonging to the order below. The other columns are management (high-input or low-input) and location within the orchard (edge, 25m and 50m). Boxes contain overall total count as the first number, and mean (1SE) for each taxa by treatment and location. It should also be noted that some taxa listed are not predaceous, however pest DNA was detected in the PCR screening (Bostrichidae, Chrysomelidae, Curculionidae, Mordellidae, Nitidulidae, Scarabaeidae, Bibionidae, Simuliidae and Tephritidae).

Taxa	low			high		
	edge	25m	50m	edge	25m	50m
Araneae (total)	**285,5.7(0.63)**	**294,5.55(0.68)**	**312,6.24(0.86)**	**466,7.4(0.96)**	**342,5.61(0.65)**	**313,5.13(0.57)**
Blattodea (total)	**0**	**0**	**0**	**1**	**0**	**0**
Ectobiidae	0	0	0	1,0.02(0.02)	0	0
Coleoptera (total)	98	84	126	237	168	121
Anthicidae	15,0.3(0.19)	0	3,0.06(0.03)	50,0.79(0.33)	12,0.2(0.08)	15,0.25(0.12)
Bostrichidae	1,0.02(0.02)	0	0	0	0	0
Carabidae	1,0.02(0.02)	0	0	0	0	0
Chrysomelidae	4,0.08(0.06)	4,0.08(0.06)	4,0.08(0.05)	33,0.52(0.39)	47,0.77(0.33)	10,0.16(0.06)
Coccinellidae	57,1.14(0.33)	60,1.13(0.23)	88,1.76(0.41)	109,1.73(0.38)	80,1.31(0.27)	71,1.16(0.24)
Curculionidae	0	0	1,0.02(0.02)	0	0	0
Cybocephalidae	0	0	0	0	4,0.07(0.05)	3,0.05(0.04)
Elateridae	17,0.34(0.12)	16,0.3(0.14)	25,0.5(0.24)	42,0.67(0.29)	18,0.3(0.14)	18,0.3(0.11)
Lampyridae	0	0	0	1,0.02(0.02)	0	0
Meloidae	0	0	2,0.04(0.03)	0	0	0
Mordellidae	0	0	0	0	1,0.02(0.02)	0
Nitidulidae	0,0(0)	3,0.06(0.06)	2,0.04(0.04)	0,0(0)	3,0.05(0.03)	4,0.07(0.03)
Scarabaeidae	3,0.06(0.06)	0	0	1,0.02(0.02)	0	0
Staphylinidae	0	1,0.02(0.02)	1,0.02(0.02)	1,0.02(0.02)	3,0.05(0.03)	0
Dermaptera (total)	**0**	**0**	**0**	**1**	**2**	**1**
Anisolabididae	0	0	0	0	1,0.02(0.02)	1,0.02(0.02)
Forficulidae	0	0	0	0	1,0.02(0.02)	0
Labiduridae	0	0	0	1,0.02(0.02)	0	0
Diptera (total)	**5**	**5**	**2**	**11**	**16**	**22**
Asilidae	0	0	0	1,0.02(0.02)	1,0.02(0.02)	0
Bibionidae	1,0.02(0.02)	0	0	0	0	0
Cecidomyiidae	0	0	0	0	3,0.05(0.03)	1,0.02(0.02)
Ceratopogonidae	0	0	0	0	0	1,0.02(0.02)
Dolichopodidae	3,0.06(0.03)	3,0.06(0.03)	0	1,0.02(0.02)	2,0.03(0.02)	3,0.05(0.03)
Muscidae	1,0.02(0.02)	1,0.02(0.02)	0	4,0.06(0.04)	2,0.03(0.02)	2,0.03(0.02)
Sarcophagidae	0	1,0.02(0.02)	0	1,0.02(0.02)	0,0(0)	1,0.02(0.02)
Simuliidae	0	0	2,0.04(0.04)	0	0	0

Table 3 Continued						
Syrphidae	0	0	0	1,0.02(0.02)	0	0
Tephritidae	0	0	0	0	0	1,0.02(0.02)
Tipulidae	0	0	0	3,0.05(0.03)	8,0.13(0.05)	12,0.2(0.07)
Hemiptera (total)	**53**	**80**	**69**	**78**	**66**	**85**
Alydidae	1,0.02(0.02)	0	0	0	0	0
Anthocoridae	34,0.68(0.36)	62,1.17(0.55)	49,0.98(0.48)	39,0.62(0.2)	36,0.59(0.2)	52,0.85(0.33)
Cicadellidae	0	0	1,0.02(0.02)	0	0	0
Coreidae	0	0	1,0.02(0.02)	0	0	0
Geocoridae	0	0	0	4,0.06(0.03)	5,0.08(0.04)	4,0.07(0.04)
Miridae	2,0.04(0.04)	1,0.02(0.02)	0	0	0	0
Nabidae	1,0.02(0.02)	4,0.08(0.05)	1,0.02(0.02)	6,0.1(0.08)	7,0.11(0.04)	5,0.08(0.04)
Pentatomidae	5,0.1(0.07)	0	4,0.08(0.05)	2,0.03(0.02)	0	1,0.02(0.02)
Reduviidae	10,0.2(0.07)	13,0.25(0.08)	13,0.26(0.08)	27,0.43(0.11)	18,0.3(0.09)	23,0.38(0.1)
Hymenoptera (total)	**139**	**140**	**151**	**121**	**87**	**83**
Crabronidae	0	0	0	0	0	1,0.02(0.02)
Formicidae	135,2.7(0.31)	140,2.64(0.24)	148,2.96(0.34)	101,1.6(0.26)	75,1.23(0.19)	72,1.18(0.2)
Parasitoid	4,0.08(0.04)	0	2,0.04(0.03)	18,0.29(0.08)	11,0.18(0.07)	10,0.16(0.06)
Pompilidae	0	0	1,0.02(0.02)	0	0	0
Tiphiidae	0	0	0	1,0.02(0.02)	1,0.02(0.02)	0
Vespidae	0	0	0	1,0.02(0.02)	0	0
Mantodea (total)	1	0	2	1	0	0
Mantidae	1,0.02(0.02)	0	2,0.04(0.03)	1,0.02(0.02)	0	0
Neuroptera (total)	**72**	**49**	**47**	**55**	**67**	**73**
Chrysopidae	69,1.38(0.52)	44,0.83(0.27)	47,0.94(0.27)	53,0.84(0.19)	64,1.05(0.18)	70,1.15(0.21)
Hemerobiidae	3,0.06(0.04)	5,0.09(0.08)	0	2,0.03(0.02)	3,0.05(0.03)	3,0.05(0.03)
Opiliones	**1,0.02(0.02)**	**0**	**0**	**0**	**0**	**0**
Psocoptera	**3,0.06(0.03)**	**2,0.04(0.03)**	**1,0.02(0.02)**	**0**	**1,0.02(0.02)**	**1,0.02(0.02)**

Table 2.4. Canopy arthropods screened and their proportion positive for key pest DNA. The 'taxa' column is organized with the arthropods order in bold, with the taxa belonging to the order below. 'Total count' column is the total number of the taxa screened. The other columns are the key pest that was screened for in the PCR; 'PC' plum curculio, 'SJS' San Jose scale, 'BMSB' brown marmorated stink bug, and 'Lepidoptera' as an order. The numbers are the predator proportion positive for the specific pest. It should also be noted that some taxa listed are not predaceous, however pest DNA was detected in the PCR screening (Bostrichidae, Chrysomelidae, Mordellidae, Nitidulidae, Scarabaeidae, Simuliidae).

