ECOLOGICAL DYNAMICS OF ARTHROPODS IN PECAN CANOPIES: INSIGHTS INTO COMMUNITY STRUCTURE, SPATIOTEMPORAL PATTERNS, AND FARMING-INDUCED STRESS

by

PEDRO FELIPE SEGURO DE TOLEDO

(Under the Direction of Jason M. Schmidt)

ABSTRACT

Understanding how arthropod communities are structured and how environmental stressors such as farming management practices act as ecological filters in this process is essential to advancing sustainable arthropod management. In this dissertation, I used the pecan system as a model to answer basic and applied questions related to how structural and chemical interventions influence arthropod communities in agroecosystems. This work integrates experimental, observational, and molecular approaches. Initially, I examined shifts in arthropod population abundances in response to canopy hedge pruning, revealing that pest and natural enemy groups responded in a case-specific manner. Building on these patterns, I evaluated how farming-induced stressors influenced multi-trophic species interactions, demonstrating that predation and parasitism dynamics were shaped not only by natural enemy abundance, but also by their spatial and temporal alignment with prey, which is affected by common farming practices. Further, I characterized hidden arthropod communities within *Phylloxera*-induced galls in pecan leaves, employing dissection and DNA metabarcoding to uncover the diversity and structuring of these enclosed systems. Communities inhabiting galls were diverse, and their structure was shaped by gall phenology, providing insight into the ecology of gall systems and how these microhabitats respond to farming-induced stress. These findings also position *Phylloxera* galls as a promising model for studying community assembly processes under managed conditions. Finally, I applied ecological insights gained from the pecan canopy system to investigate the temporal and spatial dynamics of Auchenorrhyncha herbivores, a group relevant to disease transmission in pecans. Their abundance reflected seasonal and vertical stratification patterns, with farming practices interacting with their activity over time and across canopy layers. Together, these studies contribute to a broader understanding of how management-induced environmental changes interact with habitat structure to shape community composition, multi-trophic interactions, and ecological function across multiple scales in agroecosystems.

INDEX WORDS: canopy arthropod communities, community structuring, ecological filtering by farming practices, predator-prey interactions, *Phylloxera* gall community ecology, DNA metabarcoding of arthropods, agroecosystem management, *Carya illinoinensis*

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

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2025

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August 2025

DEDICATION

I dedicate this dissertation to my parents, Wilma A. Seguro de Toledo and José E. de Toledo, whose love, sacrifices, and unwavering support made this journey possible. Their belief in me has always been the foundation of my academic and personal growth.

I also dedicate this dissertation to my wonderful siblings, Guilherme, Letícia, and Gabriela, whose constant inspiration and encouragement have motivated me to improve and reach higher.

Finally, I dedicate this dissertation to my PhD advisor and friend, Dr. Jason M. Schmidt. His mentorship has shaped my development as a scientist and instilled in me the values of integrity, curiosity, perseverance, and ethical research. His genuine passion for science and thoughtful guidance have left a lasting impact on both my career and personal life.

ACKNOWLEDGEMENTS

The path to completing my doctoral studies has involved significant challenges and was made possible by the support of many individuals who influenced my academic and personal development.

I am deeply grateful to my advisor, Dr. Jason M. Schmidt, for his guidance, patience, and support throughout my doctoral journey. I extend sincere appreciation to my committee members, Dr. Angelita Acebes-Doria, Dr. Ted Cottrell, and Dr. William Snyder, for their constructive feedback, encouragement, and guidance throughout this process.

I gratefully acknowledge the University of Georgia for providing a world class academic environment and resources. I am thankful to the Sustainable Agriculture Research and Education (SARE) Program for financial support. I also wish to express my deep appreciation to the United States Department of Agriculture (USDA), particularly the researchers and staff at the Southeastern Fruit and Tree Nut Research Laboratory in Byron, GA, for their scientific collaboration, technical assistance, and support, all of which contributed significantly to my doctoral research.

I am also thankful to the mentors who have supported me along the way. My master's advisor, Dr. Eugênio Oliveira, provided early guidance that laid the foundation for my career. To my colleagues and lab mates, past and present, thank you for the discussions, collaborations, and shared experiences that made this journey intellectually stimulating, fun, and rewarding.

Finally, I am forever indebted to my parents, family, and friends for their endless support, patience, and encouragement.

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GENERAL INTRODUCTION

Understanding the structure, dynamics, and interactions of insect communities is fundamental to advancing ecological approaches to arthropod management for both pest control and conservation purposes. Arthropod community composition and ecological interactions are influenced by habitat-specific environmental conditions, which often vary across spatial and temporal scales (Cadotte & Tucker, 2017; Kraft et al., 2015). For instance, plant-associated arthropod communities are often structured by resource distribution (*e.g.*, across vertical strata or plant tissue), which can shift with plant phenology (Lawton, 1983; Ulyshen, 2011). Therefore, trophic interactions within a canopy environment depend on synchrony between species requirements and spatiotemporal resource availability. However, identifying patterns and predicting community response to environmental changes remain challenging, particularly in systems that are continually reshaped by anthropogenic interventions (i.e., management).

Agroecosystems, such as mature pecan (*Carya illinoinensis*) orchards, are examples of complex environments where natural ecological processes are constrained to habitat features and on-farm management decisions (Crowder et al., 2010; Gomiero et al., 2011; Landis et al., 2000; Raven & Wagner, 2021; Redhead et al., 2020). For instance, mature pecan orchards feature tall, stratified canopies that support vertically structured arthropod assemblages (Cottrell et al. 2017, 2024; Edelson & Estes, 1983; Slusher et al., 2022), likely explained by heterogeneous microclimates and resource distributions across the canopy vertical strata.

Structured canopy communities and species interactions can be differently affected by common agricultural practices, such as pruning and pesticide applications. These commonly used practices can interact with and modify the canopy environment, imposing different habitat structures, disrupting resource availability, shifting species dominance, and altering patterns of community assembly and trophic interactions (Brenard et al., 2020; Fonte et al., 2023; Grechi et al., 2008; Guedes et al., 2016; Knapp et al., 2023; Pennington et al., 2019; Stark & Banks, 2003).

Furthermore, pecan trees provide an interesting agricultural model to answer basic and applied ecological questions as the managed system contains both open arthropod communities and concealed habitats, such as *Phylloxera*-induced galls. Gall-inducers restructure plant tissues into specialized environments that house herbivores, parasitoids, and inquilines (Mani, 1964; Sanver & Hawkins, 2000). Although Hymenoptera- and Diptera-induced gall communities have been relatively well studied (Abrahamson & Weis, 2020; Hayward & Stone, 2005), galls induced by hemipterans, such as *Phylloxera* spp., remain comparatively underexplored. Recent advances in DNA metabarcoding now offer powerful tools to reveal the hidden diversity and community structure within these enclosed systems (Deiner et al., 2017; Kaartinen et al., 2010).

Thus, to advance our understanding of how management-driven stressors shape insect communities across multiple ecological scales, this dissertation addresses four primary objectives: **Chapter 1** examines how canopy pruning influences the abundance of key pest and natural enemy groups in pecans, including scorch mites, aphids, *Phylloxera* spp., parasitic wasps, predatory mites, and soil-dwelling entomopathogens. **Chapter 2** investigates how farming-induced stressors reshape species interactions, focusing on aphid predation and parasitism through field experimentation and PCR-based gut content analysis. **Chapter 3** explores the assembly of arthropod communities within Phylloxera-induced galls, using classical dissection and DNA metabarcoding to examine the effects of gall phenology, vertical canopy position, and pesticide exposure. **Chapter 4** assesses the spatiotemporal dynamics of Auchenorrhyncha in response to pruning and chemical management, providing insights into the ecology of an abundant herbivore group that includes plant disease vectors.

Together, these studies provide a multi-scale perspective on how management-driven stressors influence insect community structure, mediate species interactions, and shape hidden community assembly processes within tree agroecosystems.

CHAPTER 1

CANOPY HEDGE PRUNING IN PECAN PRODUCTION DIFFERENTIALLY AFFECTS GROUPS OF ARTHROPOD PESTS AND ASSOCIATED NATURAL ENEMIES[1]

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[1] Toledo, P.F.S.; Phillips, K.; Schmidt, J.M.; Bock, C.H.; Wong, C.; Hudson, W.G.; Shapiro-Ilan, D.I.; Wells, L.; Acebes-Doria, A.L. 2024 Canopy hedge pruning in pecan production differentially affects groups of arthropod pests and associated natural enemies. Crop Protection. 176:106521. Reprinted here with permission of publisher.

Abstract

Controlling canopy growth and size through pruning techniques in perennial agroecosystems is often crucial to maximizing productivity. However, the implications of modifying canopy architecture for arthropod management are often overlooked. We studied the effects of a hedge pruning cycle on pests and natural enemies within mature pecan canopies by assessing the abundance of: 1) leaf-dwelling aphids and mites, 2) visually detectable injuries caused by black pecan aphids [*Melanocallis caryaefoliae* (Davis)] and *Phylloxera* spp., 3) aphid parasitoids and parasitized hosts (aphid mummies), and 4) predatory mites. Additionally, we assessed the prevalence of entomopathogens that dwell on the orchard floor, under the trees. Hedge pruning decreased *Phylloxera* infestation but increased pressure from scorch mites and the yellow aphid complex. Black pecan aphid abundance responded inconsistently, but their damage was consistently reduced in hedged canopies. Predatory mites were only affected by hedging in the second year when higher populations were observed. Although parasitoid wasp abundance was not affected by hedging, more parasitized aphids (mummies) were observed in trees that did not receive pruning in the first year. Also, higher activity of entomopathogens was observed in soil cores collected under hedged trees in the first year but lower in the second. Taken together, the effects of hedge pruning pecan canopies were case-specific and cannot be generalized across pests or natural enemies. Nevertheless, structural and environmental variation across seasons and within hedge pruning systems clearly have implications for crop protection in pecans and other perennial systems.

Keywords: hedging, *Carya illinoinensis,* within-canopy ecology; pest management

Introduction

Modifying canopy structure to control tree growth in perennial agroecosystems is often crucial to improve crop performance and maximize profitability (Harper and White, 1974; Martin-Gorriz et al., 2014; Mika, 1986; Moore, 1958). In pecan [*Carya illinoinensis* (Wangenh.) K. Koch], for instance, controlling the density and size of mature trees is important to prevent crowding, which ultimately impacts nut yield and quality (Lombardini, 2006; Wood, 2009). As pecan trees grow and the orchard develops, pruning strategies including hedge pruning (also known as hedging) can be implemented to regulate the size of trees.

Hedging programs in pecan orchards usually aim to prune the sides and/or top of the trees to allow for more sunlight within the canopies, and between rows, and encourage new growth (Lombardini, 2006; Wells, 2018). Consequently, hedge pruning efforts will result in the removal of major pecan branches, altering the availability of resources (*e.g.,* food and shelter) for herbivores and their natural enemies. For instance, the new foliar growth promoted by hedging or pruning (Hellwig et al., 2022) could be more attractive to pests that prefer younger, tender leaves and actively growing shoots. The gaps created in the canopies coupled with overall size reduction, likely allow for enhanced pesticide coverage into sections that were previously difficult to access, including the interior and upper strata of the pecan canopy (Bock et al., 2017; Bock and Hotchkiss, 2021). Also, a greater amount of solar radiation will likely result in an altered microclimate in the hedged canopy (*e.g.*, temperature and relative humidity) and the understory (*e.g.*, soil moisture and ultraviolet penetration) (Martius et al., 2004; Niether et al., 2018).

Arthropods are known to respond to shifts in habitat characteristics ranging from conspicuous, macro-scale alterations (*e.g.*, in diversity and abundance of resources of a landscape (Perović et al., 2021; Schowalter, 2012), to less explored, finer-scale rearrangements (*e.g.*, in the density of leaves, shape, or height of a plant (Haysom and Coulson, 1998; Lawton, 1983; Marquis et al., 2002; Neuvonen, 1999; Saudreau et al., 2013). For instance, herbivores that depend on a specific plant tissue can be directly impacted by the distribution and abundance of the tissue type within a plant, well exemplified by gall-inducing insects that rely on meristems (Espírito-Santo et al., 2007; Labandeira, 2021). In a tree canopy, the degree of branching, or simply, distances between leaves are attributes that can affect the establishment and performance of herbivores (Marquis et al., 2002; Simon et al., 2012). Moreover, plant architecture can modulate trophic interactions (and the outcomes of biological control) as in the case of chrysomelid beetles that can escape parasitism by laying egg clutches on taller plants and in the upper strata (Obermaier et al., 2008). Collectively, we hypothesized that the alterations in the pecan canopy structure exerted by hedge pruning and its aftereffects (*e.g.*, producing different canopy environments and increasing solar radiation in the floor beneath) could affect the dynamics of pests and natural enemies in both the pecan canopy and orchard floor.

Hedge pruning in pecans is frequently employed in arid production areas, such as the southwestern United States, where it has been a successful strategy for reducing issues related to shading of productive branches and alternate nut bearing tendencies (Wood and Stahmann, 2004). In the Southeast (*e.g.*, in the state of Georgia), conditions differ greatly from southwestern climates by having lower light, more rain, and higher humidity, and it is unclear whether hedge pruning in southeastern systems results in the same benefits observed in the Southwest. Current hedging studies in pecans focus primarily on effects on tree physiology (*e.g.*, water usage), resistance to wind damage from storms and hurricanes, and the severity of scab disease caused by *Venturia effusa*, with a primary interest in the potential impacts on nut yield and quality (Bock et al., 2017; Lombardini, 2006; Wells, 2018). However, to our knowledge, no studies have investigated whether hedge pruning influences insect and mite management in pecan orchards.

Here we examine the impacts of hedge pruning on pests and biological control agents by measuring arthropod abundance and/or activity-density in hedged *versus* non-hedged systems over a two-year cycle. Throughout each season, our assessments encompassed 1) Arthropod pests: pecan leaf scorch mites [*Eotetranychus hicoriae* (McGregor); Acari: Tetranychidae]; the black pecan aphid, [*Melanocallis caryaefoliae* (Davis); Hemiptera: Aphididae], and the "yellow aphid complex", a collecting term (Slusher et al., 2021b) referring to the blackmargined aphid, [*Monellia caryella* (Fitch)] and the yellow pecan aphid, [*Monelliopsis pecanis* Bissell; Hemiptera: Aphididae], 2) Economically-important pest damage: leaf injury caused by black pecan aphids, and galls induced by *Phylloxera* spp. (Hemiptera: Phylloxeridae); and, 3) Natural enemies from three functional groups: the aphid specialist parasitoid, [*Aphelinus perpallidus* (Gahan); Hymenoptera: Aphelinidae], the western predatory mite [*Galendromus occidentalis* (Nesbitt); Acari: Phytoseiidae], and soil-dwelling entomopathogens present in the orchard floor (via infection of hosts with soil cores). Additionally, we assessed potential disruptions in the biocontrol services provided by the parasitoid *A. perpallidus* through the count of parasitized aphids (*i.e.*, mummies). Thus, this research intended to provide insight into the implications of hedge pruning for pecan pest-natural enemy systems to help guide canopy management strategies for improved pest management programs in systems that undergo hedging.

Methods

Site characteristics, management, and experimental design

The study was conducted in Marshallville, GA (32.500209, -83.933302), in a 24-hectare commercial pecan orchard of mature trees of cv. Desirable, interplanted with pollinator trees of cv. Sumner. The trees were approximately 30 years old, 12 meters in height with a 15 × 12.5 m spacing. The row middles were mowed regularly and the strip beneath the tree row was maintained vegetation-free with herbicides (a 6-m wide herbicide strip). The orchard was managed using conventional pecan practices recommended for Georgia (Wells, 2019) with standard applications of insecticides and fungicides (Supplementary Table S1). Irrigation was scheduled according to the University of Georgia Cooperative Extension irrigation recommendations for pecan production (Wells, 2017). Six drip emitters (7.6 L/h) were ported to the surface 2 m from the tree trunk on each side of each tree with a distance of 1 m between emitters. The hedge pruning program was on a three-year rotating schedule. The trees were first hedged pruned in 2013 (west side of the canopy) and again in 2014 (east side of the canopy) but not in 2015. In the second cycle, the trees were hedge pruned in 2016 (west side of the canopy) and 2017 (east side of the canopy) but not in 2018. Our sampling was performed in 2019 when the west side of the trees was hedge pruned; and in 2020, when the east side of the trees was hedge pruned. Hedging was always performed during the winter before each season when trees were dormant. Trees were topped at 12 m and the sides were pruned at 1.85 m from the trunk using a mechanical hedge pruning machine. The study was a randomized complete block design with four blocks. Each block consisted of plots of five rows of hedge-pruned trees and five rows of non-hedged trees.

Estimating pest populations and pest damage on pecan leaves

Ten trees from the middle row of each plot (hedge pruned or non-hedged) were randomly selected for leaf sample collection. Each leaf sample consisted of two compound leaves from both the upper (sample height ~9 m) and lower canopies (sample height ~2 m) of the trees. The upper canopy samples were obtained with the aid of a hydraulic lift/pruning tower (Prune-Rite Orchard Manlift; Jack Rabbit, Ripon, CA), with a maximum platform height of 9 m (Figure 7). To standardize leaf samples and account for compound leaf size variation, only the six middle leaflets (three leaflet pairs) from each compound leaf were kept, and stored in plastic bags (26.8 × 27.3 cm) in coolers until processing in the laboratory. Leaflets were assessed under a stereomicroscope (Leica Microsystems) to count the number of immature and adult stages of three aphid species, the yellow pecan aphid (*M. pecanis*), the blackmargined aphid, (*M. caryella*), and the black pecan aphid, (*M. caryaefoliae*), and adult and immature stages (including eggs) of the pecan leaf scorch mite (*E. hicoriae*), as well as adult and immature stages (except for eggs) of the western predatory mites (*G. occidentalis*). Additionally, the number of parasitized aphids from the "yellow aphid complex" (*i.e.*, aphid mummies of *M. pecanis* and *M. caryella*), were recorded. We also evaluated arthropod-related injuries that were visually detectable, including necrotic areas caused by the feeding behavior of black pecan aphids (as the number of leaflets with damage from *M. caryaefoliae*), and galls induced by *Phylloxera* spp. infestations (as the total number of galls on the six leaflets assessed per compound leaf). Leaves were evaluated on three occasions during the 2019 and 2020 seasons corresponding to three phenological stages of the pecan trees [June (post-pollination), July (rapid nut expansion), and August (kernel filling)] (Wells and Conner, 2007).

Estimating parasitoid activity with yellow sticky cards

To track the activity of the pecan aphid parasitoid, *A. perpallidus,* in the canopy, yellow sticky card traps (7.6 × 12.7 cm, Olson Products Inc., Medina, OH) were deployed. One sticky card was placed in the lower and one in the upper canopy of two randomly selected trees in the middle row of the hedged or non-hedged trees. The cards were attached to tree branches horizontally (facing downwards) and twist ties were used to secure them (the protective layer of only the lower side was peeled off, exposing the sticky surface). Cards were left in the field for one week, and subsequently, brought to the lab where parasitoids were counted under a stereomicroscope. Traps were also deployed on three occasions (June, July, and August) in each year of this study.

Entomopathogens: beneficial nematodes and fungi beneath trees

The prevalence of entomopathogenic nematode and fungal infection was estimated based on the soil-baiting method (Shapiro-Ilan et al., 2007). Six soil cores were sampled from around each pecan tree at 1 m from the trunk, and another six cores from just inside the canopy dripline (approximately 3 m from the trunk but always inside the herbicide-treated strip) of hedged or non-hedged pecan trees. Soil samples were collected weekly for four weeks in 2019 and six weeks in 2020. Soil from each sample was mixed and sub-samples of approximately 470 ml were placed in plastic aerated cups and incubated at 25 ºC. Fifteen larvae of the greater wax moth *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) were exposed to the soil collected in the understory of the pecan trees and larval mortality due to infection caused by soil-dwelling entomopathogens was recorded 12 days after exposure. The proportion of infected larvae per tree was compared between treatments.

Data management and statistical analyses

Numbers of aphids from the yellow aphid complex, mummies, parasitoids, black pecan aphids, black aphid injury, *Phylloxera* galls, scorch mites, and predatory mites were pooled by row (sum of counts combined for trees sampled within a hedge pruned or non-hedged plot). We were specifically interested in the whole tree treatment effects and thus, sampling height (or canopy location) was not included in the analysis. Models were fit separately for each year. Linear mixed effects models (LMEs, function: lme, package nlme; Pinheiro et al., 2023), general linear models (GLMs), or generalized linear mixed effects models (GLMMs, function: glmer, family: binomial; package lme4; (Bates et al., 2015) were used to assess the effects of hedge pruning treatment, assessment date, and possible interactions between hedge pruning treatment × date. Each LME was fit to either the natural log-transformed (for yellow aphids and parasitoid wasps) or the squared root-transformed (for aphid mummies and *Phylloxera* galls) counts to resolve issues related to data normality in each year studied. Plots were used as random effects in the LMEs to account for repeated measures over time. Each GLM model was fit with a Poisson distribution for counts of black pecan aphids, black aphid injury, scorch mites, and predatory mites using hedge pruning treatment as the predictor variable. For these, only counts recorded in August of each season were used because few predatory mites and black pecan aphids were recovered, and their known seasonal peak abundance is August (Jackson et al., 1983; Tedders, 1978). The GLMM model was used to assess the effects of hedge pruning on soil entomopathogens to the binomial data of the dead larvae (killed by entomopathogen infection) as a proportion of all larvae infected with soil cores collected per tree, and sampling week was designated as a random effect. No comparison between years was performed as the environment in the trees is expected to be dynamic as the east and west sides of the trees grew out from hedge-pruning each year. All analyses were performed in R version 4.3.0 “Already Tomorrow” (RCoreTeam, 2023).

Results

Occurrence of yellow aphid complex species and parasitized individuals

Yellow aphid complex species (*M. pecanis* and *M. caryella*) had statistically greater abundance in hedged trees in both seasons studied. We also captured date effects as the aphid population changed throughout the season (Table 1, Figures 2A and 2B), and although there appears to be an interaction with much higher numbers of aphids in August for hedged pecans, overall, the main effect of higher abundance in hedged trees was significant in both years (Table 1). For parasitized aphids (mummies), their numbers increased over time, yielding a significant effect of assessment date in both seasons; however, effects of hedge pruning were only observed in the first year of the hedge pruning cycle (2019) where non-hedged trees had higher mummy abundance (Table 1, Figures 2C and 2D).

Parasitoid activity measured with yellow sticky cards

No effects of hedge pruning or interactions between hedging and assessment date were observed for parasitoid wasps (*Aphelinus perpallidus*) in either season studied. However, assessment date effects were found as parasitoid abundance increased between June and July in both years (Table 1, Figure 3A and 3B).

Black pecan aphids and related injury

We rarely found black pecan aphids or evidence of their injury in the first two assessment dates (June and July) for either year (*i.e.*, 95% of leaflet samples had 0 counts of aphids or damage), therefore, our analysis focused on the August sampling. Black pecan aphids were less abundant in the hedged trees in August of 2019, but more in August of 2020 (Table 2, Figure 4A). However, there was a reduction in aphid damaged to pecan leaflets assessed in hedged trees in both years studied (Table 2, Figure 4B).

Pecan leaf scorch mites and western predatory mites

Like observations of black pecan aphids, we rarely captured pecan leaf scorch mites or their natural predators (western predatory mites) in the first two assessment dates (June and July) for either year (*i.e.*, 95% of leaflet samples had 0 counts of mites), therefore, our analysis focused on the August samples. Hedge pruned trees had a higher abundance of pecan leaf scorch mites in both seasons (Table 2, Figure 5A). Western predatory mites were not affected by hedge pruning treatment in 2019. However, in the second year, 2020, hedge pruned trees had significantly higher predatory mite abundance compared to trees that did not receive pruning (Table 2, Figure 5B).

Galls induced by* Phylloxera *spp. on pecan leaves

Hedge pruning significantly reduced the number of galls formed by *Phylloxera* spp. on leaflets in both the 2019 and 2020 seasons. Assessment date effect or its interactions with hedge pruning treatment were not observed. (Table 1, Figure 6)

Occurrence and activity of soil entomopathogens under pecan trees

The presence and activity of soil-borne entomopathogens (fungi and nematodes), measured by the infection rate of the larval host (*G. mellonella*), varied between years. On average 11.9% more larvae died when infected with soil collected under hedge pruned trees in 2019 (Z_{45} = -3.25; $P = 0.001$, Figure 7A), while in 2020, 11.4% more larvae died when infected soil cores collected from underneath trees that did not receive pruning (Z_{45} = 0. 42; $P < 0.001$, Figure 7B). Also, the majority of the activity in the first year was related to fungi infection while in the second year, activity from both fungi and nematodes was similarly observed in the soil samples.

Discussion

Here we show that hedge pruning cycles in pecan systems can result in species-specific effects on arthropod pests, reducing infestation/damage in some instances (*e.g.*, *Phylloxera,* and injury caused by black pecan aphids), while increasing pest pressure in other instances (*e.g.*, scorch mites, and the yellow aphid complex species). Additionally, we observed inconsistent effects of hedge pruning as the system progressed. Entomopathogens in the orchard soil seemed to benefit from hedge pruning only in the first year after hedge pruning, whereas predatory mites only in the second. Indeed, the effects of habitat alterations on arthropods, which are better understood on larger spatial scales but less so within tree canopy environments, are often case-specific and cannot be generalized across organisms or systems (Karp et al., 2018; Lawton, 1983; Simon et al., 2007). Nonetheless, determining how pests and natural enemies respond to sequential architectural alterations may assist in more assertive crop protection strategies.

Hedge pruning favored the establishment of yellow aphid complex species on hedge pruned trees which may be partially explained by aphid feeding preference and/or dispersal capabilities within the different tree architectures. Yellow pecan aphids have a preference for younger leaves, characterized by softer tissue and smaller veins (Tedders, 1978). These conditions are more likely to be found in hedge pruned trees due to the enhanced growth stimulated by the pruning cuts (Hellwig et al., 2022). Additionally, other studies comparing different training systems in fruit trees have found that aphids exhibit a preference for actively growing tree shoots as opposed to non-growing ones (Fonte et al., 2023; Grechi et al., 2008; Simon et al., 2012). In the present study, damage from the yellow aphid species complex was not quantified since it would require more complex assessments such as of the tree physiology. Thus, we cannot ascertain whether the higher aphid abundance would necessarily result in greater negative impacts on hedge pruned systems. However, it is worth mentioning that unusually high infestations of yellow aphids have been reported to lead to decreased yield in subsequent seasons, and trees may require a few years to recover from the physiological damage inflicted (Barnes and Moffitt, 1978; Dutcher et al., 1984).

Aphid control in commercial pecan agroecosystems relies on applications of insecticides (Slusher et al., 2021a); however, when infestations are low, naturally-occurring biological control usually suffices to maintain aphid populations below economic damage thresholds (Wells and Conner, 2007). Many aphid natural enemy species are known to occur in pecan trees and the specialist parasitoid wasp, *A. perpallidus,* is acknowledged for its contributions to yellow aphid species complex control (Bueno Jr and Stone, 1983; Bueno Jr and Van Cleve, 1997; Bueno and Stone, 1985). Here, we revealed that hedge pruning did not affect the presence of *A. perpallidus* wasps in the pecan canopy. Yet, during the first year, fewer parasitized aphids (mummies) were found in hedge pruned trees, suggesting that their ecological services may have been constrained. It is worth noting that even though we generally found fewer hosts (*i.e.*, yellow aphid complex) in non-hedged rows, the more branchy habitat may indirectly facilitate parasitoid movement within non-hedged canopies, potentially leading to higher parasitism.

In contrast to our findings concerning the yellow aphid complex species, the first year of hedge pruning decreased the abundance of black pecan aphids and, consequently, their damage to leaflets. Interestingly, during the second year, despite an increase in the abundance of black pecan aphids in hedge pruned rows, there was still a decrease in leaflet damage as a result of hedge pruning. The dissimilar response of black pecan aphids compared to the yellow aphid species complex, which consistently favored hedge pruned trees throughout the seasons, may be attributed to their distinct life history strategies. Unlike the yellow aphid complex, immature and adult stages of black pecan aphids disperse less after establishment as they rely on developing chlorotic spots on the leaves (caused by their feeding behavior) at single feeding locations for their development (Cottrell et al., 2009). This reliance on induced leaf chlorosis, coupled with the fact that younger leaves typically possess greater metabolic defenses, may elucidate the reason behind the reduction in damage caused by hedge pruning observed in our study. Furthermore, there have been no reports suggesting that black pecan aphids display preferences for younger leaves, as appears to be the case for yellow pecan aphids.

Hedge pruning resulted in an overall increase in the abundance of scorch mites within the pecan canopy. This could be attributed to the mites' preference for warmer or dryer environments, whereby the augmented sunlight penetration in the canopy of hedge pruned trees (Lombardini, 2006) likely facilitated a more favorable microclimate for their establishment and development. Moreover, it is recognized that tetranychid mites, such as *E. hicoriae*, may experience enhanced fitness when exposed to pesticide applications in pecans (Ball, 1982; Wells and Conner, 2007) and other systems via processes such as hormesis (Croft and Van De Baan, 1988; Guedes and Cutler, 2014) and/or displacement of their natural enemies (Wells and Conner, 2007). Consequently, the potential improvement in pesticide coverage resulting from the creation of a more open canopy due to hedge pruning could have also contributed to the higher scorch mite abundance observed in hedge pruned trees.

Hedge pruning did not affect predatory mite (*G. occidentalis*) abundance in the first year, but higher numbers of these natural enemies were observed in the hedge pruned trees during the second year. Throughout both seasons, the counts of predatory mites remained relatively low regardless of the pruning treatment, except for August 2020, when significantly higher numbers were detected in the hedge pruned trees. This outcome can be reasonably attributed to a direct response to the elevated abundance of their prey, scorch mites, also observed in the hedge pruned trees during that specific season. These findings suggest that the potential numerical response of predatory mites, driven by the availability of prey, outweighed the absence of observable effects resulting from hedge pruning during the initial year of the study.

Hedge pruning reduced the number of *Phylloxera* spp. galls on the trees. Gall-inducing insects usually rely on the number of meristems present in a plant for gall formation (Espírito-Santo et al., 2007; Larson and Whitham, 1997). Therefore, we hypothesize that the reduction in pecan branches and their available meristems in hedge pruned trees resulted in the reduction in galls formed. It is worth noting that in the first assessment of 2019 (in June), gall numbers were similar between hedge pruned *vs* non-hedged trees. Considering that *Phylloxera* spp. may have only one (or two) generations during the pecan season, our results suggest that greater differences captured later in the season (July and August) could have resulted from other factors (*e.g.*, defoliation) rather than solely insect activity. The exact implications of gall formation for pecan production are not fully understood; however, the presence of *Phylloxera* galls negatively impacts the nutritional status and photosynthetic rates of gall-adjacent tissues and impairs normal metabolic function (Andersen and Mizell, 1987). Furthermore, galls formed by *Phylloxera* can serve as a host site for the hickory shuckworm [*Cydia caryana* (Fitch); Lepidoptera: Tortricidae] early in the season (Boethel et al., 1974; Dinkins and Reid, 1988) which is a critical pest affecting the quality of nuts. Hence, if further elucidated, the decrease in gall formation induced by hedge pruning could be advantageous in areas where *Phylloxera* control poses challenges.

Hedge pruning improves sunlight penetration into orchards as trees become smaller and narrower (Lombardini, 2006). Given that ultraviolet radiation is harmful to entomopathogens (*e.g.*, fungi and nematodes) (Shapiro-Ilan et al., 2007), we expected a decrease in entomopathogen prevalence in the orchard soil under hedge pruned trees. Moreover, the increased light radiation in the understory could reduce soil moisture and alter the dynamics of soil-borne entomopathogens, despite the orchard being irrigated. In contrast, environmental manipulations within the orchard that increase shading and soil moisture, such as cover cropping, have been reported to enhance the prevalence of endemic entomopathogenic fungi in pecan orchards (Shapiro-Ilan et al., 2012). Here, the impact of hedge pruning on the presence and pathogenicity of soil entomopathogens was found to be modest and inconsistent. Soil cores collected beneath hedge pruned trees infected and killed more larval hosts (*G. mellonella*) in the first year, but less in the second year when compared to non-hedged trees. These divergent responses highlight the significance of background environmental variations in shaping the outcomes of management practices across different seasons. For instance, the microbial activity observed in infected larvae indicates a distinct composition of entomopathogens in the soil during each season, with fungi being the predominant cause of mortality in the first year, while similar contributions from fungi and nematodes were observed in the second year. Also, such differences may have resulted from the specific hedge pruning program implemented during the study. Since only one side of the trees was pruned each year, it is plausible to assume that greater sunlight penetration occurred in 2020, potentially leading to the anticipated detrimental effects. However, the slight differences observed in larval death show that the effects of hedging on entomopathogens were limited, but deserves further attention.

In other tree systems (*e.g.*, macadamia), similar hedging efforts have been demonstrated to increase natural enemy diversity and reduce pests (Gutierrez-Coarite et al., 2018). However, these positive outcomes were partially attributed to the presence of understory plants benefiting from increased sunlight penetration, which in turn supported the recruitment and establishment of natural enemies. In our study, the absence of such understory plants at the orchard site could have limited the potential benefits of hedge pruning. It is also important to consider that our experiments were performed in a commercial orchard where pesticides are routinely applied to protect trees from weed competition, pests, and diseases. These pesticide applications may have masked or modulated some of the hedge-pruning effects (or lack of effects) observed here. Additionally, even though we did not intend to evaluate the seasonal activity pattern of these organisms, the fluctuating populations could have, similarly, resulted from pest control methods being applied. Further studies are still needed to elucidate whether the effects of hedge pruning in pecan orchards would be more pronounced in systems that employ environmentally friendly crop protection methods or reduce the reliance on conventional pesticide applications.

In closing, our investigation on some of the most economically significant pests of pecan crops in the Southeastern US, along with their natural enemies, revealed that a hedge-pruning cycle can lead to a multitude of small-scale and species-specific effects over the seasons that can benefit or hinder the activity of arthropods in the system. While our study did not specifically examine species interactions within the canopy strata (except for counts of aphid mummies), understanding these interactions could provide valuable insights into the mechanisms driving the observed shifts resulting from hedge pruning. It is also worth underscoring that canopy architecture was not the same in 2019 and 2020 as only one side of the canopy was hedge pruned each year. The resulting different environments could have impacted the response patterns of the organisms assessed in this study, suggesting that longer term studies in hedged systems may be desirable. We emphasize, however, the importance of considering the differential activity of pests and their natural enemies, as revealed by our findings. Dynamics of pests and their natural enemies have important implications for the development of effective and sustainable crop protection strategies in hedge pruned pecans and other perennial tree crops that undergo frequent structural alterations.

Acknowledgments

We thank Stacy Byrd, Jacob Horton, Shardaya Weems, Kirsten Flinn, Caitlin Crawford, Pamela Halliday, E. Kyle Slusher, and Jamal Hunter for technical assistance. The project was funded by SSARE Project LS20-340. This work was supported, in part, by the University of Georgia, USDA-NIFA Multistate Hatch Project GEO00884-S1073. CHB and DIS received additional support from USDA-ARS projects 6606-21220-014–00D and 6042-22000-024-000-D, respectively. Mention of a trademark or proprietary product is solely to provide specific information and does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, or the University of Georgia, and does not imply its approval to the exclusion of other products that may also be suitable. In short, reference to any agrochemical

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Table 1.1 Summary statistics from the linear mixed effect models (LMEs) used to analyze the effects of hedge pruning treatment, assessment date, and their interaction on the yellow aphid species complex (*Monelliopsis pecanis* and *Monellia caryella*), parasitoid wasps (*Aphelinus perpallidus*), parasitized aphids (mummies), and galls formed by *Phylloxera* spp. in pecan trees during the 2019 and 2020 seasons.

Sources of variation		**2019 Season**			**2020 Season**		
		$df_{\text{num}}/df_{\text{den}}$	F	P	$df_{\text{num}}/df_{\text{den}}$	F	P
Yellow aphid complex	Hedge pruning (H)	1 /15	**8.56**	**0.0104***	1 /15	**18.31**	**0.0002***
	Date (D)	2 /15	**10.82**	**0.0012***	2 /15	**13.09**	**0.0065***
	H x D	2 /15	1.36	0.2855	2 /15	2.96	0.0826
Mummies	Hedge pruning (H)	**1 /15**	**4.92**	**0.0424***	1 /15	0.46	0.5083
	Date (D)	2 /15	**23.27**	**0.0001***	2 /15	**23.40**	**0.0001***
	H x D	2 /15	2.17	0.1492	2 /15	0.61	0.5564
Parasitoid wasps	Hedge pruning (H)	1 /15	2.79	0.1158	1 /15	0.02	0.8983
	Date (D)	2 /15	**31.49**	**0.0001***	2 /15	**9.65**	**0.0020***
	H x D	2 /15	1.28	0.3062	2 /15	2.08	0.1601
***Phylloxera* galls**	Hedge Pruning (H)	1 /15	**5.08**	**0.0395***	1 /15	**6.56**	**0.0217***
	Date (D)	2 /15	0.25	0.7808	2 /15	0.50	0.6188
	H x D	2 /15	0.72	0.5021	2 /15	0.30	0.7449

* Significant at $P < 0.05$ (highlighted in bold font)

Table 1.2 Summary statistics from generalized linear models (GLMs) with Poisson distribution used to analyze the effects of hedge pruning treatment on black pecan aphid (*Melanocallis caryaefoliae*), their injury to leaves, pecan leaf scorch mite (*Eotetranychus hicoriae*) and predatory mite (*Galendromus occidentalis*) in pecan trees in August of the 2019 and 2020 seasons.

Sources of variation		**2019 Season**			**2020 Season**		
		df	χ^2	*P*	*df*	χ^2	*P*
Black pecan aphids	Hedge pruning (H)	1	**34.57**	**4.115E^{-09}***	1	**9.86**	**0.0017***
Black pecan aphid injury	Hedge pruning (H)	1	**20.44**	**6.139E^{-06}***	1	**5.49**	**0.0191***
Scorch mites	Hedge pruning (H)	1	**16.76**	**4.234E^{-05}***	1	**260.92**	**2.2E^{-16}***
Predatory mites	Hedge pruning (H)	1	2.14	0.1439	1	**36.96**	**1.207E^{-08}***

* Significant at $P < 0.05$ (highlighted in bold font)

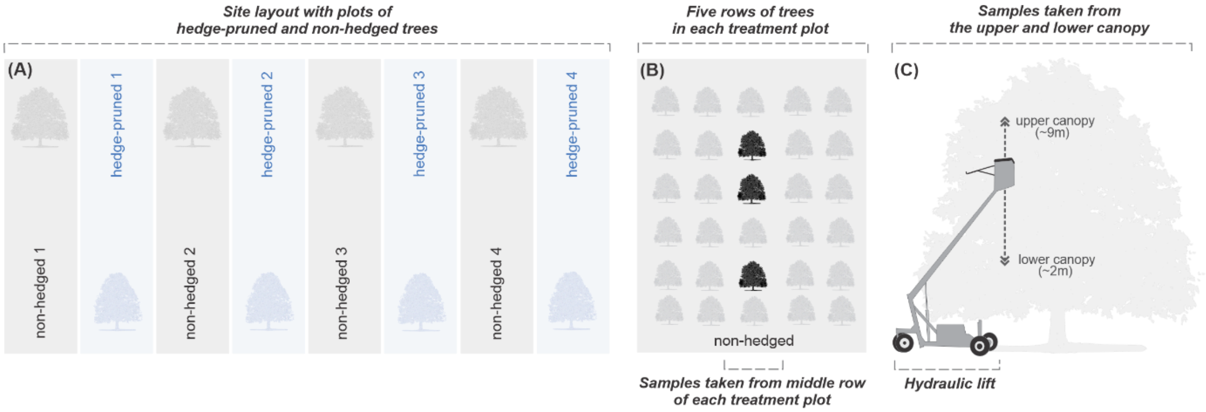


Figure 1.1 Site layout **(A)** and sampling process **(B)** illustrating sample trees in the middle row of each treatment block, and **(C)** sampling equipment and sample heights in the pecan tree canopies using a hydraulic lift.