Predator Taxa	Total count	PC	SJS	BMSB	Lepidoptera
Coleoptera (total)	**744**	**0.004**	**0.1022**	**0**	**0.004**
Anthicidae	94	0.24	0.03	0	0
Bostrichidae	1	0	0	0	0
Carabidae	1	0	0	0	0
Chrysomelidae	20	0	0.05	0	0
Coccinellidae	460	0.13	0.13	0	0.01
Cybocephalidae	7	0	0.43	0	0
Elateridae	136	0	0.05	0	0
Lampyridae	1	1	0	0	0
Meloidae	2	0	0	0	0
Mordellidae	1	0	0	0	0
Nitidulidae	12	0.17	0.08	0	0
Scarabaeidae	4	0	0.25	0	0
Staphylinidae	5	0	0.2	0	0
Hymenoptera (total)	**697**	**0**	**0.0473**	**0.0014**	**0.0158**
Crabronidae	1	0	0	0	0
Formicidae	648	0	0.0494	0.0015	0.0167
Parasitoid	44	0	0.0227	0	0
Pompilidae	1	0	0	0	0
Tiphiidae	2	0	0	0	0
Vespidae	1	0	0	0	0
Hemiptera (total)	**407**	**0.0025**	**0.0516**	**0**	**0.0197**
Anthocoridae	270	0	0.0111	0	0
Formicidae	1	0	0	0	0
Geocoridae	9	0	0.4444	0	0.2222
Miridae	3	0	0	0	0
Nabidae	22	0	0.0454	0	0
Reduviidae	102	0.0098	0.1176	0	0.049
Neuroptera (total)	**363**	**0.0028**	**0.2645**	**0**	**0.0248**

Table 4 Continued	347	0.0029	0.268	0	0.0259
Chrysopidae	347	0.0029	0.268	0	0.0259
Hemerobiidae	**16**	**0**	**0.1875**	**0**	**0**
Diptera (total)	**58**	**0**	**0.0517**	**0**	**0**
Asilidae	2	0	0	0	0
Bibionidae	1	0	0	0	0
Cecidomyiidae	4	0	0	0	0
Ceratopogonidae	1	0	0	0	0
Dolichopodidae	11	0	0.0909	0	0
Muscidae	10	0	0.1	0	0
Sarcophagidae	3	0	0	0	0
Simuliidae	2	0	0	0	0
Syrphidae	1	0	0	0	0
Tipulidae	23	0	0.0435	0	0
Dermaptera (total)	**4**	**0**	**0.25**	**0**	**0**
Anisolabididae	2	0	0	0	0
Forficulidae	1	0	1	0	0
Labiduridae	1	0	0	0	0
Mantodea (total)	**2**	**0**	**0**	**0**	**0**
Mantidae	2	0	0	0	0

Chapter 2 Figures:

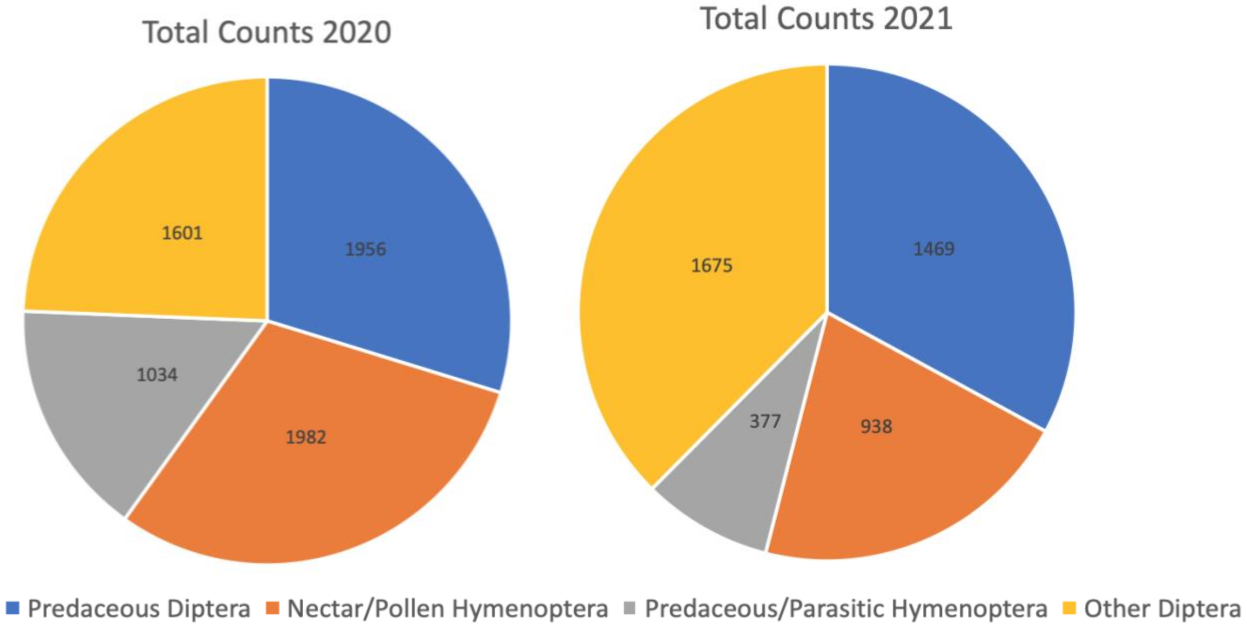

Figure 2.1.
Total community raw sum of flower-visiting insect functional groups for 2020 and 2021. Functional group colors are: Predaceous Diptera (Blue), Nectar/pollen feeding Hymenoptera (Orange), Predaceous/parasitic Hymenoptera (Grey), Other Diptera (Yellow).

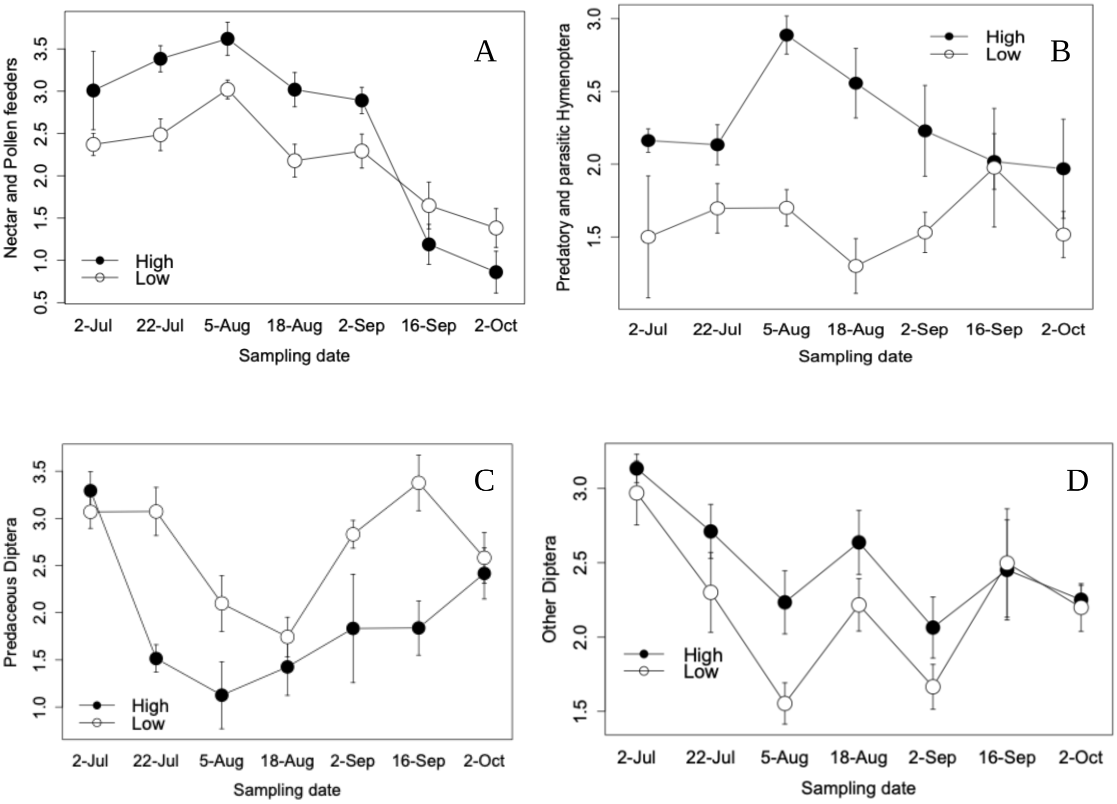

Figure 2.2. 2020 seasonal activity of flower-visiting insect functional groups (A) nectar and pollen feeders, (B) predaceous and parasitic Hymenoptera, (C) predaceous Diptera, (D) other Diptera. Y axis log transform mean counts per bowl +/-1 SE. X axis is sample date.