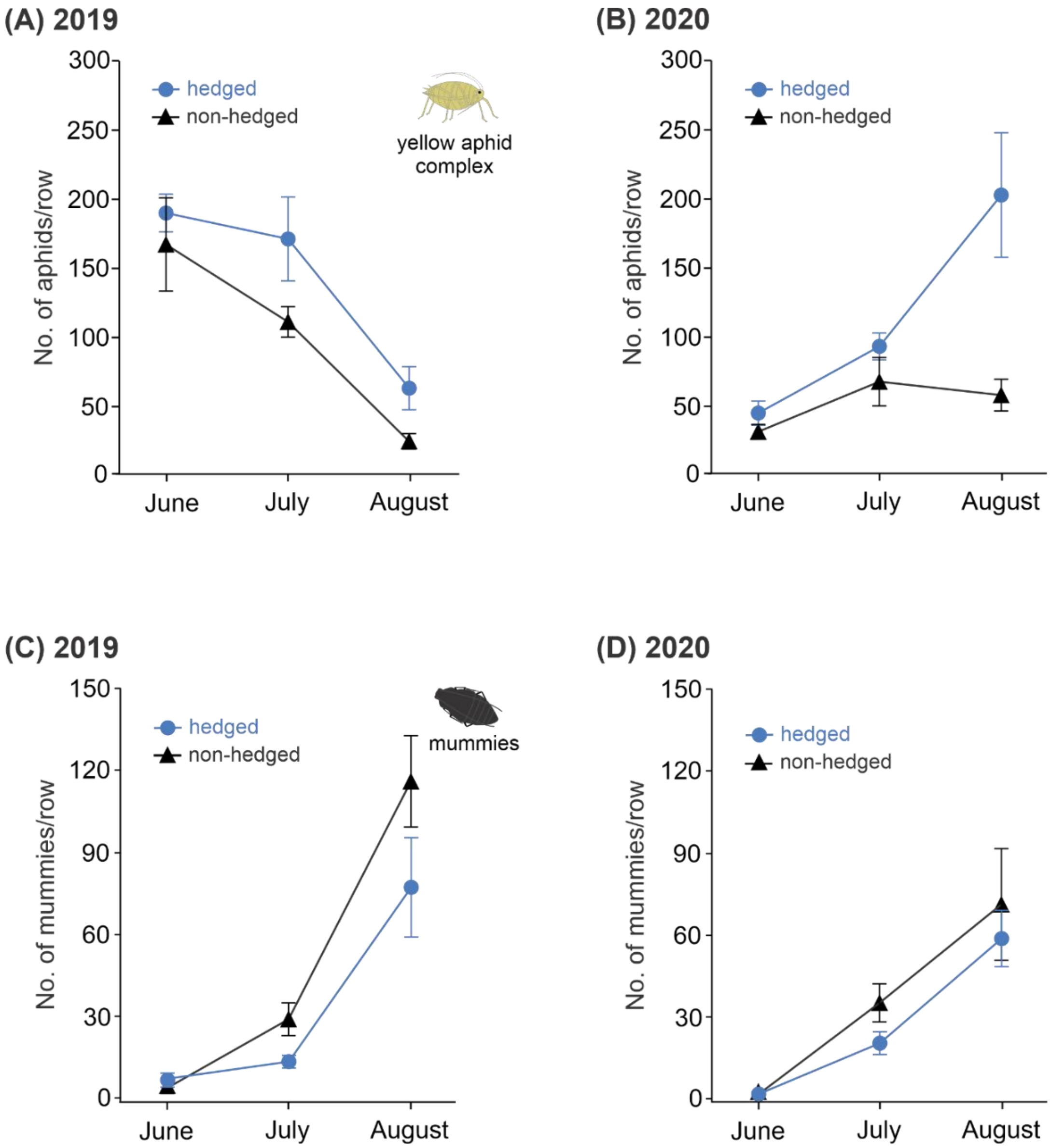


Figure 1.2 Abundance of yellow aphid complex species (**A and B**), and mummies (**C and D**) in relation to hedge pruning treatment (hedged *vs* non-hedged) over time. The number of aphids and mummies was assessed at three different time points in both the 2019 and 2020 seasons. Symbols with error bars represent the mean ± 1 SEM number of aphids or mummies per row (n = 4). No comparison between the years was performed.

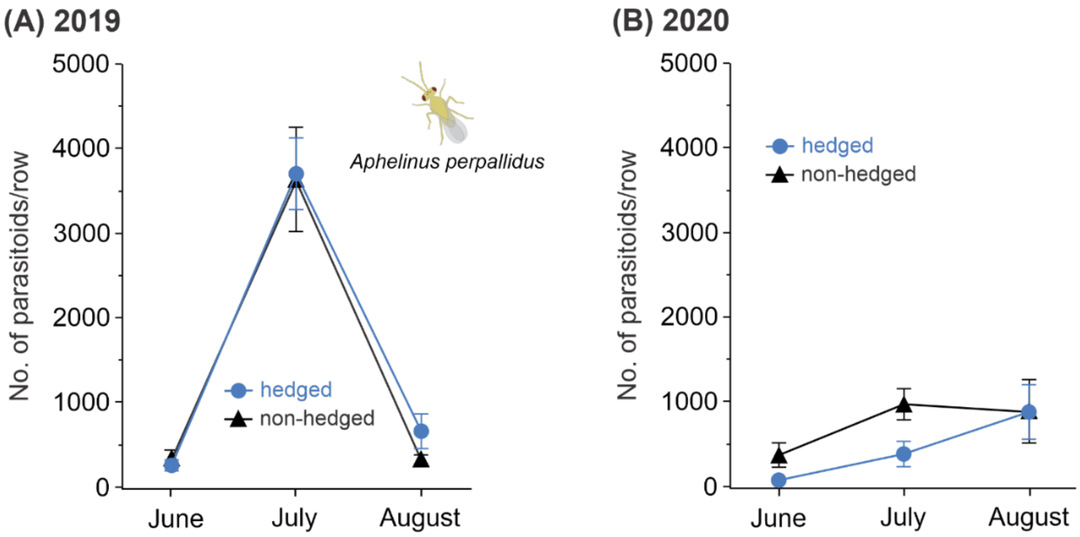


Figure 1.3 Activity of the parasitoid wasp, *Aphelinus perpallidus,* captured in yellow sticky cards in both the 2019 **(A)** and 2020 **(B)** seasons in relation to hedge pruning treatment (hedged *vs* non-hedged). Parasitoid activity was assessed at three different time points using yellow sticky cards. Symbols with error bars represent the mean ± 1 SEM of parasitoids captured per row (n = 4). No comparison between the years was performed.

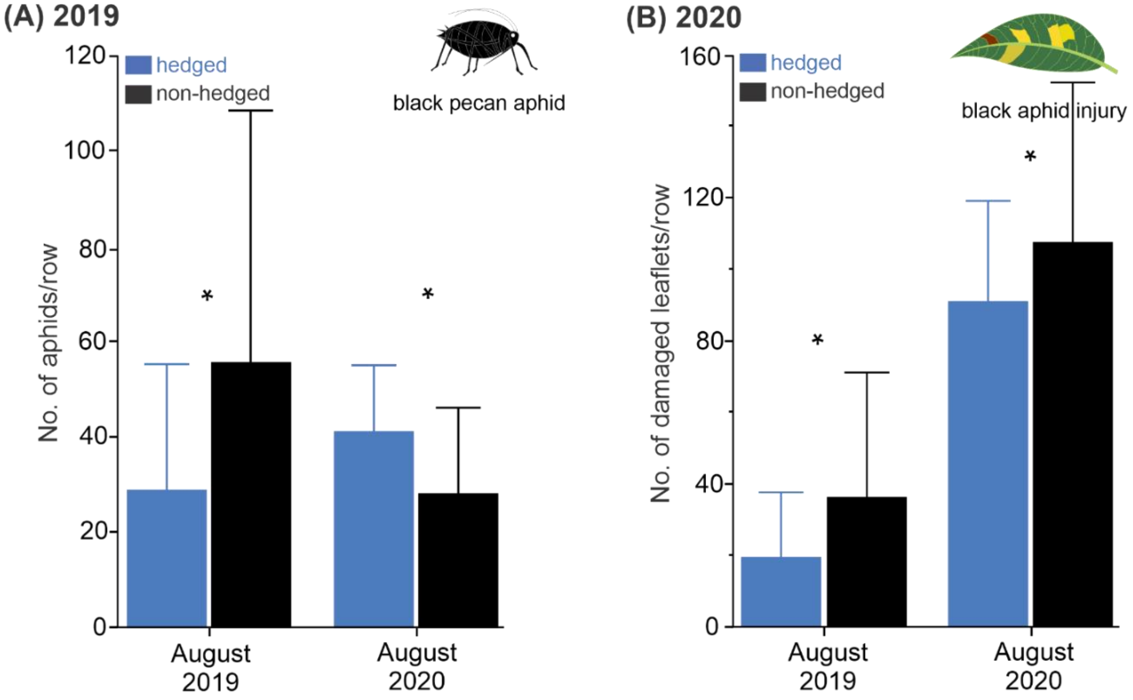


Figure 1.4 Abundance of black pecan aphids **(A)** and black pecan aphid-related injury **(B)** in relation to hedge pruning treatment (hedged *vs* non-hedged trees) in August of 2019 and 2020. Bars represent the mean ± 1 SEM. Asterisks represent differences between treatments. No comparison between years was performed.

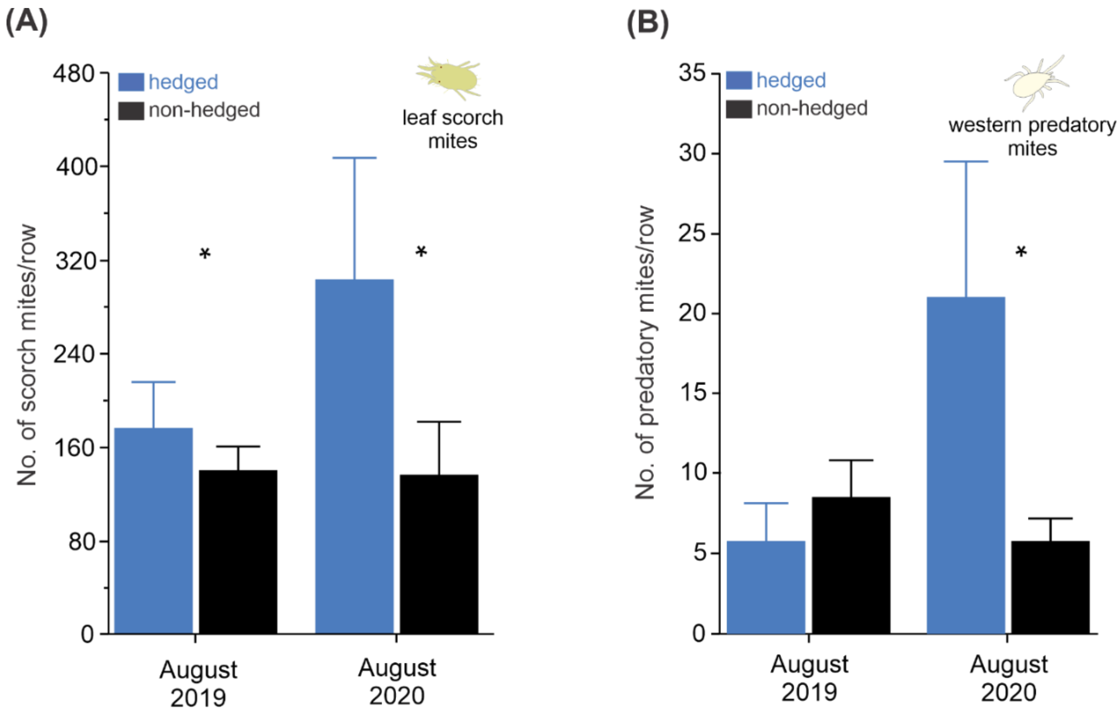


Figure 1.5 Abundance of pecan leaf scorch mites **(A)** and western predatory mites **(B)** in relation to hedge pruning treatment (hedged *vs* non-hedged trees) in August of 2019 and 2020. Bars represent the mean ± 1 SEM. Asterisk represent differences between treatments. No comparison between years was performed.

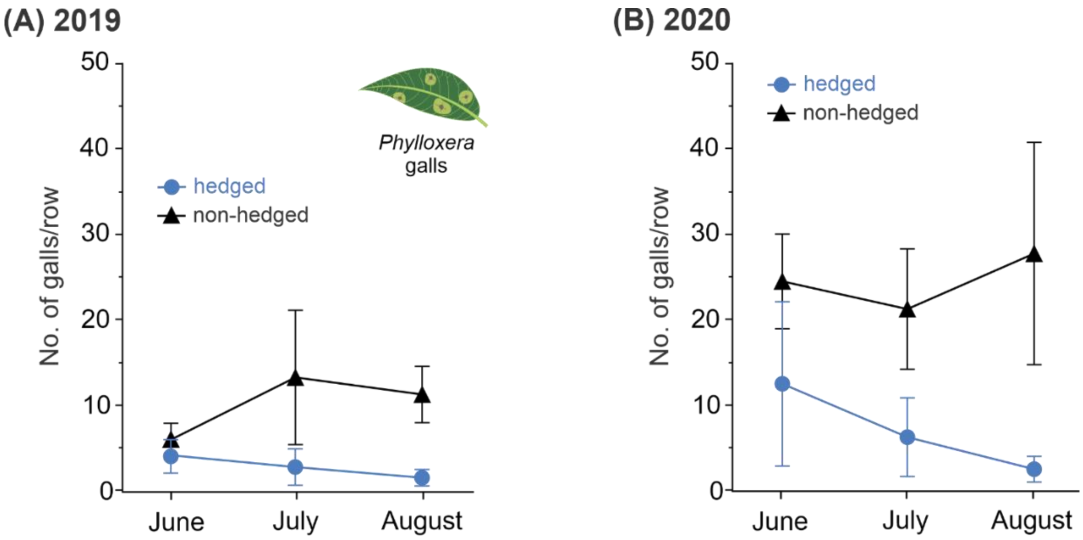


Figure 1.6 Number of galls formed by *Phylloxera* spp in both 2019 **(A)** and 2020 **(B)** seasons in relation to hedge pruning treatment (hedged *vs* non-hedged). The number of galls was assessed at three time points. Symbols with error bars represent the mean ± 1 SEM number of galls per row (N = 4). No comparison between the years was performed.

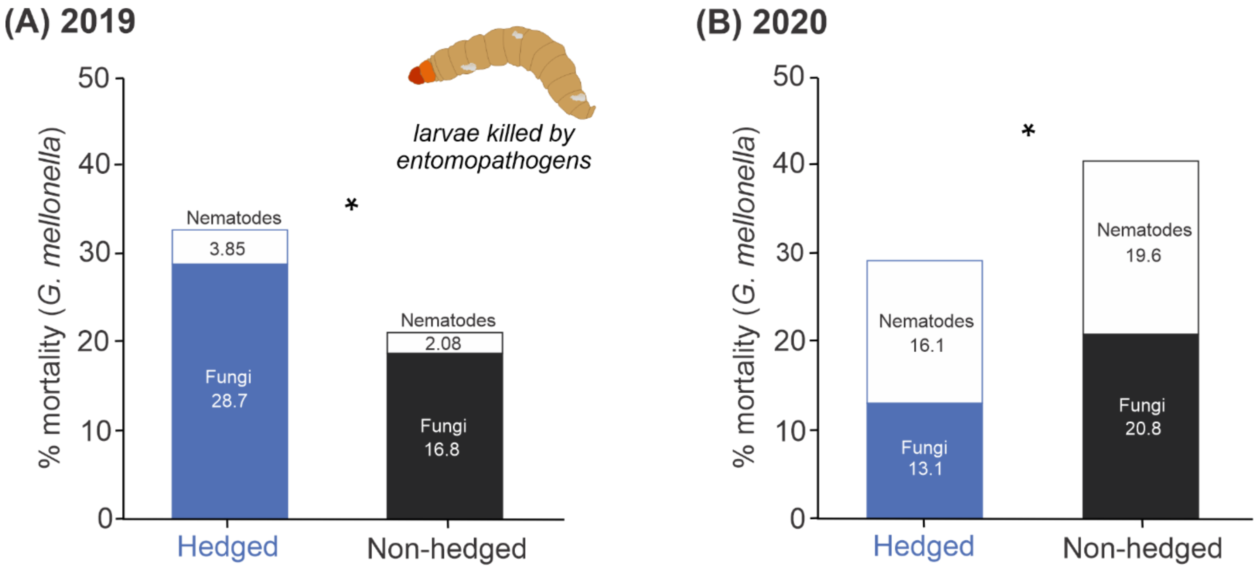
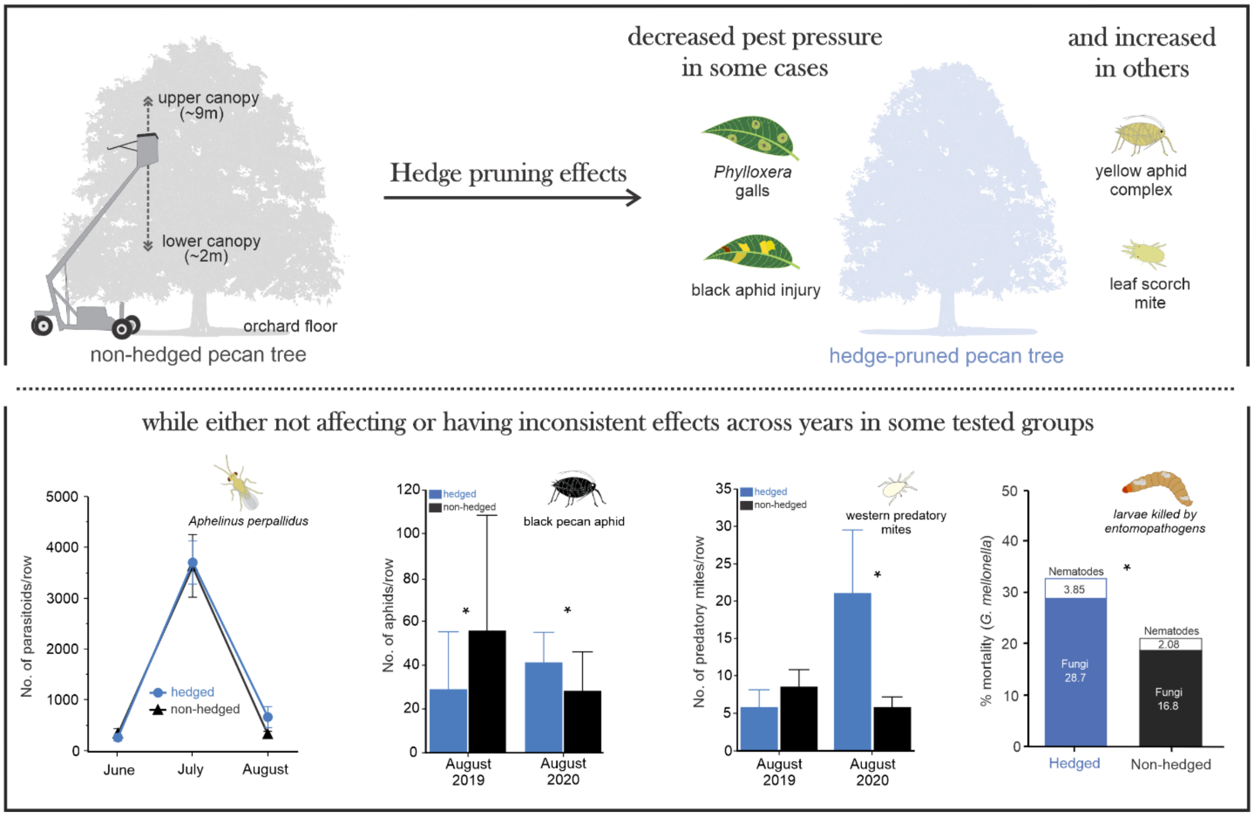


Figure 1.7 Mortality of larval hosts, *Galleria mellonella,* infected by entomopathogens (fungi and nematodes) from soil samples collected under hedge pruned or non-hedged pecan trees during the 2019 **(A)** and 2020 **(B)** seasons. Stacked bars show the percentage of mortality caused by each pathogen group. No comparison between years was performed.

Graphical Abstract



Highlights:

- Modifying canopy architecture can affect arthropod populations in pecan systems
- Hedging increased tree susceptibility to the yellow aphid complex and scorch mites
- Hedging reduced *Phylloxera* infestation and injury caused by black pecan aphids
- Natural enemies of arthropod pests responded inconsistently to hedge pruning
- Hedging cycles may implicate differential pest management decisions across seasons

Supplementary Table S1.1 Pesticide spray records during the sampling period for both 2019 and 2020 seasons are based on the in-farm personal management schedule at the Marshallville site.

	Date	Pesticide[†]	Type[‡]	Active ingredient	Rate (L/ha)	Method[§]
2020 Season	May 25	Super Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
		Interprid 2F	Insecticide	Methoxyfenozide	0.44	AirBlast Sprayer
	June 6	Super Tin	Fungicide	Triphenyltin hydroxide	0.88	Airplane
		Elast 400	Fungicide	Dodine	1.83	AirBlast Sprayer
	June 29	Ag-Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
	July 2	Elast 400F	Fungicide	Dodine	1.83	AirBlast Sprayer
	July 9	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	0.95	Airplane
	July 13	Ag-Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
		Elast 400F	Fungicide	Dodine	1.83	AirBlast Sprayer
	July 27	Ag-Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
	July 30	Elast 400F	Fungicide	Dodine	1.83	AirBlast Sprayer
		Transform	Insecticide	Sulfoxaflor	0.04	AirBlast Sprayer
	Aug 4	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	0.95	Airplane
	Aug 10	Elast 400	Fungicide	Dodine	1.83	AirBlast Sprayer
	Aug 13	Ag-Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
		Transform	Insecticide	Sulfoxaflor	0.04	AirBlast Sprayer
	Aug 21	Abba Ultra	Insecticide/Miticide	Abamectin	N/A	Irrigation
		N/A	Insecticide	Bifenthrin	N/A	Irrigation
2019 Season	May 20	Absolute 500SC	Fungicide	Tebuconazole/Trifloxystrobin	6 oz	AirBlast Sprayer
	June 10	Elast 400	Fungicide	Dodine	1.83	AirBlast Sprayer
		Ag-Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
	Jun 12	Elast 400	Fungicide	Dodine	1.83	AirBlast Sprayer
	June 24	Elast 400	Fungicide	Dodine	1.83	AirBlast Sprayer
		Ag-Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
		Macho 4.0	Insecticide	Imidacloprid	0.88	AirBlast Sprayer
	June 27	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	0.95	Airplane
	July 8	Elast 400	Fungicide	Dodine	1.83	AirBlast Sprayer
		Ag-Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
	July 22	Elast 400	Fungicide	Dodine	1.83	AirBlast Sprayer
		Ag-Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
	July 31	Elast 400	Fungicide	Dodine	3.65	AirBlast Sprayer
	Aug 5	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	0.95	Airplane
	Aug 8	Timectin 0.15EC	Insecticide/Miticide	Abamectin	N/A	Irrigation
	Aug 19	Ag-Tin	Fungicide	Triphenyltin hydroxide	0.88	AirBlast Sprayer
	Aug 23	N/A	Insecticide	Bifenthrin	N/A	Irrigation
		Transform	Insecticide	Sulfoxaflor	0.04	AirBlast Sprayer

[†] Trade name of product used. [‡] Pesticide category. [§] Method of application.

CHAPTER 2

ROUTINE FARMING PRACTICES IN TREE AGROECOSYSTEMS SYSTEMATICALLY INFLUENCE THE FATE OF ECOLOGY-BASED PEST MANAGEMENT[2]

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[2] Toledo, P.F.S. & Schmidt J.M. To be submitted to a peer-reviewed journal.

Abstract

Farming practices act as ecological filters, reshaping arthropod community structure and modifying species interactions, yet field-based evidence linking these processes to pest regulation remains limited. In a tall-canopy pecan orchard, we tested how two routine farming practices, canopy pruning and chemical management for disease and pests, affect aphid suppression by natural enemies. Using a factorial field experiment in a tall-canopy orchard, we integrated vertical stratified sampling, microclimate monitoring, and molecular diagnostics to quantify how pruning and pesticide inputs reshape aphid–enemy dynamics. Pruning reduced canopy height, altered microclimate, and increased aphid pressure, but was associated with lower aphid predation, particularly early in the season. Pesticide application suppressed predation in early months but enhanced aphid parasitism, especially within hedged canopies. Parasitism was vertically stratified, greater in the lower canopy layers. In contrast, predation was vertically uniform. These responses between parasitoids and predators reflect differing sensitivity to structural and pesticide-induced disturbances, highlighting how management practices alter the ecological context in which biological control unfolds. Pest suppression was influenced not only by natural enemy abundance but also by their spatial and temporal alignment with prey. Our findings offer a system-level perspective on how farming-induced stressors filter natural enemy communities and modify interaction dynamics, providing empirical support for more predictive and ecologically grounded approaches to biological control in perennial tree systems.

Keywords: Predator-prey interactions, farming-driven disturbance**,** molecular gut-content analysis, perennial cropping systems

Introduction

Ecology-based pest management strategies offer a promising pathway to reduce chemical inputs and improve sustainability in agriculture. Yet, designing tactics aimed at favoring natural enemy communities over herbivore populations is constrained by variable outcomes across systems and landscapes (Gurr et al., 2017; Karp et al., 2018). While tactics such as habitat diversification can increase top-down pressure and improve pest control in some contexts, their success remains difficult to predict (Perović et al., 2021; Tscharntke et al., 2016). A key limitation is often our poor understanding of how pest–natural enemy communities and the ecological processes driving pest suppression are structured across differently managed agroecosystems (Deguine et al., 2021).

Management systems filter pest-enemy interactions by species capable of tolerating given environments which determines species persistence and interactions across time and space (Cadotte and Tucker, 2017; Keddy, 1992). Temporally, herbivorous pest species exhibit phenological patterns (*e.g.*, predicted by degree day), becoming active during different periods of the season depending on their thermal requirements, developmental timing, and ecological roles (Rincon et al., 2024). Spatially, the structure of vegetation can create gradients in light exposure, temperature, and foliage density, affecting insect distribution and contributing to within-plant stratified arthropod communities (Marquis et al., 2002; Pincebourde et al., 2007). Consequently, the effectiveness of pest suppression will depend not only on the presence and abundance of natural enemies, but also on their alignment or synchrony with prey across both space and time.

Because pest-enemy interactions depend on spatiotemporal alignment, their sensitivity to changes in environmental conditions, including common farming interventions, likely impact success. Practices such as pruning, pesticide application, and cover cropping can restructure the environment, altering microclimate, vegetation architecture, and the distribution of resources (Bowers et al., 2021; Finke and Denno, 2006; Keddy, 1992; Thies et al., 2008, 2005; Waage et al., 1985). For instance, pruning reduces canopy volume and structure, which can shift arthropod distributions and potentially decrease spatial overlaps in the pest-natural enemy equation (Brenard et al., 2020; Riihimaeki et al., 2006). Chemical control through pesticide applications is also a strong ecological filter of arthropod communities, killing or displacing species, and shifting ecological dominance (Cordeiro et al., 2014; Guedes et al., 2022). Often, natural enemies are found to be more sensitive to insecticides than are pests (Sánchez-Bayo, 2021), which can disrupt predator-prey interactions and reduce biocontrol potential (Desneux et al., 2007; Guedes et al., 2022, 2016). Furthermore, the effects of management may interact. For example, pruning can enhance pesticide coverage by reducing foliage density, and, consequently, increasing non-target effects on natural enemies that take shelter in the canopy interior (Yeary et al., 2018).

Although farming practices are known to restructure arthropod communities, most studies focus primarily on changes in abundance of species (Benton et al., 2002; Raven and Wagner, 2021; Tscharntke et al., 2005) offering limited information about interactions. However, recent advances in molecular ecology, such as PCR-based gut-content analysis and DNA metabarcoding offer an avenue to estimate interactive effects of management on pest-natural enemy populations and predator-prey (Schmidt et al., 2014) and host-parasitoid (Lefort et al., 2020; Slusher et al., 2024) interactions.

To investigate how common agricultural practices *(i.e.*, pruning and chemical control) restructure prey-enemy dynamics in vertically complex systems, we used mature pecan orchards (*Carya illinoinensis*) as a model. Pecan systems feature canopies exceeding 15 meters in height, forming distinct vertical strata that may structure arthropod distributions and interactions. For example, both aphid pests and their natural enemies exhibit stratification within pecan canopies (Cottrell, 2017; Edelson and Estes, 1983; Slusher et al., 2022). Additionally, such perennial tree production systems are routinely subjected to management interventions such as pruning and pesticide applications (Wells, 2024, 2021, 2018; Wood, 2009), providing opportunities for studying prey-enemy dynamics under differentially managed canopies.

Thus, we test how hedge pruning and chemical management interact to shape insect population dynamics and trophic interactions. Specifically, we (1) assessed whether there is microclimatic gradient in the pecan canopy and how pruning alters microclimatic canopy conditions; (2) examined the spatiotemporal distribution of aphid pests, their predators, and parasitoids under contrasting management regimes; (3) used PCR-based gut-content analysis to detect aphid predation and quantified aphid parasitism rates through mummified aphids; and (4) evaluated how the strength of biological control processes vary across time, canopy strata and management combinations. By integrating field-based experimentation and molecular diagnostics, we demonstrate a mechanistic framework for understanding farm practices that act as ecological filters influencing pest regulation in perennial agroecosystems.

Material and Methods

Managing canopy structure and pest-disease pressure: A factorial approach in a pecan orchard

This study was conducted in an experimental pecan orchard (32°39'55.1"N, 83°43'45.7"W) at the USDA, ARS, Southeastern Fruit and Tree Nut Research Station in Byron, Georgia. The orchard consisted of 40-year-old ‘Pawnee’ pecan trees, approximately 17 meters tall, spaced 12 meters apart within rows and 24 meters between rows. The space between tree rows was regularly mowed, while a 6-meter-wide herbicide strip beneath the trees was kept free of vegetation. No supplemental irrigation was provided, relying solely on natural rainfall. To evaluate the effects of pruning and chemical management on herbivore populations, natural enemy activity, and their trophic interactions, we implemented a 2×2 factorial design across 36 trees. From the ~110 trees in the orchard, we randomly selected 12 groups, each consisting of three consecutive trees. Half of these groups underwent hedge pruning, with three receiving chemical management for pests and diseases and three left untreated. Hedge pruning was performed manually using chainsaws to a height of ~9.5 meters, simulating mechanical hedge pruning. The north side of trees was pruned in winter 2020, and the south side in winter 2021. The remaining six groups were not pruned but followed the same chemical management. Each treatment combination (pruned + sprayed, pruned + unsprayed, unpruned + sprayed, unpruned + unsprayed) was replicated three times. To capture the seasonal dynamics of herbivore and natural enemy communities, sampling was conducted over two consecutive growing seasons (2021 and 2022). Four assessments per year were performed, aligning with key phenological stages from early to late season: June (post-pollination), July (rapid nut expansion), August (kernel filling), and September (pre-harvest shuck split) (Wells and Conner, 2007). Sampling was only conducted on the middle tree in each group, with the two adjacent trees serving as buffers.

Environmental context: post-pruning canopy structure and microclimate

To account for potential background variation introduced by the hedge pruning cycle on the structure and microclimatic conditions of the canopy, we measured canopy height, temperature, and humidity in hedge-pruned and unpruned trees. Canopy height was measured using an optical rangefinder (Opti-Logic Insight 400 XL, Opti-Logic Corporation, Tullahoma, TN, USA). To position the rangefinder at the appropriate height, we used a telescopic crawler boom lift (JLG 660SJC, JLG Industries, Inc., McConnellsburg, PA, USA), which could reach up to ~20 meters. The lift platform was raised and leveled with the highest branches of the canopy, allowing the rangefinder to be aimed at the ground for measurement. For each tree, height was recorded three consecutive times to ensure accuracy, and the average was used as a replicate. To monitor temperature and relative humidity, we deployed data loggers (HOBO MX2301A, Onset Computer Corporation, Bourne, MA, USA) within the canopy. Loggers were placed near the central axis of the tree (closer to the interior) at both upper and lower canopy sections of hedge-pruned and unpruned trees. Measurements were recorded hourly throughout the sampling period to capture microclimatic conditions within the canopy.

Estimating herbivores and natural enemy activity across canopy environments

To quantify aphid and natural enemy communities in the pecan canopy, we conducted suction sampling using a reverse leaf blower (STIHL BG 86, Andreas Stihl AG & Co. KG, Waiblingen, Germany) operated for 30 seconds per sample, moving gently around pecan branches within the operator’s reach from a hydraulic lift basket. A telescopic crawler boom lift was used to access the upper canopy. Sampling was conducted at three vertical canopy strata (lower, middle, and upper), with two samples per height per tree, taken from opposite sides (north and south), totaling six vacuum samples per tree. Lower canopy samples were taken from the lowest accessible branches, while upper canopy samples were collected from the highest branches. Middle canopy samples were consistently taken at ~9.5 meters from the ground, aligning with the height of hedge pruning. Because tree height varied between pruned and unpruned trees, this middle level served as a fixed reference point for vertical comparisons. Counts from both sides of each tree were summed to obtain a single abundance value per canopy stratum. Samples were stored in gallon-sized resealable plastic bags, transported to the laboratory, and kept at −20°C until processing.

A total of 576 suction samples were processed over two years. Within each sample, we counted immature and adult stages of all aphids, including the black pecan aphid (*Melanocallis caryaefoliae* [Davis]; Hemiptera: Aphididae) and the "yellow aphid complex", a collective term referring to the blackmargined aphid (*Monellia caryella* [Fitch]) and the yellow pecan aphid (*Monelliopsis pecanis* Bissell). We recorded predatory arthropods present in the samples and focused on the five most frequently encountered and numerically dominant predator groups (occurring in more than 10% of samples). These included minute pirate bugs (*Orius* sp.; Hemiptera: Anthocoridae), assassin bugs (Reduviidae; both nymphs and adults), ladybeetles (Coccinellidae; larvae and adults), lacewing larvae (Neuroptera), and long-legged flies (Diptera: Dolichopodidae; adults). These groups were retained for downstream analyses focused on their distribution across canopy strata and their response to management practices.

Because the pecan aphid parasitoid *Aphelinus perpallidus* (Gahan, 1924) is rarely captured in suction samples due to its small size and fragility, an additional monitoring method was used to assess its presence. Yellow sticky card traps (7.6 × 12.7 cm, Olson Products Inc., Medina, OH) were deployed at the same three canopy heights. Two cards per height per tree (one per side) were attached to branches with twist ties. To prevent unintended adhesion to leaves, only the lower protective layer was peeled off, with the adhesive surface facing downward, leaving one side exposed. Sticky cards remained in the field for one week per deployment before being collected and examined in the laboratory under a stereomicroscope. Counts from both sides of each tree were summed per canopy stratum. Traps were deployed four times per year (June, July, August, and September) in both sampling years, totaling 576 sticky cards processed over two years.

Molecular-based detection of biocontrol services on aphids

To determine predator interactions with aphids, we conducted PCR-based molecular gut content analysis on predatory insects from the suction samples. Predators were sorted, identified to genus level, and stored at −20°C until DNA extraction. Prior to extraction, individuals were externally cleansed by rinsing them in 10% bleach solution, followed by molecular-grade water and 100% ethanol to minimize contamination. All predatory species present in more than 10% of the samples were screened for aphid consumption. The predator community analyzed included *Orius* spp. (Hemiptera: Anthocoridae), ladybeetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae and Hemerobiidae), assassin bugs (Hemiptera: Reduviidae), and dolichopodid flies (Diptera: Dolichopodidae). Whole-body DNA extractions were performed using the Qiagen 96-well DNeasy Blood & Tissue Kit, following the manufacturer's protocol, and eluted in 120 μL AE buffer. Predators were screened for aphid DNA using established primers targeting Diptera and Thrips prey. PCR conditions followed previously published protocols, and results were visualized using the QIAxcel Advanced system, with a positive detection threshold of >0.07 relative fluorescence units (RFUs).

Data management and statistical analysis

Linear mixed-effects models (LMEs) and generalized linear mixed models (GLMMs) were used to assess the effects of canopy management and spatiotemporal variation on microclimatic conditions and arthropod responses within the pecan canopy. All statistical analyses and base figures were produced in R (version 4.1.0). Figure icons, labels, and colors were added using CorelDRAW Graphics Suite 2021.

Microclimate in the canopy. To assess the effects of hedge pruning, vertical canopy stratification, and their interaction on temperature and relative humidity, hourly temperature or relative humidity values were averaged to daily means per tree. These were analyzed using LMEs (nlme package), with hedging, canopy location, and year (two growing seasons 2021 and 2022) as fixed effects, and date as a random effect to account for repeated logger measurements. To evaluate diurnal variation, the dataset was also partitioned into daytime (06:00–18:00) and nighttime (18:00–06:00) periods and analyzed using the same model structure.

Insect response models and model selection process. Due to limited statistical power to estimate all interactions, we applied model selection for all insect response variables. Full models included fixed effects for pruning, pesticide treatment, canopy location, sampling date, and all relevant two-way interactions (excluding date × location, which was omitted due to lower biological interest and to reduce model complexity). Year was included as a random intercept to account for repeated sampling. For each response variable, models were evaluated using the dredge() function of the MuMIn package (Barton and Barton, 2015), which ranks all nested models by corrected Akaike Information Criterion (AICc) (Burnham et al., 2011). Models within $\Delta AICc \leq 2$ of the best-fitting models were reported retained for inference (Supplementary Tables

2.1-2.3). Total aphid, yellow aphid, and parasitoid counts were analyzed using linear mixed-effects models (LMEs) on log-transformed data log (count +1). To account for heteroscedasticity across sampling dates, we applied a varIdent variance structure (nlme package). Transformations did not improve the model adequacy for black pecan aphids, so black pecan aphid counts were analyzed using GLMMs with a negative binomial distribution (glmmTMB). Dispersion was modeled as a function of sampling date. Fixed and random effects followed the structure described above. Total predator abundance was analyzed using generalized linear mixed models (GLMMs) with a negative binomial error distribution (glmmTMB) to account for overdispersion in count data. Separate models were then fit for each of the five numerically dominant predator groups: ladybeetles, assassin bugs, lacewing larvae, long-legged flies, and minute pirate bugs.

Examining biological control responses (aphid parasitism and predation)

Parasitism rates and aphid predation were analyzed as proportional responses using binomial GLMMs (glmmTMB) with a logit link function. Models contained the same fixed and random effects following the structure described above.

Results

Hedge pruning modified vertical structure and altered canopy-scale microclimate

Hedge pruning significantly reduced tree height, with pruned trees averaging 15.68 m and non-hedged trees 17.15 m ($t = –3.16$, $P = 0.0102$; Figure 2.1*a*). Mean daily temperature was higher in non-hedged trees ($\chi^2 = 156.88$, $df = 1$, $P < 0.0001$) and in the upper canopy ($\chi^2 = 308.09$, $df = 1$, $P < 0.0001$). However, a significant interaction between hedging and canopy location ($\chi^2 =$

29.58, *df* = 1, $P < 0.0001$) indicated that hedging reduced temperature more in the upper canopy (–0.24°C, $t = –19.69$, *df* = 1959, $P < 0.0001$) than in the lower canopy (–0.15°C, $t = –12.53$, *df* = 1959, $P < 0.0001$). Relative humidity was higher in hedged trees ($\chi^2 = 790.11$, *df* = 1, $P < 0.0001$) and in the lower canopy ($\chi^2 = 543.95$, *df* = 1, $P < 0.0001$). However, a significant interaction ($\chi^2 = 8.37$, *df* = 1, $P = 3.82 \times 10^{-3}$) showed that hedging increased humidity more in the lower canopy (+2.09%, $t = 28.11$, *df* = 1959, $P < 0.0001$) than in the upper canopy (+1.78%, $t = 23.18$, *df* = 1959, $P < 0.0001$).

Day-night patterns reflected unique canopy climates in pruned trees. During the day, temperature was higher in non-hedged trees ($\chi^2 = 186.10$, *df* = 1, $P < 0.0001$) and in the upper canopy ($\chi^2 = 390.60$, *df* = 1, $P < 0.0001$). The hedging × canopy interaction was not significant ($\chi^2 = 2.86$, *df* = 1, $P = 0.0908$), indicating that the cooling effect of hedging was similar across canopy positions (Figure 2.1*b*). Relative humidity during the day was higher in hedged trees ($\chi^2 = 873.53$, *df* = 1, $P < 2.2 \times 10^{-16}$) and in the lower canopy ($\chi^2 = 482.35$, *df* = 1, $P < 0.0001$; Figure 2.1c). A significant interaction ($\chi^2 = 18.37$, *df* = 1, $P < 0.0001$) indicated that hedging increased humidity more in the lower canopy (+2.25%, $t = 29.56$, *df* = 1959, $P < 0.0001$) than in the upper canopy (+1.78%, $t = 22.64$, *df* = 1959, $P < 0.0001$). At night, temperature remained higher in non-hedged trees ($\chi^2 = 39.48$, *df* = 1, $P < 0.0001$) and in the upper canopy ($\chi^2 = 68.40$, *df* = 1, $P < 0.0001$; Figure 2.1*b*). A significant interaction ($\chi^2 = 42.54$, *df* = 1, $P < 0.0001$) showed that hedging cooled the upper canopy more (–0.25°C, $t = –15.16$, *df* = 1959, $P < 0.0001$) than the lower canopy (–0.10°C, $t = –6.28$, *df* = 1959, $P < 0.0001$). Nighttime humidity was higher in hedged trees ($\chi^2 = 450.12$, *df* = 1, $P < 0.0001$) and in the lower canopy ($\chi^2 = 390.29$, df = 1, $P < 0.0001$). However, the interaction was not significant ($\chi^2 = 1.33$, *df* = 1, $P = 0.249$), indicating similar increases across canopy positions (Figure 2.1c).

Aphid populations exhibited seasonal and vertical structuring under pruning and pesticide regimes

Combined aphid pressure increased in late season and varied by stratum and management. The best-fitting model (lowest AIC) for total aphid abundance (yellow aphid complex + black pecan aphids) retained sampling date, canopy location, pesticide treatment, hedging, and a date × hedging interaction (Supplementary table 2.1). Sampling date had the strongest effect ($F_{3,276}$ = 83.97, $P < 0.0001$; Table 2.1) and aphid populations were higher in September (Figure 2.2*a*-*b*). Total aphid abundance was significantly lower in the upper canopy (Figure 2.2*b*) and higher in pesticide-treated trees (Table 1); A significant main effect of hedging was observed, and its effect varied by sampling date (date × hedging: Table 2.1). Pairwise contrasts showed that hedged trees hosted significantly more aphids than non-hedged trees in June (t_{276} = 4.35, $P <$ 0.0001; Figure 2*a*), with no significant differences detected in July, August, or September.