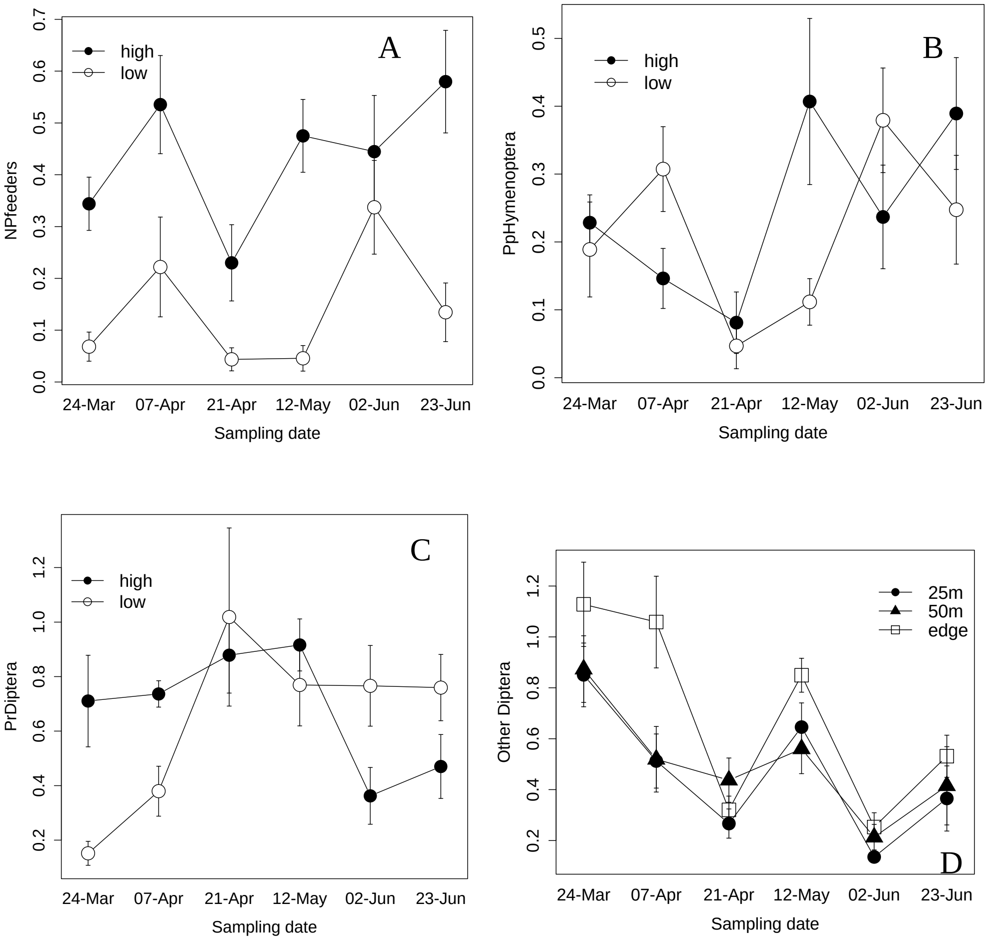

Figure 2.3. 2021 seasonal activity of flower-visiting insect functional groups (A) nectar and pollen feeders, (B) predaceous and parasitic Hymenoptera, (C) predaceous Diptera, (D) other Diptera. Y axis log transform mean counts per bowl +/-1 SE. X axis is sample date.

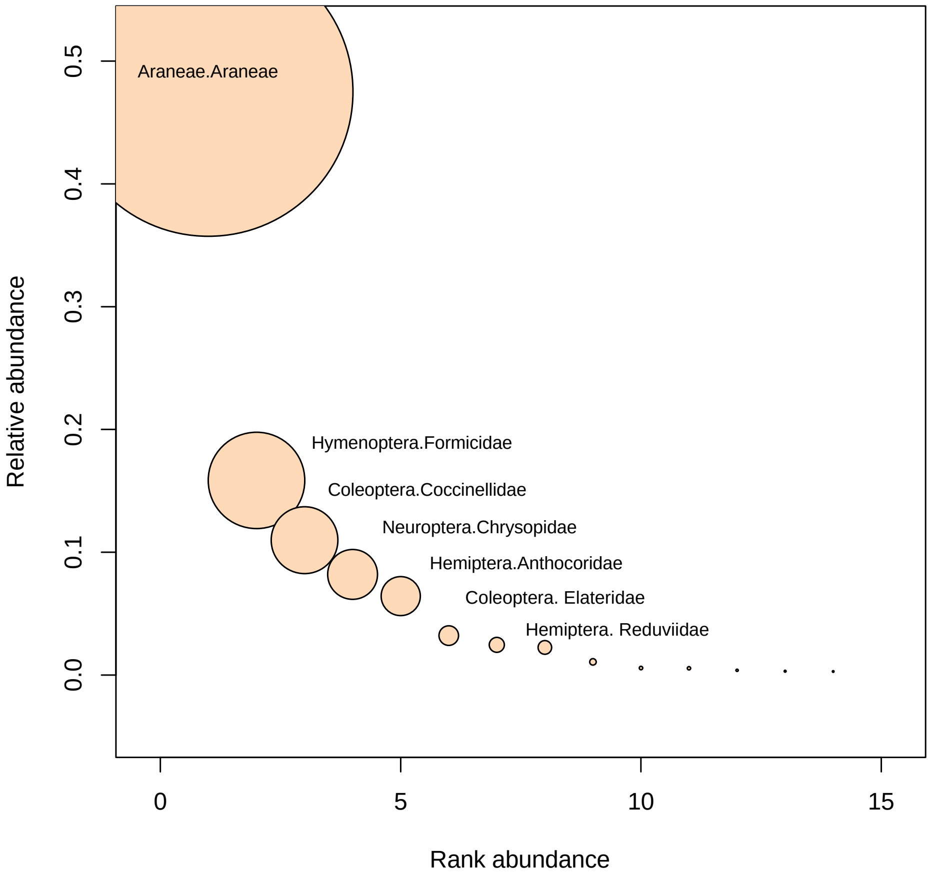

Figure 2.4. Rank abundance for total peach canopy predators (4440) over two years. Only 13 taxa were present in >4% of all samples, of which 6 taxa were the most common. Y axis is the relative abundance of the taxa relative to the others. X axis is the order of abundance (rank).