Early yellow aphid complex outbreaks in hedged trees reflected seasonal and vegetation structural effects. Model selection retained sampling date, canopy location, pesticide treatment, hedging, and a date × hedging interaction as predictors of yellow aphid abundance. The sampling date was the strongest predictor (Table 2.1), with higher abundance in September and lowest counts in July (Figure 2.2*c*). Abundance was significantly lower in the upper canopy (Table 2.2; Figure 2.2*d*) and higher in pesticide-treated trees (Table 2.1; Figure 2.2*e*). A significant main effect of hedging was also detected, but its influence varied across sampling periods, as indicated by a significant date × hedging interaction (Table 2.1). Yellow aphid complex abundance was significantly higher in hedged trees during June ($t = 4.60$, $df = 276$ $P < 0.0001$ Figure 2.2*c*), while no significant differences were detected in July, August, or September.

Black pecan aphid suppression by pesticides was offset by upper canopy increases under hedge pruning. Model selection identified sampling date, pesticide treatment, hedging, and a hedging × canopy location interaction as important predictors of black pecan aphid abundance (Supplementary table 1). Sampling date was the strongest driver (Table 2.1), with the lowest counts in June and July, and higher in September (Figure 2.2*e*). Pesticide application significantly reduced black pecan aphid abundance (Table 2.1). Hedge pruning increased black pecan aphid abundance, and a significant hedging × location interaction (Table 2.1; Figure 2.2*f*) showed that black pecan aphid abundance was significantly higher in hedged trees within the upper canopy ($z = 5.02$, $P < 0.0001$), while no differences were observed in the middle or lower canopy.

Overall parasitoid and predator populations were correlated with pesticide use and canopy structure

Parasitoid activity density fluctuated over the season and responded to pesticide inputs and canopy structure. Model selection retained sampling date, hedging, canopy location, pesticide treatment, and the interactions date × treatment, hedging × location, and location × treatment as predictors of aphid parasitoids (Supplementary table 2.1). Abundance of *Aphelinus* sp. on yellow sticky cards varied significantly across sampling dates (Table 2.1, Figure 2.3*a*). Parasitoid abundance was influenced by pesticide treatment, and this effect varied across sampling dates (Table 2.1; Figure 2.3*a*). Abundance was significantly higher in pesticide-treated trees compared to controls in September ($t = –5.44$, $P < 0.0001$) and August ($t = –3.30$, $P = 0.0011$), but not in June or July. The treatment × location interaction was not significant. Hedging had a marginal, non-significant effect ($F_{1,272} = 3.49$, $P = 0.063$), and this effect varied marginally by canopy position ($F_{2,272} = 2.92$, $P = 0.056$). Abundance was significantly lower in the lower canopy of hedged trees compared to non-hedged trees ($t = –3.04$, $P = 0.0026$; Figure 2.3*c*), while no difference was detected in the middle or upper canopy.

Total predator abundance increased with hedge pruning but declined with pesticides. Predator communities in pecan canopies were composed of five numerically dominant insect taxa. Combined for both years, Coccinellidae comprised the largest percentage of the predator community (33.4%), followed by Reduviidae (21.6%), *Orius* sp. (20.8%), Neuroptera (12.3%), and Dolichopodidae (11.9%). These groups were tested for aphid predation across both years (Table 2.2, Supplementary Figure 2.1). A total of 1,956 insect predators were identified and subjected to PCR-based gut-content analysis. For the initial analysis, we explored the overall effects of design elements on total predator abundance.

The best fitting model for total predator abundance retained the main effects of sampling date, hedging, and pesticide treatment, as well as the interactions date × hedging and date × treatment (Supplementary table 2.2). Predator abundance varied significantly across sampling dates ($\chi^2 = 67.99$, $df = 3$, $P = 1.15 \times 10^{-14}$), with higher abundance observed in June ($z = 4.39$, $P = 1.12 \times 10^{-5}$) and September ($z = 5.43$, $P = 5.52 \times 10^{-8}$) relative to August, while no difference was detected in July and August (Figure 2.4*a-b*). Hedge pruning significantly influenced predator counts ($\chi^2 = 19.72$, $df = 1$, $P = 8.97 \times 10^{-7}$), though this effect was dependent on sampling date, as indicated by a significant date × hedging interaction ($\chi^2 = 15.39$, $df = 3$, $P = 0.0015$; Figure 2.4*a*). Pairwise comparisons revealed that hedged trees supported significantly more predators in June ($z = 5.27$, $P < 0.0001$) and September ($z = 2.45$, $P = 0.0143$), whereas no hedging effect was detected in July or August. Pesticide treatment had a marginally significant effect ($\chi^2 = 2.93$, $df = 1$, $P = 0.087$), but its role was better captured through the significant date ×

treatment interaction ($\chi^2 = 8.03$, *df* = 3, $P = 0.045$; Figure 2.4*b*). Post hoc comparisons showed that predators were significantly more abundant in control plots than in pesticide-treated plots in September ($z = 3.13$, $P = 0.0018$), while no treatment effects were detected in June, July, or August. Following overall predator abundance analysis, subsequent analyses attempted to tease apart predator group specific responses, and we grouped predator responses, where possible, by common statistical outcomes.

Predator group specific responses to pesticide use and hedging

Assassin bugs increased in abundance in response to pruning in the lower canopy but declined under late-season pesticide use. The best fitting model for assassin bugs (Reduviidae) abundance retained the main effects of sampling date, hedging, canopy location, and pesticide treatment, as well as the interactions date × treatment, hedging × location, and location × treatment (Supplementary table 2.3). Assassin bug counts varied significantly across sampling dates (Table 2.2), with lower abundance in June ($z = -4.22$, $P = 2.41 \times 10^{-5}$) and July ($z = -3.13$, $P = 0.0017$) relative to August, and higher abundance in September ($z = 3.53$, $P = 0.0004$; Figure 2.5a). Hedge pruning increased assassin bug abundance (Table 2.2), with significantly fewer individuals observed in non-hedged trees ($z = -3.30$, $P = 0.00097$), but this effect depended on canopy location (Figure 2.5b). The interaction between hedging and canopy location was also significant (Table 2.2), with hedging increasing assassin bug abundance specifically in the lower canopy ($z = 3.30$, $P = 0.0010$), while no significant differences were observed in the middle or upper canopy (Figure 5b). Assassin bug populations were stratified in the canopy (Table 2.2), with lower abundances in the middle ($z = -4.02$, $P = 5.83 \times 10^{-5}$) and upper canopy ($z = -3.37$, $P = 0.00075$) compared to the lower canopy. Pesticide treatment significantly affected assassin bug abundance (Table 2.2), though this effect was context dependent. A significant interaction between sampling date and pesticide treatment (Table 2.2) indicated that assassin bugs were significantly more abundant in control plots compared to pesticide-treated plots in September ($z = 3.89$, $P = 0.0001$), whereas no treatment effects were detected in June, July, or August (Figure 2.5a). A location × treatment interaction (Table 2.2) showed that the effect of pesticide treatment was strongest in the lower canopy, where abundance was significantly reduced in treated trees relative to controls ($z = 3.49$, $P = 0.0005$; Figure 2.5c); no significant differences were found in the middle or upper strata.

Orius sp. and ladybeetle abundance responded to hedge pruning and exhibit stratified distributions. The best fitting model for minute pirate bug (*Orius* sp.) abundance retained the main effects of sampling date, hedging, and canopy location (Supplementary table 2.3). Minute pirate bug counts varied significantly across sampling dates (Table 2.2), with higher abundance in June ($z = 8.67$, $P < 2 \times 10^{-16}$), July ($z = 2.79$, $P = 0.0052$), and marginally in September ($z = 1.69$, $P = 0.0915$) relative to August. Hedge pruned trees had higher *Orius* abundance (Table 2.2), with significantly lower counts observed in non-hedged trees (Figure 2.5*d*). Canopy location also influenced abundance (Table 2), with significantly more *Orius* sp. individuals in the upper canopy compared to the lower canopy ($z = 2.59$, $P = 0.0097$), while no differences were observed between middle and lower canopy. The top-ranked model for ladybeetle abundance retained the main effects of sampling date, hedging, and canopy location (Supplementary table 2.3). Ladybeetle counts varied significantly across sampling dates (Table 2), with higher abundance in July ($z = 3.05$, $P = 0.0023$), September ($z = 9.22$, $P < 2 \times 10^{-16}$), and marginally higher abundance in June ($z = 1.94$, $P = 0.0525$) relative to August (Figure 5e). Hedged pruned canopies had higher abundance of ladybeetles and canopy location was a significant factor ($\chi^2 =$

6.97, *df* = 2, $P = 0.0306$), where the middle canopy had higher abundance compared to the upper canopy ($z = 2.63$, $P = 0.0234$) but no differences were captured between lower and middle or lower and upper (Table 2.2; Figure 2.5*e-f*).

Canopy position and seasonality influenced distributions of immature lacewing and adult dolichopodid flies. The best fitting model for long-legged flies (Dolichopodidae) abundance retained the main effects of sampling date and canopy location (Supplementary table 2.3). Dolichopodid counts varied significantly across sampling dates (Table 2.2), with lower abundance in June compared to August ($z = -5.29$, $P < 0.0001$), while no differences were detected in July or September (Figure 2.5g). Vertical stratification also influenced dolichopodid fly abundance with higher abundance in the middle (z = 3.03, $P = 0.0025$) and upper canopy ($z = 4.29$, $P = 1.79 \times 10^{-5}$) compared to the lower canopy (Table 2.2, Figure 2.5g). The top-ranked model for lacewing larvae (Chrysopidae and Hemerobiidae) abundance retained the main effects of sampling date and canopy location (Supplementary table 2.3). Lacewing counts varied significantly across sampling dates (Table 2.2), with lower abundance in July ($z = -2.32$, $P = 0.0203$), and higher abundance in June ($z = 4.03$, $P = 5.69 \times 10^{-5}$) and September ($z = 4.42$, $P = 9.74 \times 10^{-6}$) compared to August (Figure 2.5h). Canopy location also significantly affected lacewing distribution (Table 2.2), with fewer individuals observed in the middle ($z = -2.41$, $P = 0.0159$) and upper canopy ($z = -4.46$, $P = 8.38 \times 10^{-6}$) relative to the lower canopy (Figure 2.5h).

Aphid parasitism and predation were mediated by pesticide use which was canopy location specific

Parasitism increased under pesticide input and appeared higher in the lower canopy. Model selection retained sampling date, hedging, canopy location, pesticide treatment, and the interactions date × treatment, hedging × treatment, hedging × location, and treatment × location as significant predictors of parasitism rate (Supplementary table 2.4). Parasitism rates varied significantly over time, and across canopy positions, with higher parasitism observed in the lower canopy compared to the upper canopy (Table 2.3, Figure 2.6*a*). Pesticide treatment was associated with a higher overall parasitism rate, but this effect varied by sampling date, hedging treatment, and canopy location (Table 2.3, Figure 2.6*a*). Specifically, parasitism was significantly higher in pesticide-treated trees compared to controls in September ($z = 15.64$, $P < 0.0001$), but no significant differences were detected in June, July, or August (Figure 6*a*). Within hedged trees, parasitism was elevated in the middle ($z = 5.04$, $P < 0.0001$) and lower canopy ($z = 2.89$, $P = 0.0038$), but not in the upper canopy. Additionally, parasitism was significantly higher in pesticide-treated trees relative to controls within hedged canopies ($z = 7.28$, $P < 0.0001$), whereas no difference was observed in non-hedged canopies. The significant treatment × location interaction (Table 2.3) indicated that pesticide application increased parasitism in the lower ($z = 6.14$, $P < 0.0001$) and middle canopy ($z = 7.31$, $P < 0.0001$), but not in the upper canopy (Figure 2.6*a*).

Hedge pruning decreased late-season predation while early-season pesticide use disrupted biological control. Overall predation frequencies varied among groups. Immature Coccinellidae exhibited the highest aphid detection rates (47.3% to 100%), followed by adult Coccinellidae (11.1% to 95.3%) and Reduviidae (59.1% to 81.0%). Lacewing larvae also showed high aphid detection (42.5–67.4%). In contrast, *Orius* sp. and Dolichopodidae had markedly lower detection frequencies, with *Orius* adults ranging from 0–26.4% and Dolichopodids from 16.7–57.4%. The top-ranked model for aphid predation (pooled) retained the main effects of sampling date, hedging, and pesticide treatment, as well as a date × treatment interaction, with year included as a random intercept (Supplementary table 2.4). Aphid predation rates varied significantly across sampling dates (Table 2.3, Figure 2.6*b*), with lower predation in June ($z = -2.67$, $P = 0.0075$) relative to September, while no differences were detected in July ($z = -1.18$, $p = 0.238$) or August ($z = -0.78$, $P = 0.433$). Predation was higher in September ($z = 10.56$, $P < 2 \times 10^{-16}$). Hedging decreased aphid predation (Table 2.3, Figure 2.6*b*), with higher rates observed in non-hedged trees ($z = 3.56$, $P = 0.0004$). Pesticide treatment had a marginal non-significant effect ($\chi^2 = 2.77$, $df = 1$, $P = 0.096$), but its influence varied over time, as indicated by a significant date × treatment interaction (Table 2.3). Predation rates were significantly higher in treated plots than in controls in June ($z = -2.26$, $P = 0.0239$) and July ($z = -2.04$, $P = 0.0414$), whereas no differences were observed in August or September (Figure 2.6*b*).

Discussion

In this study, we empirically demonstrate how stress induced by routine farming practices lead to cascading ecological effects across herbivore, predator, and parasitoid guilds. During our study, such interventions modified canopy microclimatic conditions, reshaped spatial patterns of herbivore abundance, filtered predator and parasitoid communities across vertical strata, and led to divergent outcomes for aphid suppression via parasitism and predation. In other words, effects propagate through bottom-up (Han et al., 2022) and top-down pathways, ultimately shaping insect community structure and interaction dynamics. Together, our findings exemplify theoretical models of habitat filtering and disturbance-driven community assembly (Cadotte and Tucker, 2017; Keddy, 1992; Kraft et al., 2015), useful frameworks to interpret how farming practices mediate species abundance, distribution and aphid biocontrol across time and space.

Structural alterations reshape vertical microclimates in tall canopies. Hedge pruning significantly altered tree structure (volume and height), resulting in smaller, cooler, and more humid canopies. Although hedge pruning was performed laterally, it removed tall, outward-angled branches that contributed to overall tree height. This reduction in canopy volume led to modified microclimate across vertical strata. As expected, the upper layers of the canopy were warmer compared to the lower, likely a result of the increased sunlight exposure in upper branches. However, contrary to our initial assumption that hedge pruning would increase canopy temperature by improving sunlight penetration (Lombardini, 2006), hedge pruned trees became cooler and provided a more humid environment. Such resulting microclimate patterns likely emerged from increased air circulation and shading by the rapidly growing lateral leaf flush induced by pruning cuts (Hellwig et al., 2022; Wood, 2009). The new flush replaced outward open branches, likely increasing shading to the canopy interior, where environmental data loggers were placed. These natural and induced microclimatic gradients can influence insect behavior, development, and survival, shaping arthropod distributions and interaction zones. Such gradients are likely to contribute to spatially variable herbivore and natural enemy activity recorded in the strata of pecan canopies (Cottrell, 2024, 2017; Dutcher et al., 2012; Edelson and Estes, 1987, 1983; Slusher et al., 2022)

Pecan aphid populations are temporally dynamic, spatially structured, and management responsive. Yellow aphid complex species (*Monellia caryella* and *Monelliopsis pecanis* combined) were numerically dominant across sampling periods, with higher abundance observed in June and September. In contrast, *Melanocallis caryaefoliae* (the black pecan aphid) remained at low densities through early season, with significant population increases occurring in September. These patterns align with their known phenological dynamics, where yellow aphids tend to exhibit bimodal peaks (or season-long presence) while black aphids increase gradually toward the end of the season (Cottrell, 2022; Dutcher et al., 2012; Slusher et al., 2021; Tedders, 1978). Additionally, both aphid groups were generally less abundant in the upper canopy compared to the lower strata, contributing to lower total aphid pressure at the top.

Yellow aphid complex species were more abundant in hedge pruned trees during June, suggesting an increased vulnerability of pruned systems that creates favorable conditions for yellow aphid colonization. This pattern supports our initial observations in a commercial orchard (Toledo et al., 2024) and may reflect benefits from the differential microclimate (Kaakeh and Dutcher, 1993) and their preference for leaves with softer tissue and smaller vein structures (Tedders, 1978). Hedge pruning stimulates new vegetative growth, increasing the availability of such tissues (Hellwig et al., 2022). Additionally, black pecan aphid population increased in the upper canopy of hedged trees, as indicated by a significant interaction between pruning and canopy location. Black pecan aphids usually disperse less compared to the yellow aphid complex species and begin colonization in lower branches before moving upwards as black aphid populations increase and the season progresses (Paulsen et al., 2013). The smaller canopy size in pruned trees may have facilitated colonization towards the upper strata. Moreover, although we did not tease apart which aphid species were consumed by predators, black pecan aphids may experience lower predation due to the tendency of their immature stages to occupy both adaxial and abaxial leaf surfaces. In contrast, yellow aphids primarily occupy the abaxial surface across life stages, where predatory natural enemies are more commonly encountered. Black pecan aphids may also be a less preferred food source than members of the yellow aphid complex. Interestingly, chemical management produced opposite effects on the two aphid groups. Yellow aphid complex species abundance increased under pesticide treatment, which may be a result of the reduced enemy populations, and resistance mechanisms that have been suggested for this aphid group (Wells, 2024). On the contrary, black pecan aphid abundance declined in treated canopies. These contrasting responses may reflect differential susceptibility to chemical compounds and inherent differences in feeding niche and colony behavior between aphid groups. Such differential sensitivity to pesticides could have contributed to yellow aphid complex dominance (Cordeiro et al., 2014; Mohammed et al., 2019). Because yellow aphids were numerically dominant for most of the season, total aphid pressure closely followed their dynamics. For instance, the June increase in total aphid counts in hedged trees was entirely attributable to yellow aphid complex species, as black pecan aphids were virtually absent during this period. Collectively, these results show how pruning and chemical management may influence herbivore species composition, and vertical distribution in a canopy.

Canopy management restructures natural enemy community dynamics. The natural enemy community studied here was composed of the parasitoid species *Aphelinus perpallidus*, a specialist endoparasitoid of pecan aphids (Slusher et al., 2024; Toledo et al., 2024), and five generalist predator taxa: assassin bugs (Reduviidae), minute pirate bugs (*Orius* sp.), ladybeetles (Coccinellidae), lacewing larvae (Neuroptera), and long-legged flies (Dolichopodidae). These groups differed in their seasonal dynamics and vertical distributions, likely reflecting their phenology and niche differentiation, where species may partition resources within the canopy strata, minimizing overlap (Finke and Denno, 2006; Finke and Snyder, 2008; Janssen et al., 2007).

Minute pirate bugs, *Orius* sp., were most abundant in June, particularly in hedged trees, and declined sharply as season progressed. In contrast, ladybeetles and assassin bugs increased later in the season, with both reaching highest abundance in September, likely responding to increased aphid densities (Symondson et al., 2002). Lacewing larvae were present throughout the season, with moderate abundance early, a decline in midsummer, and an increase toward September. Lacewing seasonal abundance and location in the canopy also seem to overlap with aphid populations (Kunkel and Cottrell, 2007). Higher predator counts in hedged trees, particularly in June and September, coincided with elevated aphid densities. While this pattern may reflect improved foraging access in pruned canopies, it is also consistent with a numerical response to prey abundance. In contrast, chemical management reduced predator abundance in September, with the negative impacts observed in Reduviidae predators, particularly in the lower canopy, where pesticide coverage by airblast sprayers is optimal (Bock et al., 2023; Bock and Hotchkiss, 2021). Generalist predators dwelling in pecan canopies are vulnerable to broad-spectrum insecticide (*e.g.,* pyrethroids and sulfoxamines) applications (Oliveira-Hofman et al., 2021; Wells, 2021), which can reduce predators' activity or survival and weaken biological control during periods of pest outbreak (Desneux et al., 2007).

The parasitoid *A. perpallidus* was abundant in June and July, declined in August, and increased again in September, but only in trees receiving pesticide treatment. No consistent effect of hedging was detected overall; however, parasitoid counts in the lower canopy were reduced in hedged trees. This reduction may reflect altered microclimatic conditions or increased exposure to predators in pruned canopies (Snyder and Ives, 2003) . The abundance increase in pesticide-treated trees in September may be explained by a partial release from intraguild predation (Lucas et al., 1998), a response to higher aphid density (Heimpel and Casas, 2008), or both. Despite periods of high activity, the vertical distribution of *A. perpallidus* (middle-upper canopy) did not align with the location of aphid aggregations (middle-lower canopy), suggesting spatial constraints (Slusher et al., 2022; Tylianakis and Morris, 2017). These findings indicate that predator and parasitoid contributions to aphid regulation are shaped by species-specific responses to temporal and spatial variation in resource availability. While hedging may enhance access to prey, its primary effect may be mediated through increased aphid abundance. In contrast, pesticide applications disrupt predator communities during periods of high pest pressure.

Biocontrol outcomes as emergent properties of management-structured systems. In perennial cropping systems like pecan orchards, biocontrol efficacy is not solely determined by the presence of natural enemies but emerges from the broader habitat structure. Hedge-pruning, for instance, is commonly used to control canopy volume, enhance light penetration and airflow and maintain or improve nut yield over time (Lombardini, 2006; Wells, 2018; Wood, 2009). Like in many other systems, decisions regarding pruning in pecans are not intended as a pest management strategy or constrained by it. Likewise, most pesticide applications target *Venturia effusa* (G. Winter) Rossman & W.C. Allen, the fungal pathogen responsible for pecan scab, rather than arthropod pests. However, the combination of canopy pruning and chemical inputs has downstream effects on aphid populations and their natural enemies (Toledo et al., 2024). Here, we show that these practices can reshape both habitat structure and community composition, altering the ecological context in which predator-prey and host-parasitoid interactions unfold.

Aphid parasitism increased over time, was higher in September, and in the lower and canopy. Parasitism rates were significantly higher in pesticide-treated trees, particularly within hedged canopies. These patterns were not explained by temporal shifts in parasitoid presence, as *A. perpallidus* was already active earlier in the season. Instead, these findings may reflect context-dependent increases in parasitoid efficacy, potentially driven by reduced intraguild predation pressure (from pesticide-suppressed predators) and/or higher host aggregation within pruned canopies, both of which could enhance host detectability and successful parasitism (Heimpel and Casas, 2008; Wajnberg et al., 2008). In contrast, aphid predation was higher in non-hedged trees, particularly in June and July, when aphid populations were beginning to rise. This indicates that hedging may disrupt early-season predator activity, possibly by simplifying canopy structure or altering the microclimatic conditions that facilitate predator foraging (Finke and Snyder, 2008). Predation was also significantly reduced by pesticide applications during June and July, suggesting that even pesticides targeting fungal pathogens can negatively affect predator-mediated biocontrol during critical early stages of aphid colonization. Unlike parasitism, predation did not vary significantly across canopy strata, which may reflect broader foraging ranges among predators or compositional differences in vertical distribution. However, these explanations remain speculative without an assessment of taxon-stratified predation data.

Together, these results show that natural enemy responses to management are neither uniform nor additive. While pesticide use appeared to enhance parasitism, it simultaneously reduced predator activity in the early season, which is a critical period for suppressing initial aphid buildup. In addition, pruning was associated with higher parasitism but lower predation, reinforcing the idea that management practices restructure ecological interactions in taxon-specific and temporally variable ways. In conclusion, we show that aphid-enemy population dynamics in tall trees are complex, and herbivore suppression via parasitism and predation emerges from context-dependent interactions shaped by vertical stratification, species-specific responses, and seasonal shifts in resource availability. Importantly, predator and parasitoid efficacy reflects not only their abundance but their alignment with prey distributions and the spatial configuration of the habitat. Our work provides information for a better understanding of agricultural stress in arthropod communities, and empirical evidence essential for advancing predictive, ecology-based pest management.

Acknowledgments

We thank Bryce Vaughn, Kirsten Flinn, Rachel Ibbetson, Kirby Moncrief and Ashley Allgary for technical assistance. The project was supported by the Agriculture and Food Initiative, project award no. 2023-67013-40765, from the U.S. Department of Agriculture's National Institute of Food and Agriculture, a SSARE award no. LS20-340, and a SSARE graduate student award no. GS21-236. This work was supported, in part, by the University of Georgia, and USDA-NIFA Multistate Hatch Project GEO00884-S1073. CHB received additional support from USDA-ARS projects 6606-21220-014–00D and 6042-22000-024-000-D, respectively. The mention of any trademark or proprietary product is for informational purposes only and does not constitute a guarantee, warranty, or endorsement by the U.S. Department of Agriculture or the University of Georgia. Likewise, references to specific commercial agrochemical products used in this study do not imply endorsement, recommendation, or preference by the authors or their respective institutions over other potentially suitable products.

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Table 2.1 Summary of fixed effects retained in the best-fitting linear mixed-effects models (LMEs) describing variation in yellow aphid complex (*Monelliopsis pecanis* and *Monellia caryella*) abundance, total aphid abundance (*Monelliopsis pecanis*, *Monellia caryella and Melanocallis caryaefoliae*), and *Aphelinus* sp. parasitoid counts across pecan canopy strata. The best-fitting model for black pecan aphid was analyzed using a generalized linear mixed-effects model (GLMM). Models were selected using Akaike's Information Criterion (AIC).

Sources of variation		$df_{\text{num}}/df_{\text{den}}$	***F***	***P***
	date	3/276	83.967	<0.0001*
Total Aphids	hedging	1/276	10.328	0.0015*
	location	2/276	9.766	0.0001*
	treatment	1/276	9.062	0.0029*
	date:hedging	3/276	3.867	0.0098*
Yellow aphid complex	date	3/276	81.266	<0.0001*
	hedging	1/276	7.262	0.0075*
	location	2/276	5.192	0.0061*
	treatment	1/276	7.097	0.0082*
	date:hedging	3/276	5.243	0.0016*
Black Pecan Aphids$^{\Delta}$	date	3	179.206	<0.0001*
	hedging	1	14.742	0.0001*
	location	2	3.631	0.1627
	treatment	1	14.778	0.0001*
	hedging:location	2	11.838	0.0027*
***Aphelinus* sp.**	date	3/272	49.597	<0.0001*
	hedging	1/272	3.493	0.0627
	location	2/272	15.503	<0.0001*
	treatment	1/272	13.394	0.0003*
	date:treatment	3/272	9.403	<0.0001*
	hedging:location	2/272	2.917	0.0558
	location:treatment	2/272	2.465	0.0869

*Effects with $P < 0.05$ are considered statistically significant and are highlighted.

$^{\Delta}$Black pecan aphid abundance was estimated with generalized linear models (GLMM) and test statistics is χ^2 and not F.

Table 2.2 Summary of fixed effects retained in the best-fitting generalized linear mixed-effects models (GLMMs) describing variation in the activity of dominant predator groups, assassin bugs (Reduviidae), long-legged flies (Dolichopodidae), ladybeetles (Coccinellidae), Lacewings (Chrysopidae + Hemerobiidae) minute pirate bugs (*Orius* sp.), and the pooled predator community across pecan canopy strata. Models were selected using Akaike's Information Criterion (AIC).

Sources of variation		*df*	χ^2	*P*
Reduviidae	date	3	106.944	<0.0001*
	hedging	1	4.097	0.0430*
	location	2	8.840	0.0120*
	treatment	1	8.634	0.0033*
	date:treatment	3	9.143	0.0275*
	hedging:location	2	8.492	0.0143*
	location:treatment	2	7.650	0.0218*
Lacewing	date	3	44.897	<0.0001*
	location	2	18.668	<0.0001*
Dolichopodidae	date	3	55.534	<0.0001*
	location	2	20.132	<0.0001*
Coccinelidae	date	3	108.717	<0.0001*
	hedging	1	5.4406	0.0197*
	location	2	6.9729	0.0306*
***Orius* sp**	date	3	249.737	<0.0001*
	hedging	1	34.453	<0.0001*
	location	2	7.2468	0.0267*
Total predator abundance	date	3	67.990	<0.0001*
	hedging	1	19.719	<0.0001*
	treatment	1	2.928	0.0871
	date:hedging	3	15.387	0.0015*
	date:treatment	3	8.034	0.0453*

*Effects with P < 0.05 are considered statistically significant and are highlighted.

Table 2.3 Summary of fixed effects retained in the best-fitting generalized linear mixed-effects models (GLMMs) describing variation in parasitism rates and overall predation activity within the pecan canopy strata. Models were selected using Akaike's Information Criterion (AIC).

Sources of variation		*df*	χ^2	*P*
Parasitism	date	3	122.265	<0.0001*
	hedging	1	83.171	0.4747
	location	2	281.118	<0.0001*
	treatment	1	305.176	<0.0001*
	date:treatment	3	91.679	<0.0001*
	hedging:location	2	11.727	0.0109*
	hedging:treatment	1	23.017	<0.0001*
	location:treatment	2	28.161	<0.0001*
Predation	date	3	433.458	<0.0001*
	hedging	1	12.683	0.0004*
	treatment	1	2.768	0.0961
	date:treatment	3	8.743	0.0329*

*Effects with $P < 0.05$ are considered statistically significant and are highlighted.

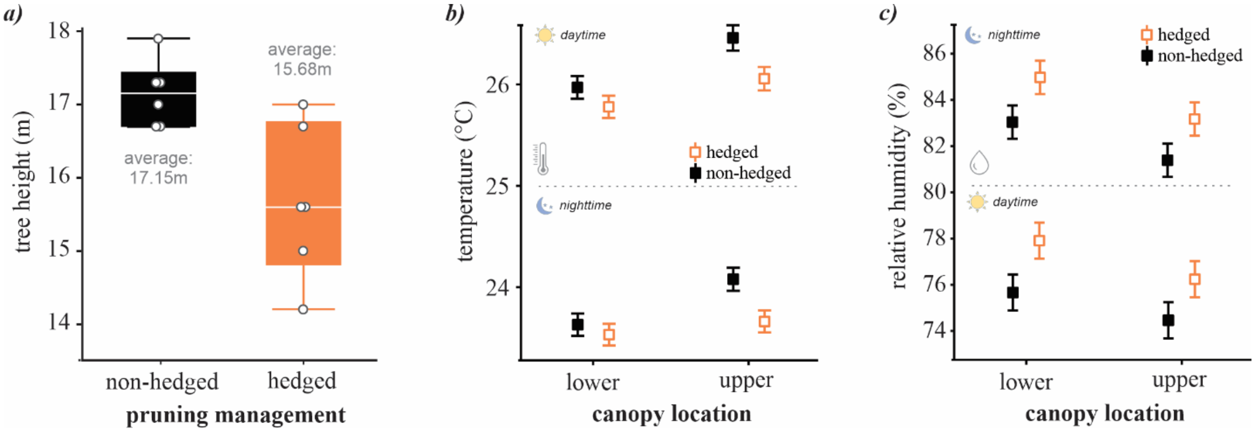

Figure 2.1 Effects of hedge pruning on tree height and canopy temperature. ***(a)*** Boxplots showing reduced tree height in hedged trees (orange) compared to non-hedged trees (black). Each dot represents an individual tree, and the white line indicates the median. ***(b)*** Mean canopy temperature (± SE) during the day and night across lower and upper canopy. ***(c)*** Mean canopy humidity (± SE) during the day and night across lower and upper canopy. Colors and symbols represent hedged trees (orange squares) and non-hedged trees (black squares).

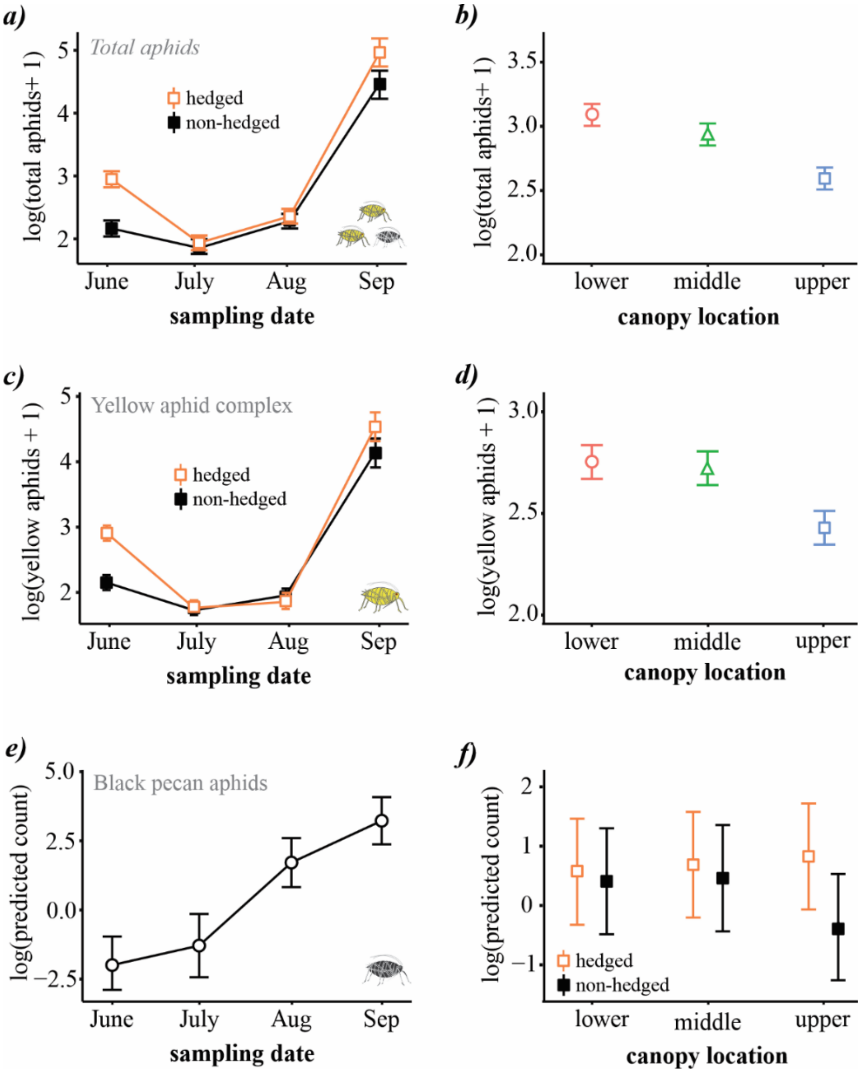

Figure 2.2 Aphid responses to canopy pruning (hedging), chemical management (pesticide), and canopy location across the growing season. ***(a, b)*** Total aphids, ***(c, d)*** yellow aphid complex, and ***(e, f)*** black pecan aphids. For total aphids and yellow aphid complex, values represent predicted means from linear mixed-effects models (LME) fitted to log-transformed data (log(abundance + 1)), with vertical bars indicating ±1 standard error (SE). For black pecan aphids ***(e-f)***, predictions were generated from a generalized linear mixed-effects model (GLMM) with a negative binomial distribution and are shown on the log scale with 95% confidence intervals.

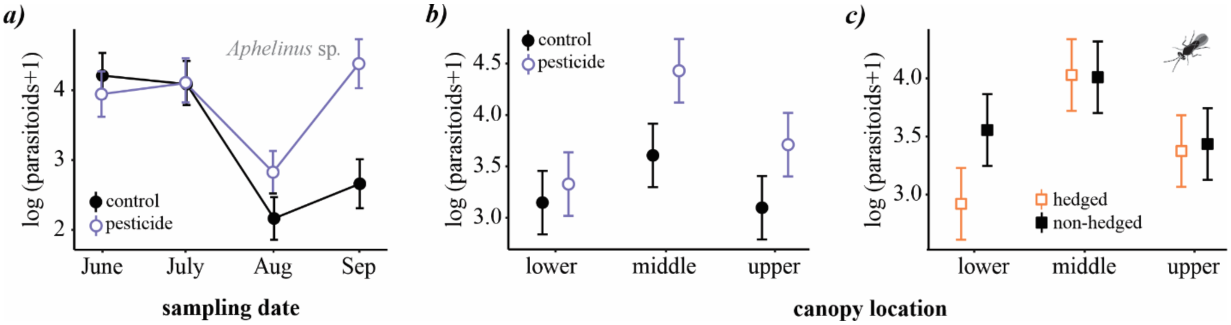

Figure 2.3 Aphid parasitoid abundance in response to chemical management, canopy location, and canopy pruning across sampling dates. All values were derived from a linear mixed-effects model (LME) fitted to log-transformed data with vertical bars indicating standard error (±1 SE). ***(a)*** Seasonal dynamics by pesticide treatment; ***(b)*** variation across canopy strata; and ***(c)*** hedging effects across the canopy. All estimates represent effects retained in the final model following model selection.

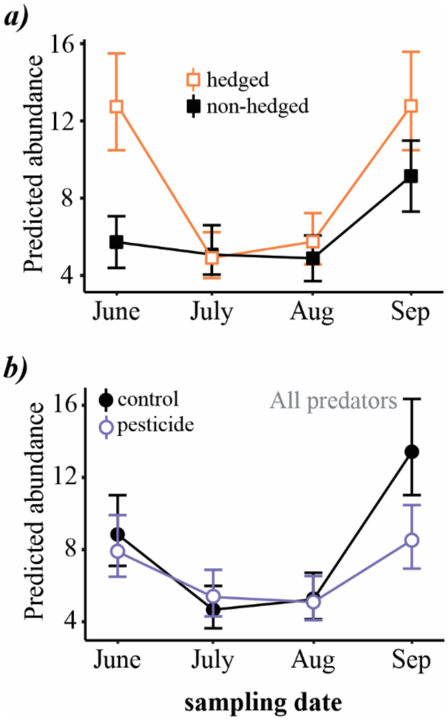

Figure 2.4 Abundance of the total predator community (predators pooled) in response to chemical management and canopy pruning across the growing season. ***(d)*** Temporal dynamics by pesticide treatment; ***(e)*** temporal dynamics by hedging treatment. Values represent predicted abundance from a generalized linear mixed-effects model (GLMM) with a negative binomial distribution, shown with 95% confidence intervals. All predictions reflect effects retained in the final model following model selection.

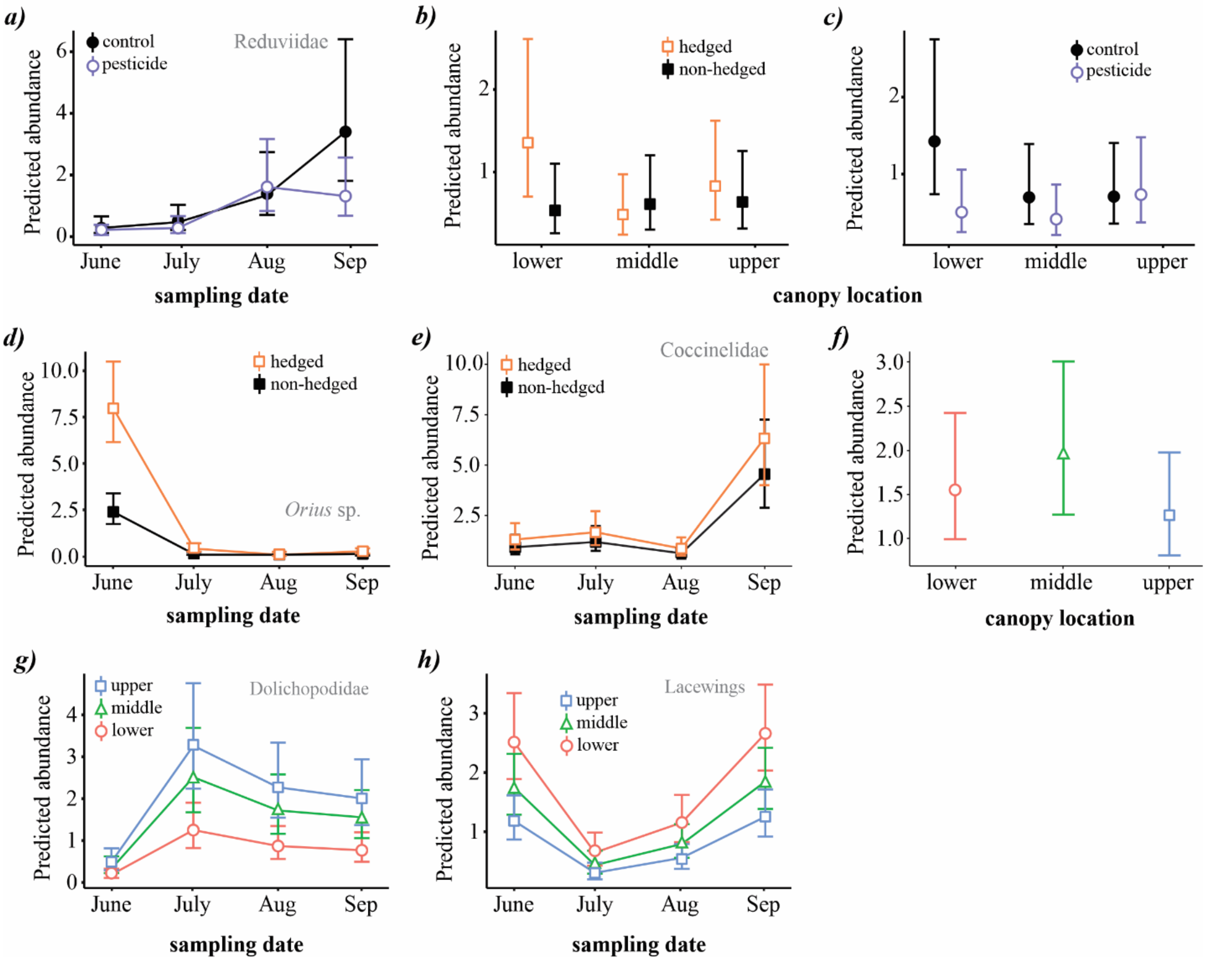

Figure 2.5 Predicted abundance (± 95% confidence intervals) of key natural enemy groups across the growing season, derived from generalized linear mixed-effects models (GLMMs) fitted with a negative binomial distribution. All predictions correspond to effects retained in the best-fitting models following model selection. (***a–c***) Reduviidae (assassin bugs): (***a***) seasonal trends by pesticide treatment; (***b***) hedging effects across canopy strata; (***c)*** pesticide effects across canopy strata. (***d***) Seasonal dynamics of *Orius* sp. by hedging treatment; ***(e)*** Coccinellidae by hedging treatment; (***f***) canopy stratification of Coccinellidae abundance. ***(g)*** seasonal trends by canopy location for Dolichopodidae; (***h***) seasonal trends by canopy location for lacewing larvae.