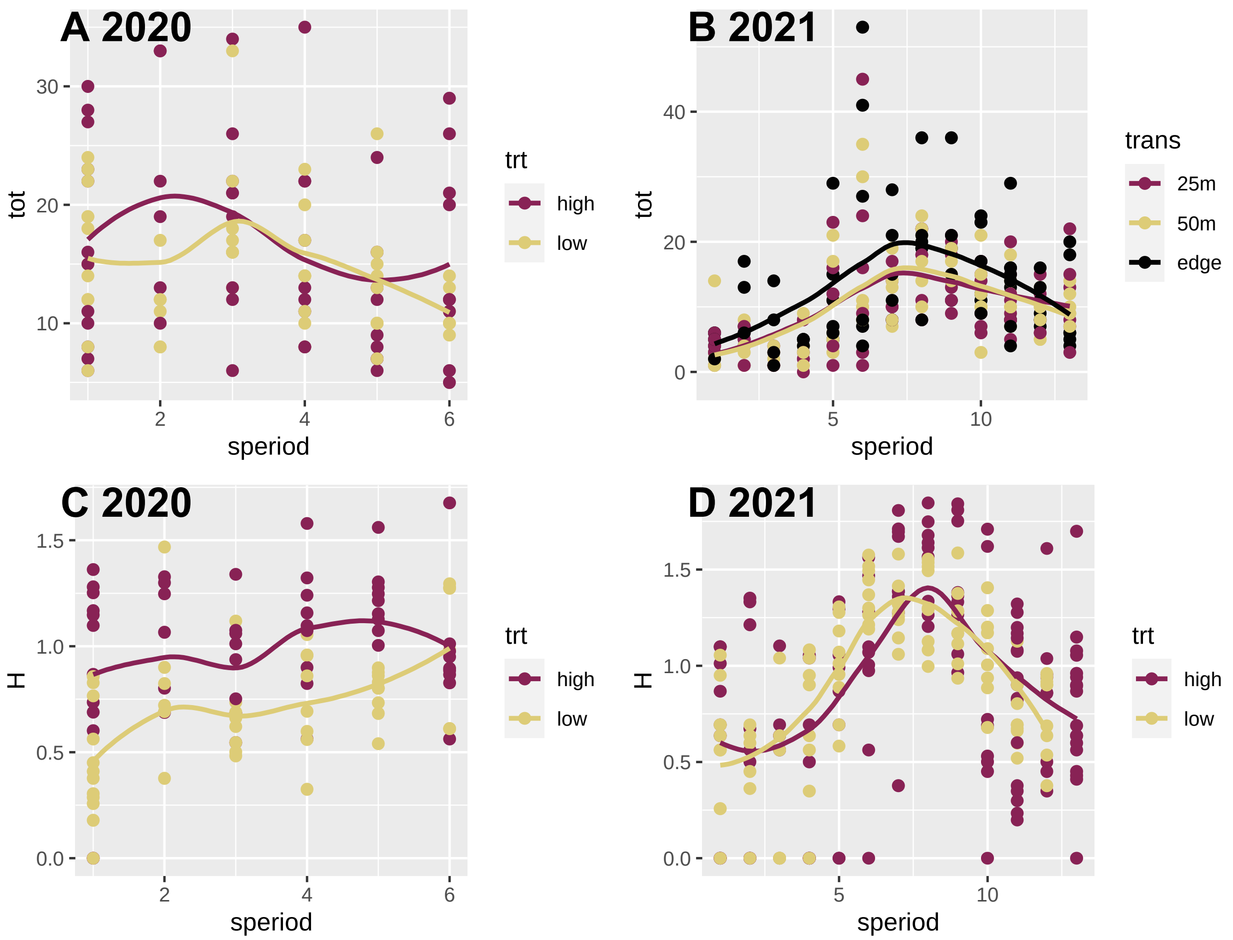

Figure 2.5. Seasonal patterns of canopy predators for 2020 abundance (A), 2021 abundance (B), 2020 diversity (C), and 2021 diversity (D). Y axis are abundance 'tot' and diversity 'H'. X axis is converted sample date to sample period; Meaning the value on the X axis corresponds the number of sampling dates for each month. For 2020, July1,2, August 3,4, September 5,6. For 2021, March1, April2,3, May5,6, June7,8, July9,10, August10,11, September12, October13.

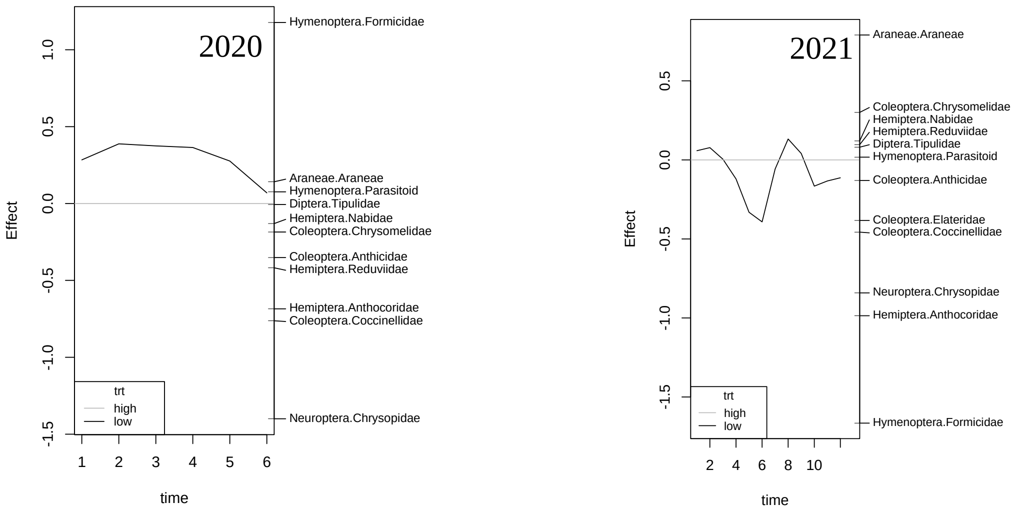

Figure 2.6. Management effects over time on community structure (left: 2020, right:2021). Y axis is the effect of low-input on the taxa relative to the control (high-input). X axis is converted sample date to sample period; Meaning the value on the X axis corresponds the number of sampling dates for each month. For 2020, July1,2, August 3,4, September 5,6. For 2021, March1, April2,3, May5,6, June7,8, July9,10, August10,11, September12, October13.

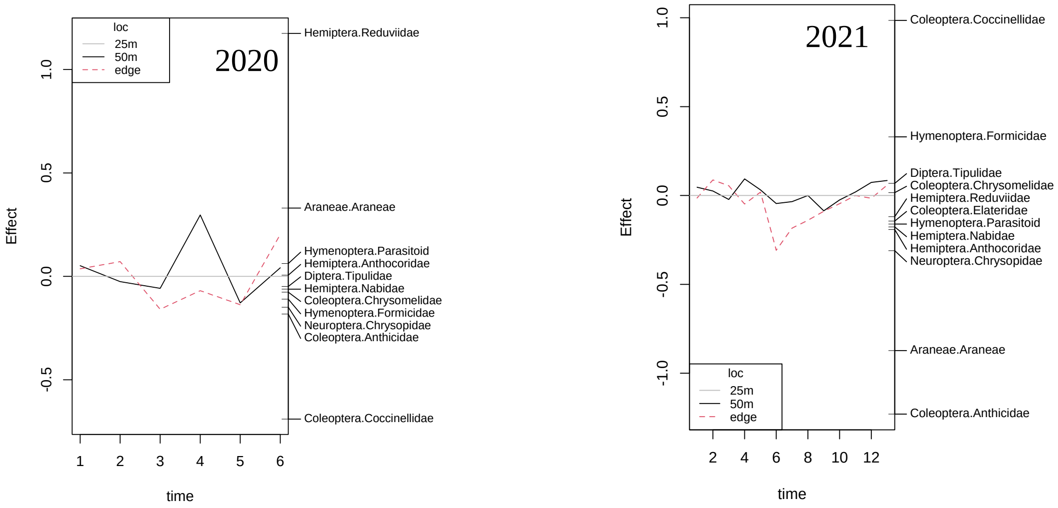

Figure 2.7. Location effects on canopy community location over time (left:2020, right:2021). Y axis is the effect of location, where taxonomic group locations on the edge and 50m interior are compared to the control (25m). X axis is converted sample date to sample period. Meaning the value on the X axis represents the number of sampling dates. For 2020, we sampled twice in July, so two sample dates in July, we sampled twice in August, so two sample dates in August, two sample dates in September (July1,2, August 3,4, September 5,6). For 2021, we sampled once in March, so one sample date in March, we sampled twice in April, two sample dates in April, and so on, for 13 total sampling dates (March1, April2,3, May5,6, June7,8, July9,10, August10,11, September12, October13).

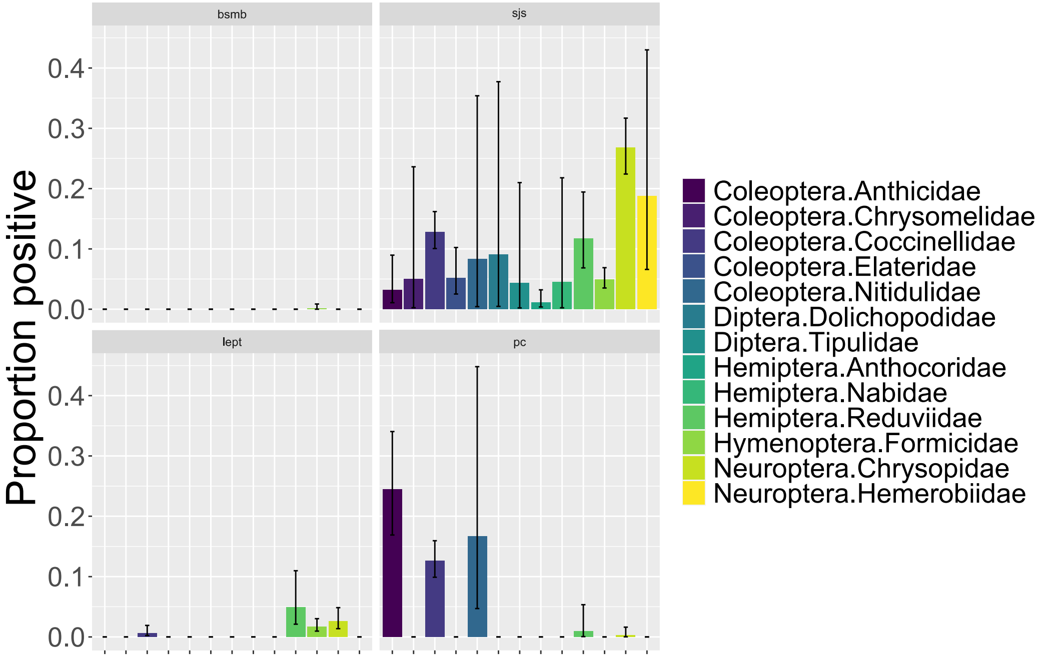

Figure 2.8. Proportion positive for taxa present in peach orchards. The taxa listed on the right had abundance >10 screened. The quadrants represent the four main peach pests; Brown Marmorated Stink Bug (bmsb), San Jose scale (SJS), Lepidoptera (lept), and Plum Curculio (pc). Y axis is proportion positive of taxa screened, with 95% CI in variability of proportion positive.