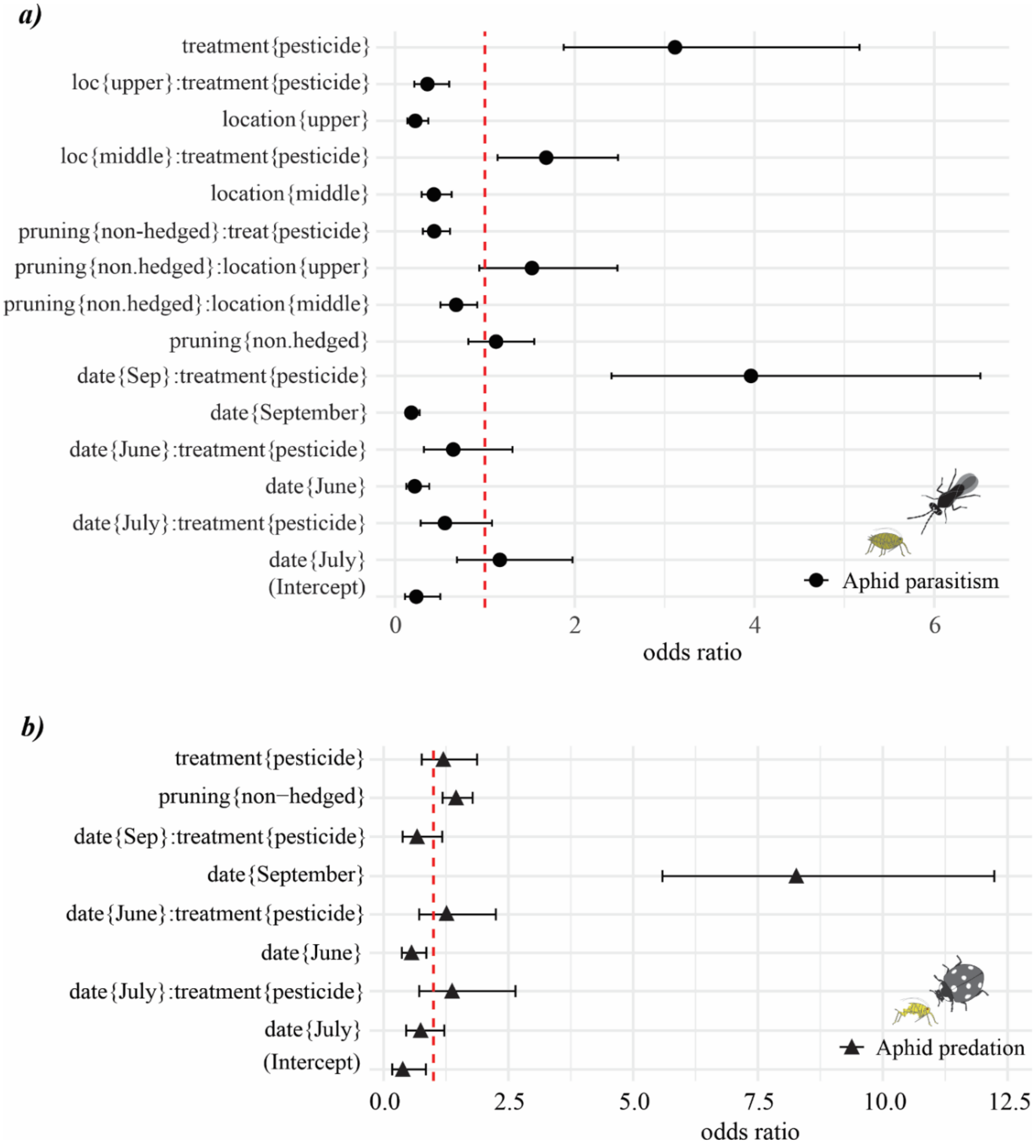

Figure 2.6 Effects of agricultural management and spatiotemporal factors on aphid biological control in pecan canopies. Odds ratios (±95% CI) were derived from binomial generalized linear mixed-effects models (GLMMs) predicting ***(a)*** aphid parasitism and ***(b)*** aphid predation. Forest plots illustrate terms retained in the best-supported models used for inference. Shifts away from the red dashed line (odds ratio = 1) indicate increased or decreased likelihood of parasitism or predation relative to reference conditions.

Supplementary Table 2.1 Chemical management (fungicide and insecticide) records during both seasons of the experiment. Pesticides were sprayed using air blast sprayers. The schedule was based on standard recommendations for the state of Georgia (Wells, 2021).

	Date	Pesticide†	Type‡	Active ingredient	Rate (L/ha)
2021 Season	May 04	Propiconizole	Fungicide	Propiconizole	0.58
	May 24	DynaPhite	Fungicide	Phosphorous Acid	4.68
	May 26	Reliant	Fungicide	Phosphorous Acid	4.68
	June 02	Intrepid	Insecticide	Methoxyfenozide	0.29
	June 08	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	1.00
	June 15	Intrepid	Insecticide	Methoxyfenozide	0.29
	June 23	Absolute	Fungicide	Tebuconazole/Trifloxystrobin	0.51
	July 09	Elast	Fungicide	Dodine	0.51
	July 29	SuperTin	Fungicide	Triphenyltin hydroxide	0.51
	Aug 06	Intrepid	Insecticide	Methoxyfenozide	0.29
		Closer	Insecticide	Sulfoxaflor	0.11
	Aug 12	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	1.00
	Aug 18	Closer	Insecticide	Sulfoxaflor	0.11
		Carbaryl	Insecticide	Carbaryl	4.68
		ProGibb	Insecticide	Gibberellic acid	0.37
	Aug 25	Elast	Fungicide	Dodine	0.51
	Sep 02	Brigade	Insecticide	Bifenthrin	0.37
		Closer	Insecticide	Sulfoxaflor	0.11
		ProGibb LV Plus	Insecticide	Gibberellic acid	0.37
2022 Season	April 12	Kphite	Fungicide	Phosphorous Acid	4.70
	May 11	Propiconizole	Fungicide	Propiconizole	0.58
	May 24	Absolute	Fungicide	Tebuconazole/Trifloxystrobin	0.51
	June 07	Kphite	Fungicide	Phosphorous Acid	4.70
	June 17	Intrepid Edge	Insecticide	Methoxyfenozide/Spinetoram	0.29
	June 27	Elast	Fungicide	Dodine	3.65
	July 18	SuperTin	Fungicide	Triphenyltin hydroxide	0.88
	Aug 03	Elast	Fungicide	Dodine	3.65
	Aug 04	Intrepid Edge	Insecticide	Methoxyfenozide/Spinetoram	0.29
		Closer	Insecticide	Sulfoxaflor	0.11
	Aug 15	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	1.21
	Aug 26	Closer	Insecticide	Sulfoxaflor	0.11
		Carbaryl	Insecticide	Carbaryl	4.68
		ProGibb	Insecticide	Gibberellic acid	0.37
	Sep 07	Brigade	Insecticide	Bifenthrin	0.37
		Closer	Insecticide	Sulfoxaflor	0.11
		ProGibb LV Plus	Insecticide	Gibberellic acid	0.37

†Trade name of product used. ‡Pesticide category.

Supplementary Table 2.2 Model selection results using the MuMIn package (dredge function), assessing how multiple factors influence the presence or absence of the (I) yellow aphid complex (II) black pecan aphids (III) parasitoids. The table presents the candidate models, including their Akaike Information Criterion (AIC) values, the number of model parameters (k), and ΔAIC values.

	Terms in the model[a]	**AIC value**	**k**	**ΔAIC**
	I. Yellow aphid complex			
1	~ date + hedging + location + treatment + date:hedging	724.3	16	0
2	~ date + hedging + location + treatment + date:hedging + hedging:treatment	726.4	17	2.14
3	~ date + hedging + location + treatment + date:hedging + treatment:location	726.6	18	2.35
	II. Black pecan aphids			
1	~ date + hedging + location + treatment + hedging:location	1116	15	0
2	~ date + hedging + location + treatment + hedging:location + hedging:treatment	1118.2	16	2.18
3	~ date + hedging + location + treatment + hedging:location + treatment:location	1118.4	17	2.35
	III. Aphid parasitoids (*Aphelinus* sp.)			
1	~date + hedging + location + treatment + date:treatment + hedging:location + location:treatment	879.4	20	0
2	~date + hedging + location + treatment + date:treatment + hedging:location	879.7	18	0.3
3	~date + hedging + location + treatment + date:treatment + location:treatment	880.8	18	1.4
4	~date + hedging + location + treatment + date:treatment	880.9	16	1.5
5	~date + hedging + location + treatment + date:treatment + hedging:location + hedging:treatment + location:treatment	881.3	21	1.9
6	~date + hedging + location + treatment + date:treatment + hedging:location + hedging:treatment	881.6	19	2.2

Supplementary Table 3.2 Model selection results using the MuMIn package (dredge function), assessing how multiple factors influence the counts of (I) Reduviidae (II) Coccinelidae (III) *Orius* sp. (IV) Dolichopodidae and (V) Lacewing and (VI) All predators pooled. The table presents the candidate models, including their Akaike Information Criterion (AIC) values, the number of model parameters (k), and ΔAIC values. Models displayed are within a change of <3 in AIC.

	Terms in the model[a]	AIC value	k	ΔAIC
I.	**Reduviidae (Assassin bugs)**			
1	~date + hedging + location + treatment + date*treatment + hedging:location	790.4	17	0
2	date + hedging + location + treatment + date*treatment + ~hedging:location + hedging:treatment + location:treatment	792	18	1.6
3	~date + hedging + location + treatment	792.4	14	2
II.	**Coccinelidae (Ladybeetles)**			
1	~date + hedging + location + treatment	1055	9	0
2	~date + hedging + location + treatment + treatment	1055.1	10	0.1
3	~date + hedging + location + treatment + location*treatment	1056.1	12	1.1
III.	***Orius* sp. (Minute pirate bugs)**			
1	~date + hedging + location + treatment	597.9	9	0
2	~date + hedging + location + treatment + treatment	600	10	2.1
3	~date + hedging + location + treatment + date*treatment	600.1	13	2.2
IV.	**Dolichopodidae (Long-legged flies)**			
1	~date + location	895.1	8	0
2	~date + location + treatment	897.1	9	2
3	~date + hedging + location + treatment	897.1	9	2
V.	**Neuroptera (Lacewings)**			
1	~date + location	849.8	8	0
2	~date + location + treatment	850.2	9	0.4
3	~date + hedging + location + treatment	851.4	9	1.6
VI.	**All Predators (pool)**			
1	~date + hedging + treatment + date*hedging + date*treatment	1700.1	14	0
2	~date + hedging + treatment + date*hedging	1701.4	11	1.3
3	~date + hedging + treatment + date*treatment	1702.1	10	2

Supplementary Table 2.4 Model selection results using the MuMIn package (dredge function), assessing how multiple factors influence biological control (parasitism and predation) in the canopy. The table presents the candidate models, including their Akaike Information Criterion (AIC) values, the number of model parameters (k), and ΔAIC values. Models displayed are within a change of <3 in AIC.

	Terms in the model[a]	AIC value	k	ΔAIC
I.	**Aphid parasitism**			
1	~ date + hedging + location + treatment + treatment:date + hedging:location + hedging:treatment + location:treatment	1202.5	17	0
2	~ date + hedging + location + treatment + treatment:date + hedging:location + hedging:treatment + location:treatment + date:hedging	1203.2	20	0.7
3	~ date + hedging + location + treatment + treatment:date + hedging:treatment + location:treatment +	1209.7	15	7.3
II.	**Aphid predation**			
1	~ date + hedging + treatment + date:treatment	940.1	10	0.00
2	~ date + hedging + treatment + location + date:treatment	941.7	12	1.60
3	~ date + hedging + treatment + date:treatment + hedging:date	942.3	11	2.12

CHAPTER 3

MOLECULAR AND OBSERVATIONAL DATA REVEAL COMPLEX STRUCTURING OF GALL-ASSOCIATED ARTHROPOD COMMUNITIES IN A MANAGED ORCHARD[3]

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[3] Toledo, P.F.S. & Schmidt J.M. To be submitted to a peer-reviewed journal.

Abstract

Insect-induced galls represent complex microhabitats that support diverse arthropod assemblages and intricate species interactions. Despite their ecological relevance, the community dynamics within galls induced by Hemiptera: Phylloxeridae remain largely undocumented. Here, we integrate classical dissection methods with DNA metabarcoding to characterize the composition and structuring of arthropod communities inhabiting *Phylloxera*-induced galls on pecan (*Carya illinoinensis*) leaves in the southeastern US. We examined how gall phenology (active vs. senescent), vertical stratification within the canopy, and pesticide-induced stress influence community structure. A total of 1,150 galls were dissected across canopy strata (*i.e.* lower to upper) in July and September. Dissections revealed that active galls were primarily occupied by Hymenoptera larvae, while senescent galls contained mostly arthropod frass, and *Phylloxera* were rarely detected. DNA metabarcoding of whole galls identified over 30 arthropod taxa, predominantly Hymenoptera and mites, with few reads for *Phylloxera*. Active and senescent galls supported distinct assemblages early in the season, which converged over time. Spraying the pecan canopy with pesticides slightly increased Hymenoptera presence in the galls and altered the overall community composition. Canopy location had limited influence on total diversity, but Hymenoptera were detected less frequently in the lower canopy. Together, these findings contribute to a broader understanding of how gall-associated arthropod communities are structured. By integrating traditional and molecular approaches, we reveal the hidden complexity in *Phylloxera*-induced galls and provide foundational insights into a promising model for studying multi-trophic community assembly processes and ecological responses to environmental stressors.

keywords: *Phylloxera* galls, environmental DNA, *Carya illinoinensis*, vertical stratification, ecotoxicology

Introduction

Arthropod communities colonizing microhabitats engineered by gall-inducing insects form complex interactions, ranging from commensal to multitrophic feeding networks (Albuquerque and Souza, 2019; Askew, 1980; Harris and Pitzschke, 2020; Sanver and Hawkins, 2000). Gall-inducers engineer microhabitats within host plant tissues that serve as sheltered refuges and food sources for the inducers and for other species (*e.g.,* parasitoids and inquilines) that exploit gall tissue or prey on gall inhabitants (Pereira et al., 2024; Price et al., 1987; Sanver and Hawkins, 2000; Shorthouse et al., 2005). As gall systems develop over the life cycle of the inducer, their physical, chemical, and biotic conditions shift, which leads to transient associated communities (Briggs and Latto, 1996; Craig et al., 1990; Joseph et al., 2011). Even after gall-inducing species complete their life cycle, structures can persist on the plant as potential habitats, therefore, supporting unique arthropod assemblages through time (Rezende et al., 2023).

Although galls may support ecologically rich communities, they remain challenging to study due to the concealed nature of the gall environment. In addition, the current knowledge of gall community structure and dynamics mostly originates from studies on hymenopteran or dipteran gall systems, where parasitoid guilds and species associations are well documented (Askew, 1980; Bunnefeld et al., 2018; López-Núñez et al., 2019; Rezende et al., 2021; Stone et al., 2002). In contrast, communities associated with galls induced by Hemipteran insects (Raman, 2012) have received considerably less attention (but see Miller, 2004; Moser, 1965).

Among hemipteran systems, Phylloxeridae-induced galls present a system of both ecological and agricultural relevance. However, research on gall forming Phylloxeridae (*e.g.*, *Daktulosphaira* sp. and *Phylloxera* sp.) has focused almost exclusively on their associations with host plants, gall morphology, and developmental biology (Andersen and Mizell, 1987; Kellow et al., 2004; Nabity et al., 2013; Stoetzel, 1985; Stoetzel and Tedders, 1981; Witiak, 2007). Only a few studies focused on the potential communities supported by these gall systems (Boethel et al., 1974; Dinkins and Reid, 1988; Payne and Schwartz, 1971). A notable exception is a pioneering study conducted four decades ago, which documented the arthropod fauna inhabiting *Phylloxera* spp. leaf galls in western pecan orchards through gall dissections to compile a list of species utilizing galls (Mitchell et al., 1984). However, subsequent research on this topic appears to be scarce.

Traditional methods for studying enclosed systems such as gall-associated communities, rely on dissections and direct observations, which can provide valuable taxonomic insights for common and morphologically distinct adult taxa (de Araújo et al., 2021). However, dissections are labor-intensive and often fail to detect cryptic species interactions, and challenging to identify life stages (*e.g.,* eggs or larvae). Recent advances in molecular tools, particularly DNA sequencing approaches like metabarcoding, have transformed how we assess arthropod communities (Deiner et al., 2017; Kaartinen et al., 2010; Sow et al., 2020). Metabarcoding combined with high throughput sequencing (HTS) methods offer an approach for studies where ecological context is limited and DNA material provides a traceable record of arthropod presence and associated interactions. HTS is suitable for reconstructing interaction histories from many types of environmental material as sources of eDNA (Pawlowski et al., 2021; Thomsen and Willerslev, 2015). For example, within concealed and difficult to monitor niches such as plant structures (Johnson et al., 2023; Thomsen and Sigsgaard, 2019; Weber et al., 2024), fungal bodies (Lunde et al., 2022), soil cores (Oliverio et al., 2018; Saccò et al., 2022), fecal material, and others (Taberlet et al., 2018). Therefore, metabarcoding is well suited for uncovering the hidden complexity of elusive gall-associated arthropods (Ács et al., 2010; Dittrich-Schröder et al., 2012; Gates et al., 2020; Zhang et al., 2019). Yet, to our knowledge, studies utilizing metabarcoding to unravel biological communities within galls have focused on microorganisms (*i.e.*, fungal and bacterial communities) (Fernandez-Conradi et al., 2019; Michell and Nyman, 2021; Pyszko et al., 2024; Turco et al., 2024).

Here, to advance our understanding of the structuring of gall-associated arthropod communities, we investigated *Phylloxera*-induced galls on the leaves of mature pecan (*Carya illinoinensis* (Wagenh.) K. Koch) trees. Pecan orchards provide a compelling model system for exploring both natural and anthropogenic drivers of community assembly. Their tall canopies (>15 m) support vertically stratified arthropod distributions (Cottrell, 2024, 2017; Dutcher et al., 2012; Hall et al., 2007; Slusher et al., 2022, 2021), creating distinct microhabitats across canopy layers. Vertical gradients may act as ecological filters, influencing which species colonize and persist within galls (Fernandes and Price, 1992; Paniagua et al., 2009). In addition, commercial pecan orchards are regularly exposed to pesticide applications targeting various insect pests and fungal pathogens (Wells, 2021). Such anthropogenic-driven stressors can alter arthropod community structure and species interactions and may shape the composition of gall-associated communities in the canopy. As such, pecan orchards offer a novel environment to refine ecological monitoring frameworks by using gall systems as tractable indicators of canopy-level arthropod community responses.

Our study objectives were to: 1) assess the composition and diversity of arthropod communities inhabiting *Phylloxera*-induced galls in pecan leaves; 2) investigate effects of host traits (gall phenology) and vertical canopy position effects on the structure of associated gall communities and their interactions; and 3) evaluate whether pesticide applications influence arthropod assemblages detected within galls. By integrating classical dissections with modern molecular methodologies, this study offers a framework for using gall-associated arthropod communities as gauges to reveal microhabitat specific shifts in species assemblages associated with environmental stress.