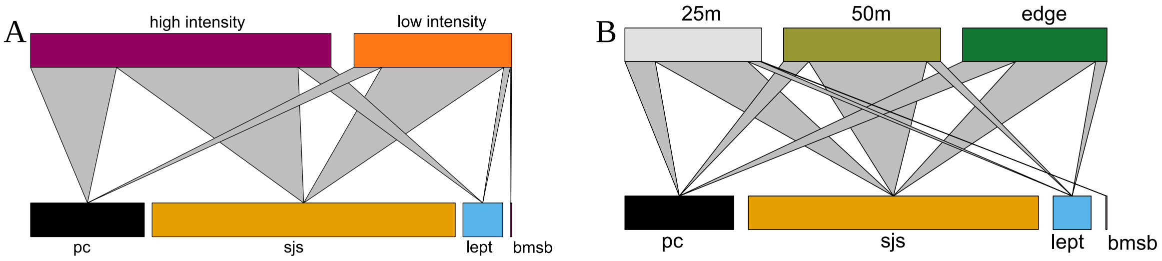

Figure 2.9. Consumer web for total catch data by treatment (A) and location (B). For treatment (A) and location (B), the bars above represent the number of screened predators from each treatment or location, respectively. The bars below represent the four major peach pests; Plum Curculio (pc), San Jose scale (SJS), Lepidoptera (lept), and Brown Marmorated Stink Bug (bmsb). The area connecting the bars indicates the relative proportion positive PCR results for the linked pest.

Chapter 3: First Documented Wild Population of the "Hunter Fly", *Coenosia attenuata* Stein (Diptera: Muscidae) in North America

Introduction

The hunter fly, *Coenosia attenuata* Stein (1903) (Diptera: Muscidae), is a widespread predatory fly often associated with greenhouses (Seabra et al. 2015). Phylogenetic studies suggest that *C. attenuata* originated in the Mediterranean region, but is now known to occur in Europe, Asia, the Middle East, Africa, South America, and North America. Across this wide distribution, the species exhibits limited mitochondrial genetic diversity, leading Seabra et al. to propose that much of its current range is due to human-mediated expansion into greenhouses worldwide. Hoebeke et al. hypothesized that the larvae are transported in potted plant soil. The larvae are soil-dwelling, polyphagous predators capable of living for 20–35 days even when prey is scarce (Ugine et al. 2010).

Coenosia attenuata is a generalist predator in both adult and larval stages and, as such, is a biological control agent of multiple larval and adult greenhouse pests (Kühne 1998; Pohl et al. 2012; Zou, Coudron, Zhang, et al. 2021; Zou, Coudron, Xu, et al. 2021). Common prey include: whiteflies (Hemiptera: Aleyrodidae), various Diptera, including fungus gnats (Sciaridae), leaf-miner flies (Agromyzidae), and pomace flies (Drosophilidae) (Bautista-Martínez, Illescas-Riquelme, and de Jesus García-Ávila 2017; Tellez et al. 2009). The hunter fly thrives in artificial greenhouse environments and has been documented in New World greenhouses in Ecuador (Martinez-Sanchez, Marcos-Garcia, and Pont 2002), Peru, Colombia (Pérez 2006), Costa Rica (Hernández-Ramírez 2008), Chile (Couri and Salas 2010), Venezuela (Solano-Rojas et al. 2017), Mexico (Bautista-Martínez, Illescas-Riquelme, and de Jesus García-Ávila 2017), Honduras (Orozco 2018), Brazil (Couri et al. 2018), Uruguay (Giambiasi et al. 2020), and the USA (Hoebeke et al. 2003). However, reports of *C. attenuata* outside of the greenhouse environment are uncommon, especially in the New World. In the first report of the species for North America, most records were greenhouse-based records, except for a single report of specimens captured in malaise traps in suburban Los Angeles, California (Hoebeke et al. 2003). The only other records of *C. attenuata* found in an open field setting in the New World are from South America, where adults were reported on baby's breath flowers (Caryophyllaceae: *Gypsophila paniculata* L.) in Northeastern Brazil in 2016 and 2017 (Couri et al. 2018), and on blueberries (Ericaceae: *Vaccinium* sp.) in Chile (Couri and Salas 2010).

During insect sampling in peach orchards in South Carolina (SC) and Georgia (GA), USA, in 2020 and 2021, 717 specimens of unknown fly species were collected in colored pan traps. The specimens were identified as *Coenosia attenuata* using morphological and molecular approaches (Figure 1.1). Here we document this new occurrence in peach orchard systems, provide a preliminary phenology for the species in peach orchards of the Southeastern USA, provide diagnostic information to assist in the recognition of this species based on the anatomy, and contribute COI barcodes to Genbank.

Materials and Methods

Field Sites

The study was conducted in peach orchards in the Southeastern USA. The climate in the Southeastern USA is highly variable, experiencing extremely high temperatures in the summer and low temperatures in the winter months. There is also considerable humidity, and an average rainfall of between 44 and 52 inches per year (Konrad and Fuhrmann 2013). The study sites were located in Byron, GA, and Monetta, SC, approximately 240 km apart (Figure 1.1). In GA, the six study sites were separated by 1–5 km, and in SC, the study sites were separated by 1–3 km. In an effort to maintain consistency, we selected orchards that were approximately the same size (1–3 ha). Peach orchards have largely sandy soil within the rows where peach trees are planted. The ground within and between rows is naturally well-lit, as trees are typically spaced 5 m apart, separated by grasses that are frequently mowed (Figure 1.1A–C). The orchards we sampled were managed with recommended practices following the 2022 Southeastern Peach Guide, with intensive commercial chemical applications or “high input” management, or “low input” (i.e., fungicide only in 2020, and organic fungicide and insecticide in 2021) management (Figure 1.1A–C). The high input orchards were treated primarily with a pyrethroid-based spray regimen, as well as supplementary fungicide sprays (Blaauw et al. 2022).

Sample Collection

We used pan traps colored with blue, white, or yellow fluorescent paint to estimate the activity of flower-visiting insects, such as Hymenoptera, Diptera, and Lepidoptera (Campbell and Hanula 2007; Schlueter and Stewart 2015). Traps were constructed of plastic bowls (7.1 cm diameter) filled with dish soap (to break surface tension) and water. Twenty-seven traps were deployed per field site and were collected after 24 h. Traps were deployed in sets of three (blue, white, and yellow grouped together) and placed under nine trees in a transect pattern. Three trees along the edge of the orchard, three at 25 m towards the interior, and three at 50 m toward the interior. A total of 2244 traps were deployed over 17 sample dates. Traps were deployed monthly between July and October in 2020, and from March–September in 2021. During this study, many other insects were collected, however here we only report on *Coenosia attenuata*.

Sample Processing

While sorting the pan traps, we discovered an unusual dipteran, and initially we sent representative specimens to E. Richard Hoebeke (University of Georgia Collection of Arthropods) and Adrian C. Pont (Oxford University) for identification. Hoebeke and Pont confirmed the identification of the fly as *Coenosia attenuata*. Remaining traps were then sorted to document all specimens of this fly, and subsequent specimens were morphologically identified by the authors using Hoebeke et al.'s guide. *Coenosia attenuata* is a member of the *tigrina*-group of *Coenosia* (*sensu* Hennig 1964) (Hennig and Lindner 1964), which includes Old World species that can be diagnosed by the occurrence of two major diverging bristles near the midlength of the anterior and anterodorsal surfaces of the hind tibia (Figure 3.1D). Two other species of this group have been introduced into North America, *Coenosia tigrina* F. (1775) and *Coenosia humilis* Meigen (1826) [2]. *Coenosia attenuata* can be distinguished from these two species based on its smaller size, having a body length of 2.5–3.0 mm for males and 3.0–4.0 mm for females, (*C. tigrina*: 4.75–5.75 mm, male and 5.75–7.0 mm, female; *C. humilis*: 3.0–3.5 mm, male and 3.0–5.5 mm, female), and by the yellow color of the femora in males (*C. tigrina* femora are black with a reddish-yellow apex; *C. humilis* femora are black). In addition, the form of the male genitalia of *C. attenuata* is diagnostic (Figure 3.1G). A detailed taxonomic description and natural history summary for *C. attenuata* are given by Hoebeke et al.

Three male specimens were identified using DNA barcoding of the cytochrome oxidase 1 (CO1) gene. Genomic DNA was extracted from individual flies (whole body extraction) using the Qiagen DNeasy Blood and Tissue kit, following the manufacturer's protocol. Extracted DNA was stored at −20 °C. A negative extraction control that contained all Qiagen buffers for extraction and Proteinase K solutions was included. The DNA barcode region of the CO1 gene was amplified using standard DNA barcoding primers (Folmer et al. 1994), following the protocol described by Cutler et al. (Cutler et al. 2015). PCR products were sent for purification and sequencing at Eurofins (©Eurofins Scientific 2021). Purified PCR products were sequenced bidirectionally. Using Codon Code Aligner version 9.0.1, forward and reverse sequences were assembled, aligned, and edited to trim the remaining primer sequences. Prepared sequences were then "blasted" against all sequence records in public databases of the Barcode of Life Data System (BOLD) and the National Center for Biotechnology Information (NCBI). A representative male and female were imaged by transferring preserved specimens with 75% ethanol to amyl acetate for 48 h before being mounted on a minute pin and air dried. Specimens were photographed using the automated image rendering of a Keyence VHX-7000 digital imaging microscope (Keyence, Itasca, IL, USA). Voucher specimens were deposited in the University of Georgia Collection of Arthropods (UGCA) in Athens, Georgia, USA.

Statistical Analyses

We fitted several statistical models to test if hunter-fly counts were influenced by sampling date, trap color, or management intensity (i.e., high or low chemical input), and to assess male:female sex ratios. In each of the models, we fitted general mixed effect models (GLMMs) using the GLIMMIX procedure. AR1 covariance structure of errors was used in testing for seasonal variation, which considered repeated measures. To test if hunter fly counts were influenced by pan trap color, we fitted a GLMM of hunter fly pooled counts by field. Trap color was the fixed effect, and transect and date were set as random effects. To ask whether hunter flies in the Southeast are multivoltine, we assessed significant seasonal variation in hunter fly counts for each year using GLMMs with date as the fixed effect and transects nested in fields used as random effects. Lastly, management intensity (i.e., high or low chemical input) in orchards may affect the abundance of hunter flies. Therefore, in similar structured models, we analyzed the effect of management and field position (i.e., transect) on hunter fly counts using GLMMs with management intensity, transect, and the interaction between management intensity and transect as fixed effect terms with date nested within field as random effects. The differences in the model structure of fixed effects and random effects were needed to test different hypotheses. In all cases, natural log transformed hunter fly counts were used as the response variable, which improved normality and homogeneity of variance. The statistical analyses were conducted in SAS version [9.4] (2013) by SAS Institute Inc., Cary, NC, USA.

Results

Coenosia attenuata is a small, light gray fly with a muscoid body form (Figure 3.1A–C). The tarsi are long with black setulae and at rest are held slightly curved, giving a raptorial appearance (Figure 3.1A,B,D). The sexes are dimorphic. Males are smaller than females (see above). The male head has a bright silvery-white pruinose vertex, frons, parafacials, and lunule (Figure 3.1E). The female head is pruinose and gray with two dark converging longitudinal stripes on the frons (Figure 3.1F). The legs are yellowish in males with slightly darker tarsi (Figure 3.1A,D). The legs of females are grayish, especially on the femora (Figure 3.1B). The abdomen is uniformly light gray in males (Figure 3.1A,C,G) or has some indistinct darker blotches, but in females, the abdomen bears three distinct black transverse stripes (Figure 3.1B,H). The morphological characteristics described above, and the COI sequence analysis confirmed the collected specimens to be *C. attenuata*. All three individuals that were barcoded yielded a 658-bp fragment (Genbank accession numbers: ON257860–ON257862), and were consistent (i.e., 100% overlap and >99% identity match) with public sequences for *C. attenuata* on both BOLD and Genbank.

Overall, we captured 609 individuals in GA and 108 in SC for a total of 717 *C. attenuata* over the two-year study. In 2020, we captured a total of 231 and in 2021, 486 C. *attenuata*. For both male and female hunter flies, in 2020 there was significant variation in counts throughout the season ($F_{6,30} = 5.57$, $p = 0.0006$; $F_{6,30} = 8.67$, $p \leq 0.0001$, respectively), with a peak in July (Figure 3.2A). In 2020, there were significantly more flies caught on July 2nd than on all other sample dates, expect for another peak in capture on September 16th (Tukey-Kramer). There was marginally significant variation in seasonal data for male and female hunter flies in 2021 ($F_{9,34} = 1.95$, $p = 0.0783$; $F_{9,34} = 1.98$, $p = 0.0734$, respectively; Figure 3.2B).

Coenosia attenuata was found at both high-input and low-input managed peach orchards during both years of the study. Our sampling efforts over two years produced a greater number of specimens from the high input sites (n = 595) than the low input sites (n = 122) (Figure 4). Trap color appears to be a significant predictor of hunter-fly counts observed in traps for both males ($F_{2,255} = 34.78$, $p \leq 0.0001$) and females ($F_{2,255} = 16.47$, $p \leq 0.0001$). A greater number of hunter flies were observed in white bowls (n = 585) as compared to either blue (n = 45) or yellow (n = 77) ($t = -7.65$, df = 255, $p \leq 0.0001$; $t = 6.70$, df = 255, $p \leq 0.0001$, respectively).

The number of male hunter flies captured was influenced by management strategy ($F_{1,179} = 16.64$, $p \leq 0.0001$; Figure 4), no significant influence of field transects ($F_{2,179} = 2.50$, $p = 0.0847$), and no interaction between management and field transects ($F_{2,179} = 1.49$, $p = 0.2272$) (Figure 4). More males were observed in high input management than in low input ($t = 4.08$, $p \leq 0.0001$) (Figure 4). For females, management strategy had a significant effect on the number of flies captured ($F_{1,179} = 13.61$, $p \leq 0.0001$), with no transect effect ($F_{2,179} = 0.98$, $p = 0.3773$) or interaction between management and field transect ($F_{2,179} = 1.12$, $p = 0.3301$). The management effect is explained by higher numbers of female hunter flies observed in the high input system ($t = 5.29$, $p \leq 0.0001$) (Figure 3.3). Lastly, abundance patterns showed male hunter flies were more commonly captured, providing evidence of male biased sex ratios with on average 3.145 males per female ($X^2 = 191.98$, $p < 0.0001$) (Figure 3.3).

Discussion

Here, we report the first wild population of *C. attenuata,* the hunter fly, in the peach agroecosystems of Georgia and South Carolina, USA. The number of flies captured varied according to management, sex ratio, as well as trap color. Overall, *C. attenuata* was present in high-input, commercially managed orchards, USDA organically certified orchards, and in low-input, fungicide-only managed research orchards. In Turkey, *C. attenuata* was monitored in a greenhouse that underwent chemical treatments to combat whiteflies. All four chemical treatments of insecticides and fungicides appeared to have low-to-no impact on hunter fly populations (Pohl et al. 2012). This suggests that *C. attenuata* may be tolerant of some insecticide and fungicide applications, demonstrating their potential value as a biological control agent in commercially managed field settings.

As a predator, adult hunter flies rely primarily on visual cues. Their hunting behavior involves perching in well-lit areas, waiting for flying insects to venture nearby, darting out to capture the prey in the air, and returning to their perch (Cicero et al. 2017; Pons 2005). This precise mid-air attack is called “hawking” hunting behavior, and is a strategy shared with other insects, such as Anisoptera (Odonata) and Asilidae (Diptera) (Cicero et al. 2017). Hunter flies can attack prey from any angle (launching from the ceiling, a wall, or the ground), minimizing their flight time and efficiently expending energy. Adults kill their prey by stabbing the cervix (neck) area with their proboscis, sometimes partially decapitating their prey, before returning to their perch to drink the nutrients (Mateus 2012). Hunter flies were recorded attacking flying insects near their perch without attempting to eat them, exhibiting almost territorial behavior (Martinez and Cocquempot 2000). Interestingly, Mateus et al. found that an attack was not provoked if prey walked near *C. attenuata;* only a nearby flight would trigger an attack. This provoked hunting behavior of adults was observed again in laboratory feeding experiments with different prey species. In a controlled laboratory experiment, hunter fly adults preyed most heavily on adult fungus gnats, followed by adult shore flies and adult whiteflies (Sensenbach 2004; Sensenbach, Wraight, and Sanderson 2005). Whiteflies are mostly sedentary when feeding, so it would follow that they are attacked less often by the hunter flies. We did not monitor the feeding habits of the *C. attenuata* in this study. However, many of the known prey taxa of *C. attenuata* were present in our pan trap bycatch. It is possible that *C. attenuata* are drawn to the prey taxa attracted to the pan traps, using these pseudo "flowers" as a hunting site (Zou et al. 2017). The feeding habits and biological control contributions of *C. attenuata* in the southeastern USA's peach orchards should be considered in future studies.

The number of hunter flies captured in peach orchards varied seasonally. Capture data suggest anecdotal peaks in activity during early April, late May, and early July (Figure 3.2). Under laboratory conditions, the lifecycle of *C. attenuata* takes 26 days to complete (Kühne 2000). The only record of a wild population of *C. attenuata* phenology suggests the fly is multivoltine in Turkish cotton fields (Pohl, Uygur, and Sauerborn 2003). Wild populations of a closely related species, *Coenosia tigrina*, in Michigan onion fields were also found to be multivoltine (Drummond et al. 1989). Similarly, species of another hunter-fly genus, *Limnophora* Robineau-Desvoidy (1830) (Muscidae: Coenosiinae), were found to be multivoltine in tufa barriers in Croatia (Ivković and Pont 2016). However, our current data do not suggest multiple statistically significant peaks in counts throughout 2020 and 2021, so we cannot conclude if *C. attenuata* are multivoltine in the southeastern part of the USA.

The sex ratio of reported male and female hunter flies differs between a greenhouse, laboratory, and field settings as well as according to sample methodology. In this study, the sex ratio of hunter flies was male-biased; 3.145 Male:Female (Figure 3.3), while greenhouse systems appeared to be female-biased; 0.25 M:F ratio (Mateus 2012). Furthermore, under optimized laboratory-rearing conditions, emerging flies had a sex ratio of 0.67 and 0.69 M:F (Martins et al. 2015; Zou et al. 2017). With the number of confounding variables between studies, it is difficult to speculate on what caused the different sex ratios. Environmental conditions may have affected the sex ratio (e.g., the moist soil in greenhouses, or coconut fiber/black peat mixture for rearing, versus the dry sandy substrate of peach farms). Alternatively, sampling methodology may affect sex-specific capture rates (e.g., suction sampling, sticky cards, and pan traps). In addition, we found that both male and female hunter flies were most abundant in the white-colored pan traps. However, factors that contribute to different sex ratios of *C. attenuata* should be explored in future studies.

Conclusions

At this time, we cannot predict the impact of the *Coenosia attenuata* establishment in the southeastern part of the USA's agroecosystems. However, based on hunter-fly biology and life history traits, this species should be considered a potential biological control agent. As such, future studies in the region should be mindful of this potentially significant addition to the local insect fauna.

Chapter 3 Figures

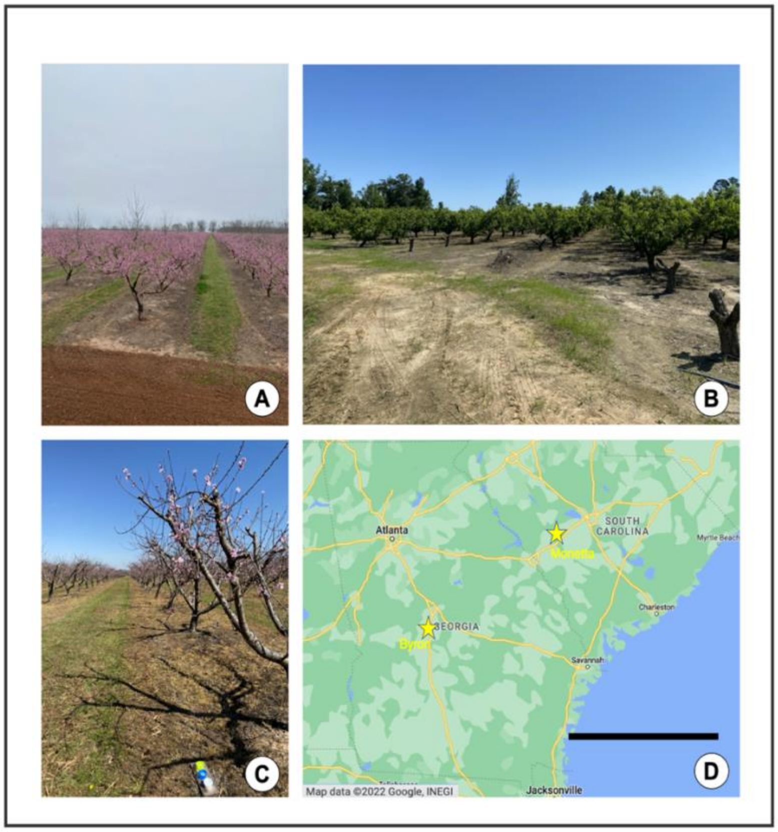

Figure 1.1. Field sites. (**A**) Commercial peach farm (high input), Byron, GA, 3/9/2021. (**B**) Organic peach farm (low input), Monetta, SC, 5/13/2021. (**C**) Commercial peach farm, Byron, GA, 3/16/2021. (**D**) Locations of field sites in Southeastern U.S. Map data © 2022 Google, INEGI (Google Maps) (Google 2022) Scale bar = 240 km.

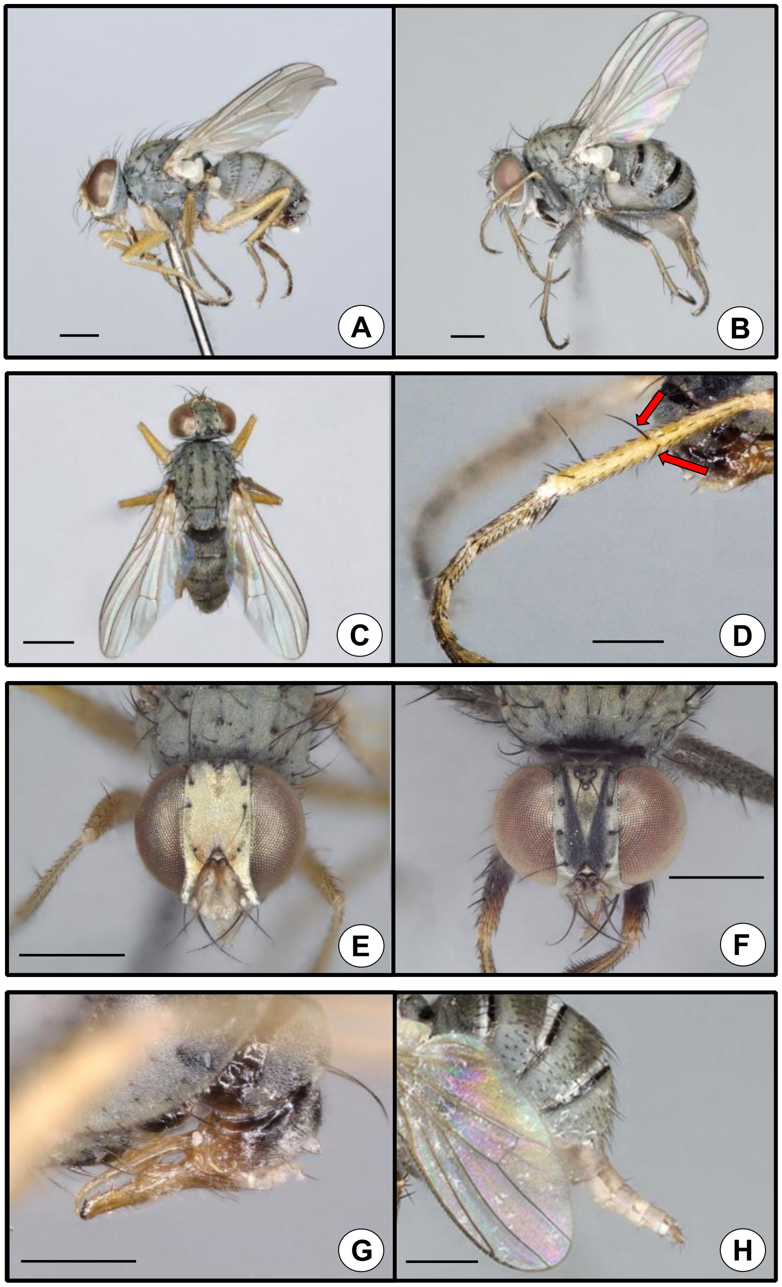

Figure 3.1. *Coenosia attenuata*. (**A**) Male, lateral. Scale bar = 0.5 mm. (**B**) Female, lateral. Note abdomen distended, exposing three black transverse stripes. Scale bar = 0.5 mm. (**C**) Male, habitus, dorsal. Scale bar = 0.5 mm. (**D**) Male, metathoracic tibia and tarsus, lateral. Arrows mark two major diverging setae at midlength. Scale bar = 0.25 mm. (**E**) Male, head, anterior. Scale bar = 0.5 mm (**F**) Female, head, anterior. Scale bar = 0.5 mm. (**G**) Male, abdominal terminalia, genitalia, lateral. Scale bar = 0.25 mm. (**H**) Female, abdominal terminalia, ovipositor, lateral. Scale bar = 0.5 mm.

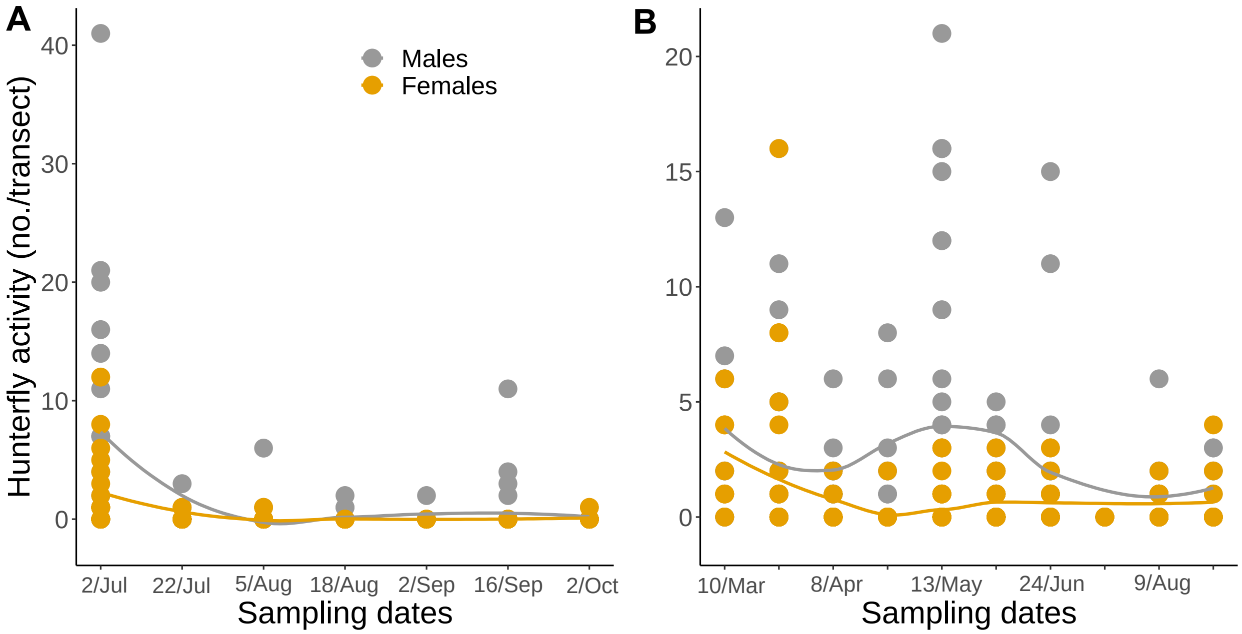

Figure 3.2. Seasonal summary of mean counts of *Coenosia attenuata* observed in pan traps combined by transect for (**A**) 2020 and (**B**) 2021. Line color indicates: Male = gray, Female = gamboge. Data points represent the mean sum total of hunter flies captured in blue, white, and yellow pan traps for a total of nine traps per field. The solid lines are loess smoothing curves with corresponding shaded 95% CI to display the estimated fluctuation in captures over the season.

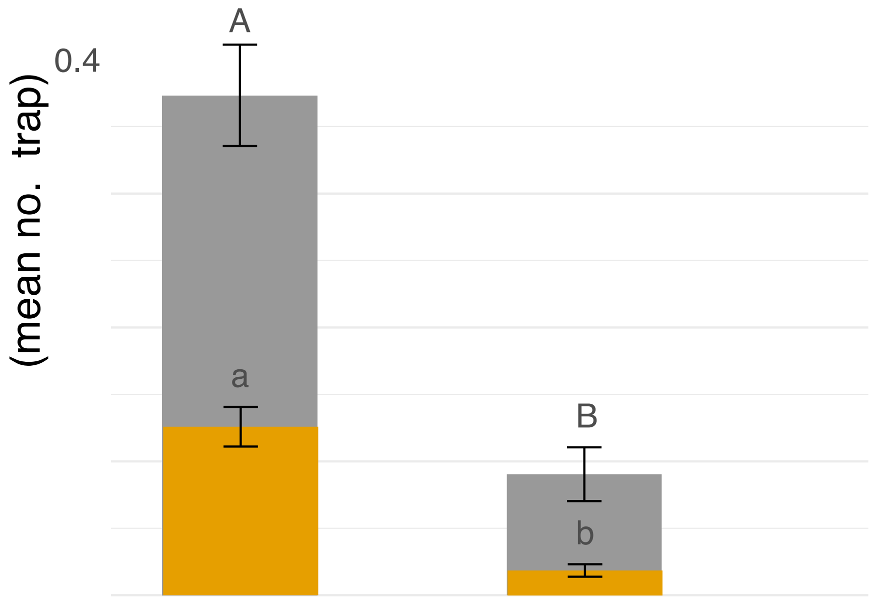

Figure 3.3. Male = grey, Female = gamboge. On the x-axis, “high” is high-input commercial chemical management, “low” is low-input fungicide-only chemical management. On the y-axis, blue, white, and yellow traps were pooled together, and displayed as average count of flies per trap location. The uppercase letters represent linear contrasts for males and lowercase letters for females in relation to management strategy ($\alpha = 0.05$).

Chapter 4: Thesis Conclusion

The long term goal of this study is to contribute knowledge toward developing IPPM programs in Southeastern peaches. Broadly speaking, IPPM is ecologically-based strategies to promote naturally occurring ecosystem services (biological control and pollination), while still maintaining control of pest populations (Biddinger & Rajotte 2015, Egan et al. 2020). But because peaches have a low economic threshold for damage, and experience heavy pest pressure, most commercial orchards are “forced” into heavy chemical management. Due to this culture of management, the community of beneficial arthropods in peach orchards is largely unknown. The toxicity of broad-spectrum pesticides may reduce or impair beneficial arthropods that contribute ecosystem services (Brittain et al. 2010, Tylianakis & Binzer 2014, Crowder & Harwood 2014). Improved delivery of ecosystem services can increase yield, reduce chemical input, and ultimately reduce cost of production (Biddinger et al. 2014, Wan et al. 2014, Bengtsson 2015). To accomplish this goal, we must start at the very foundation of the agroecosystem, and work our way up to precise IPPM. This study serves as this foundation. We estimated the communities of beneficial arthropods in managements ranging from conventional commercial to “reduced risk” and organic. We monitored the spatiotemporal activity of these communities within each management, and also used MGCA to estimate natural enemy predation rates on key pests. We now have an estimation of the most abundant and potentially important taxa for ecosystem services in the agroecosystem, as well as their spatiotemporal responses to management. With this novel information about the beneficial arthropods in peaches, more targeted studies can build off our findings and continue to refine our understanding of IPPM.

We can build on this study with both broad themes and from specific findings. For example, we found that certain pollinators had higher abundance and diversity in high-input orchards than in low-input orchards. This would call into question well-studied areas such as negative management impacts on pollinators (Tscharntke et al. 2005, Martins et al. 2015, Roquer-Beni et al. 2021), which is almost certainly not the case. Rather, it would suggest we need to adjust the scope of the study and consider how landscape context may play a role in the community assemblage in the agroecosystem (Debinski et al. 2001, Brittian et al. 2010, Tscharntke et al. 2012, Gallé et al. 2019). Another example is the result from the MGCA, which suggests that predation rates in high-input and low-input management are same. Again, this result is not refuting the well-studied impact of broad spectrum pesticides altering biological control (Tylianakis & Binzer 2014, Crowder & Harwood 2014, Bengtsson 2015). Instead, it presents an opportunity to explore specific pesticide-predator combinations, which would advance knowledge on preserving key taxa, and refine a more precise IPPM program. Finally, we have the “discovery” of the hunter fly, *Coenosia attenuata*; which is a highly abundant beneficial natural enemy in peach orchards. Peaches have been grown in the Southeastern USA for hundreds of years, but this beneficial species has gone unnoticed until this year. It shows the benefit of taking a closer look at the foundation of an ecosystem by simply asking the question “what are we working with?”, before any manipulations or targeted questions. IPPM is a never-ending process, but every question and every study moves us forward.

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