Material and Methods

Experimental orchard, study design, and gall collection

The study was conducted in an experimental pecan orchard (32°39'55.1" N; 83°43'45.7" W) at the USDA, ARS, Southeastern Fruit and Tree Nut Research Station located in Byron, Georgia. The orchard contained approximately 110 trees (cultivar Pawnee) approximately 40 years old. The space between rows was mowed regularly and the strip beneath the tree rows was maintained vegetation-free. No irrigation was performed besides rainfall. A total of 12 trees were randomly selected in the orchard to be used in the experiment where six were treated for pests and diseases during the season (both insecticides and fungicides) and six were control trees (untreated). Insecticides were used only after gall formation. For further details on chemical management records, see **Supplementary Table 3.1**. We collected leaf samples containing *Phylloxera*-induced galls from three positions in the pecan canopy: the lower (~4m), middle (~10m), and upper strata (~16m). Leaves were removed with pruning scissors and height in the canopy was reached with the aid of a Telescopic Crawler Boom Lift (660SJC, JLG Industries, Inc., McConnellsburg, PA, USA). Sampling for galls occurred on July 25th and September 5th of the 2022 pecan season.

Collection of galls for arthropod community exploration

To investigate the arthropod community associated with *Phylloxera*-induced galls in pecans, we collected 12 to 15 compound leaves containing galls from each tree stratum (lower, middle, and upper canopy). Leaves were placed in gallon-size plastic bags and stored in an ice chest within the boom lift basket during sampling. Once collected, samples were transported to the laboratory and stored at -20°C until processing. In the laboratory, galls were categorized based on their phenology/tissue condition as either active (fresh green tissue) or senescent (mature/declining tissue). Active galls were turgid, with green or yellow hues, while senescent galls were brown or reddish, often dry and wrinkled (Figure 3.1). Active galls often indicate ongoing plant manipulation, primarily by the gall inducer (*Phylloxera* sp.) or other arthropods capable of influencing gall tissue, such as Hymenopteran inquilines. In contrast, senescent galls signal the cessation of plant manipulation, often resulting from competitive interactions, natural mortality, or the completion of an inhabitant's life cycle (Costa et al., 2022; Ferreira et al., 2019). From each tree stratum, we randomly selected 20 galls (10 active and 10 senescent) for dissection and stereoscopic examination. Additionally, four galls (two active and two senescent) were set aside for molecular analysis. Gall collection was conducted on 10 trees, with five receiving pesticide treatments and five serving as untreated controls. Detailed methodologies for dissections, direct observations, and DNA sequencing are provided in the following sections.

Dissection of galls and exploration of contents

Using a scalp and forceps, 10 fresh galls and 10 senescent galls from each tree and canopy strata were opened and observed under a stereoscope, and their contents were recorded (for a total of 30 fresh and 30 senescent galls per tree). We assessed the presence of arthropods (which were identified to order) and arachnids. Additionally, arthropod eggs, remains such as exuvia or frass (an intertwined collection of insect secretion, exuvia, silk and other *Phylloxera* colony remains). The effects of habitat context (assessment date, canopy location, and gall phenology) were assessed for commonly found contents, *i.e.*, present in ≥ 5% of all galls dissected.

Molecular characterization of gall-associated communities

DNA extraction from gall inhabitants and whole galls

DNA was extracted from both individual arthropods dissected from galls and whole galls to assess gall-associated community composition. The extracted individuals consisted primarily of Hymenoptera spanning all stages of life, including eggs, larvae, pupae, and adults. Each specimen was identified to the lowest possible taxonomic level and photographed using an iPhone 11 (Apple Inc., Cupertino, CA, USA) mounted on a stereoscope (Supplementary Fig. 1). DNA extractions were performed using the Qiagen DNeasy® Blood and Tissue 96-well kit (Qiagen, Chatsworth, CA, USA), following the manufacturer's protocol. Negative controls, containing all reagents but no insect tissue, were included in each batch to monitor for potential contamination. Extracted DNA was stored at -20°C for short-term use and archived at -80°C after PCR amplification.

Sanger Sequencing of Hymenopteran Inhabitants

To establish molecular references for gall-associated Hymenoptera, Sanger sequencing was conducted on DNA extracted from dissected specimens. PCR amplifications were performed using established primers and standard protocols (Schmidt et al., 2021; Slusher et al., 2024). Each 10 µL PCR reaction contained 5 µL of Qiagen 2x Multiplex Master Mix, 0.1 µL of Bovine Serum Albumin (BSA), 1.9 µL of PCR-grade H_2O, and 0.5 µL of each primer (LCO1490 and HCO2198). Amplifications were carried out using a Bio-Rad C1000 Touch™ Thermal Cycler under the following conditions: initial denaturation at 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 53.3°C for 40 s, and 73°C for 1 min, then an additional cycle of 94°C for 30 s, 46.6°C for 1 min, and 73°C for 1:30 min, with a final extension at 72°C for 10 min. PCR products were sequenced bidirectionally using an ABI 3730 DNA Analyzer (Eurofins Genomics LLC). Forward and reverse sequences were assembled, aligned, and edited using Codon Code Aligner v4.0.4. Taxonomic identities were assigned through searches against the Barcode of Life Data Systems (BOLD) and NCBI GenBank databases. Hymenoptera samples that failed to yield high-quality sequences or could not be reliably identified were subsequently included in the metabarcoding analysis along with whole-gall extractions.

HTS metabarcoding of gall communities

To characterize the gall-associated arthropod diversity, DNA from whole galls and unidentified Hymenoptera from Sanger sequencing were subjected to high-throughput sequencing on an Illumina NextSeq 2000 platform. Library preparation followed a two-step nested DNA metabarcoding approach (Grabarczyk et al., 2025, 2022; Kitson et al., 2019; Slusher et al., 2024). In the first PCR, DNA was amplified using fwhr and bfbr primers, which target both short and long amplicon regions suitable for arthropod identification (Elbrecht et al., 2019). Primers incorporated Illumina bridge adapters to facilitate sequencing. PCR products were purified using MagBio HighPrep PCR magnetic beads (MagBio Genomics, Inc.), serving as templates for a second PCR step where Illumina dual-indexing barcodes (10 nt, IDT xGen™ primers) were incorporated to uniquely label each sample. Final libraries were standardized for concentration, pooled, and submitted to the Georgia Genomics and Bioinformatics Core (GGBC) at the University of Georgia for sequencing on an Illumina NextSeq^{TM} 2000 (Illumina, CA, USA) with P2 reagents (600 cycles).

Bioinformatics, taxonomic assignment and data filtering

Raw sequence data were demultiplexed using pheniqs v2.1.0 (Galanti et al., 2021), ensuring strict barcode index matching to separate samples. A second round of demultiplexing in pheniqs assigned raw reads to per-sample fastq files while trimming index sequences to retain only the target amplicon. Cutadapt v3.4 (Martin, 2011) was used to remove primer sequences from forward and reverse reads, with reads discarded if primers were not detected at the expected 5' position (error rate < 0.15). Merged read pairs were processed using vsearch v2.15.2 (Rognes et al., 2016), with sequences shorter than 150 bp or longer than 400 bp excluded (Edgar and Flyvbjerg, 2015). Unique sequences were clustered using the unoise3 denoising algorithm with an alpha threshold of 5, discarding sequences appearing fewer than eight times. Chimeric sequences were identified and removed using uchime3 in vsearch. Taxonomic assignment was conducted using a best-hit alignment approach against a reference database of publicly available sequences from NCBI GenBank (Benson et al., 2005). Assignments were based on semi-global pairwise alignment in vsearch, with a consensus taxonomy accepted when >90% agreement was observed across the top matches within 1% of the highest-scoring reference sequence. To improve accuracy and minimize sequencing artifacts, rare reads were filtered using a minimum detection threshold of 10 reads per taxon. Taxa with fewer than 10 reads were considered absent and removed from further analysis. Non-arthropod sequences were also excluded, and data from both primer sets were merged to improve taxonomic resolution. To standardize the dataset for community analyses, samples with fewer than 500 reads were excluded and the resulting data read matrix was rarefied (RRA, relative read abundance) (Deagle et al., 2019; Schmidt et al., 2019). This standardized dataset was used for downstream estimation of richness and diversity for ecological analyses, focusing on the effects of pesticide treatments, canopy stratification, and gall phenology on gall-associated arthropod assemblages.

Data management and statistical analysis

All statistical analyses and base figures were produced in R version 4.4.0 “Puppy Cup” (R Core Team 2024). Figure icons, labels, and colors were added using CorelDRAW Graphics Suite 2021. To study gall contents, community diversity and richness from metabarcoding-recovered reads, we fit generalized linear mixed-effects models (GLMMs) and Linear Mixed effects models (LMEs) then employed model selection using the dredge function from the MuMIn package (Barton and Barton, 2015) to select the best fitting model to explain variation (Burnham et al., 2011; Burnham and Anderson, 2004). Competing models with ΔAICc < 2 and full model summaries are available in Supplementary Table 3.2.

Hymenoptera and frass, the most frequently observed contents of dissected galls, were analyzed for presence/absence (binary response) using GLMMs with a binomial distribution (glmer, lme4 package; Bates et al., 2015). For both response variables, the models included assessment date, gall stage, pesticide treatment, and canopy location, along with all two-way interactions: date × stage, date × treatment, date × location, stage × treatment, stage × location, and treatment × location. Tree identity was included as a random intercept to control for repeated measures within trees. Rarefied read abundances from metabarcoding of whole galls were used to calculate Shannon diversity (H) and species richness (S). These metrics were analyzed using linear mixed-effects models (LMEs). For both diversity (H) and species richness (S), models included assessment date, gall stage, pesticide treatment, and canopy location as fixed effects. All two-way interactions among predictors were incorporated to evaluate their combined effects: date × stage, date × treatment, date × location, stage × treatment, stage × location, and treatment × location. Tree identity was included as a random effect to account for repeated sampling.

To assess community dissimilarity, we used a permutational multivariate analysis of variance (PERMANOVA) on the rarefied read matrix created from the metabarcoding data (adonis2 function, vegan package; Oksanen et al., 2023; Anderson 2001). Model selection was performed using AICc-based comparison (AICcpermanova2 function, AICcPermanova package; (Corcoran and Corcoran, 2023; Zuur et al., 2009) (Supplementary Table 3.3). To visualize community dissimilarity, we performed non-metric multidimensional scaling (NMDS) (metaMDS function, vegan package) using a Bray-Curtis dissimilarity matrix estimated from rarefied reads of taxa recovered from whole-gall extractions (Oksanen et al., 2023)

Results

Arthropod community and contents of dissected galls

We dissected 1,150 *Phylloxera* galls (Figure 3.1) to characterize their associated arthropod community and other contents, uncovering insects, spiders, mites, arthropod eggs, silk structures, fungal bodies, and frass (Table 3.1). *Phylloxera* sp., the gall inducer, was rarely observed, while Hymenoptera were the most frequently encountered taxa. Approximately 90% of Hymenoptera found were larvae and the sole occupants of galls. On both assessment dates, larvae observed were non-motile and consistently at the same developmental stage (fully grown larvae). Some Hymenoptera also hosted ectoparasitic Hymenoptera feeding on them (Supplementary Figure 3.1). Frass, a dominant gall content, was often intertwined with fungal structures and silk, suggesting potential Lepidopteran activity. Other arthropod orders found in lower frequencies included Coleoptera (Cleridae and Mycetophagidae), Neuroptera (Coniopterygidae and Chrysopidae), Lepidoptera immatures, Thysanoptera, mites, and spiders, as well as silk masses or web structures. Of the dissected galls, 138 (~10% of galls) were empty, while 307 contained only frass (Table 3.1). In addition, DNA sequencing using the full Folmer COI barcode approach identified five families of Hymenoptera from individuals dissected from galls: Bethylidae, Eurytomidae, Eupelmidae, Perilampidae, and Pteromalidae (Supplementary Figure 3.1; Table 3.3). Full barcode sequences were successfully generated and have been deposited in public databases (GenBank Accession Numbers to be released upon publication; currently held in BOLD Systems). Additionally, the metabarcoding of dissected Hymenoptera content that did not generate full barcodes revealed Megaspilidae as an additional Hymenoptera family.

Influence of seasonality, canopy stratification, and chemical management on gall contents

Hymenoptera were more frequently encountered in July than in September and their presence varied across canopy locations (Table 3.2), with more Hymenoptera found in the upper and middle canopy compared to the lower canopy (Figure 3.2). The effect of date on Hymenoptera presence depended on gall stage (Table 3.2) where Hymenoptera were more frequently present in active galls than in senescent galls in both July and September, but this difference was stronger in July (z = -9.921, $P < 0.0001$) than in September ($z = -2.630$, $P = 0.0085$). The effect of date depended on treatment ($\chi^2 = 4.44$, $df = 1$, $P = 0.035$). Hymenoptera presence was lower in September compared to July in both control ($z = -7.842$, $p < 0.0001$) and pesticide-treated trees ($z = -4.891$, $P < 0.0001$), but the reduction was more pronounced in control trees. Additionally, gall phenology influenced Hymenoptera presence, with active galls containing more Hymenoptera than senescent galls (Table 3.2).

Frass was more frequently found in senescent galls compared to active galls (Table 3.2). The presence of frass also varied among canopy locations (Table 3.2), with more frass found in the lower canopy compared to the upper canopy. The presence of frass was slightly higher in September than in July (Table 3.2). The effect of date on frass presence depended on canopy location and frass was more frequently present in the middle canopy in September than in July (z = 4.038, $P = 0.0001$), while no significant differences between dates were detected in the lower canopy (z = 1.198, $P = 0.2309$) or upper canopy (z = 0.487, $P = 0.6265$). The effect of date on frass presence also depended on gall phenology (Table 3.2). In active galls, frass was more frequently found in September than in July (z = 3.956, $P = 0.0001$), while in senescent galls, frass presence remained stable between dates (z = -0.101, $P = 0.9197$). In both dates frass was more frequent in senescent galls compared to active galls, but this difference was greater in July (z = -9.790, $P < 0.0001$) than in September (z = -7.164, $P < 0.0001$).

Metabarcoding of whole galls: Community structure and environmental drivers

DNA metabarcoding of whole galls using mlep/lep primers targeting a 350 bp COI sequence within the Folmer region revealed a diverse arthropod community, with over 30 taxa detected in more than 5% of analyzed gall samples (Supplementary Table 3.3). These taxa encompassed six insect orders and five arachnid orders, comprising 17 families, 26 genera, and 32 species. Hymenoptera exhibited the highest diversity, including five families, eight genera, with Eurytomidae and Eupelmidae being the most dominant. Arachnids displayed a diverse community with six families and six genera, primarily represented by Trombidiformes, Mesostigmata, and Ixodida. Mites were the most frequently detected arachnids, with Tydeidae and Macrochelidae being dominant (Supplementary Table 3.3).

Patterns of diversity and richness

The best-fitting model for species richness (S) included pesticide treatment, date, stage, and the interactions date x stage and stage x pesticide treatment. Species richness (S) varied significantly with chemical management, gall phenology and over time. Richness depended on the interaction between sampling date and gall phenology ($F_{1,135} = 4.03$, $P = 0.0466$), where richness remained stable between July and September in active galls ($t = -0.799$, $P = 0.4257$), but showed a marginal increase in senescent galls from July to September ($t = 1.945$, $P = 0.0539$, Figure 3.3*a)*. Additionally, richness was marginally higher in pesticide-treated trees ($F_{1,9} = 5.54$, $P = 0.0431$, Figure 3.3*b*).

For diversity, the null model (intercept-only) was selected as the best-fitting model, indicating lack of measurable influence of the tested predictors on diversity. Taxa diversity (H) remained stable across all treatments and sampling conditions, with no statistically measurable variation across sampling dates, gall phenology, chemical management, or canopy locations. Model selection indicated that none of the factors considered in the study contributed to explaining significant variation in diversity (Supplementary Table 3.2).

Factors influencing community composition

Model selection based on PERMANOVA comparisons retained sampling date, gall developmental stage, pesticide treatment, and the interaction between sampling date and gall stage as the best predictors of community composition. Competing models included additional interaction terms (*e.g.*, pesticide × date, canopy location × date), but these terms were either non-significant or did not improve model explanatory power. Therefore, the main results reported below are based on significant predictors from the best-fitting model; marginal effects from competing models are addressed separately. Variation in community composition was primarily explained by differences across sampling dates, which accounted for 12.0% of the observed variation ($R^2 = 0.1200$, $F_{1, 95} = 14.018$, $P = 0.001$; Figure 3.4a). Changes in gall developmental stage contributed an additional 2.7% ($R^2 = 0.0273$, $F_{1, 95} = 3.194$, $P = 0.009$; Figure 3.4b), with arthropod assemblages differing between active and senescent galls. However, the interaction between sampling date and gall developmental stage accounted for 1.9% of the variation ($R^2 = 0.0186$, $F_{1, 95} = 2.169$, $P = 0.037$; Figure 3.4c), suggesting that community differences associated with seasonal timing depend on gall stage. Pesticide treatment explained 2.1% of the variation ($R^2 = 0.0210$, $F_{1, 95} = 2.459$, $P = 0.028$).

In competing models, additional interactions were examined. The interaction between sampling date and pesticide treatment explained 1.6% of the variation ($R^2 = 0.01635$, $F_{1, 95} = 1.9621$, $P = 0.059$) but was only marginally significant. Similarly, the interaction between sampling date and canopy location explained 2.6% of the variation ($R^2 = 0.02567$, $F_{1, 95} = 1.5404$, $P = 0.080$) and was not statistically significant. Finally, canopy location alone explained 2.1% of the variation ($R^2 = 0.02083$, $F_{1, 95} = 1.2228$, $P = 0.233$) and was also not significant.

Discussion

Our study reveals previously undocumented complexity within arthropod communities inhabiting Phylloxeridae-induced galls. By integrating classical dissections with DNA metabarcoding, we characterized a diverse assemblage of arthropods and other microhabitat contents structured by seasonal progression, gall phenology, and pesticide management. While chemical inputs had a comparatively minor effect, gall developmental stage and seasonality emerged as primary ecological filters shaping community composition. Importantly, we show that later in the season, even after gall inducers have exited, these structures remain ecologically active, sustaining dynamic arthropod communities. Together, these findings support the use of *Phylloxera*-induced galls on pecan leaves as a tractable model for investigating plant structures mediating arthropod community assembly across spatial and temporal gradients, with broader implications for canopy-level ecological monitoring.

Hymenoptera larvae were the most frequent occupants of dissected galls, with *Eurytoma gigantea* being the most common species detected. Their dominance hints at a shift in internal gall ecology, transitioning from the original inducer to new occupants. A similar pattern was documented by Mitchell et al., 1984, when studying *Phylloxera* galls in pecans, also found that over 40% of galls induced by *P. russellae* contained a Hymenoptera larva, although further identification of the wasp was not pursued, likely due to the challenges when relying on morphological characteristics of immature insects. While parasitoid wasps are commonly recognized as natural enemies of gall-inducing insects (Sanver and Hawkins, 2000), the size of the Hymenoptera larvae observed in this study suggests they are unlikely to be direct parasitoids of *Phylloxera* sp., but a parasite of the gall habitat. In other gall systems, *Eurytoma* species (including *Eurytoma gigantea*) are known gall inquilines and parasitoids (Askew, 1980; López-

Núñez et al., 2019; Sanver and Hawkins, 2000; Stone et al., 2002). For instance, *E. gigantea* parasitizes the goldenrod gall fly and various cynipid gall inhabitants (Abrahamson et al., 1989; Gómez et al., 2011). This underscores the ecological plasticity of gall-associated Hymenoptera and highlights the potential for shared communities across gall systems (Aebi et al., 2007; Csóka et al., 2005; Holt and Lawton, 1993; Ronquist and Liljeblad, 2001; Van Noort et al., 2007). Additionally, some dissected *Eurytoma* larvae found in our study hosted ectoparasitic Hymenoptera (true parasitoids in our system), indicating a complex trophic network within these galls. Further behavioral observations and analysis of host-parasite interactions coupled with literature consensus (Albuquerque and Souza, 2019) would help clarify these ecological roles.

Dissections revealed that the primary gall-inducer was mostly absent at the time of sampling, consistent with their life history. Most *Phylloxera* species that utilize pecan trees as hosts have one or few generations during the year, except for *P. notabilis*, which has been reported to have multiple generations per year (Stoetzel, 1985). We were unable to pinpoint the identity of the species inducing these galls using sequencing likely due to the lack of sequence availability in public databases, a common limitation in studies of understudied insect taxa (Pawlowski et al., 2021; Taberlet et al., 2018; Zhang et al., 2019). However, their location (*i.e.*, on the leaves), morphology (*i.e.*, gall with reticulated pattern), and the general absence of *Phylloxera* within our samples suggest the southern pecan leaf phylloxera, *P. russellae* (Stoetzel, 1985), as the primary species inducing gall formation in the canopies studied here.

The primary contents of the galls, Hymenoptera larvae and frass, exhibited contrasting patterns. Hymenoptera were primarily found in active, green galls, while frass was more common in senescing ones. This suggests a successional trajectory within the gall, potentially shaped by whether or not it is colonized by secondary occupants. Hymenoptera larvae likely consume all contents of the galls, including active *Phylloxera*, remaining arthropod material, frass, and potentially gall tissue, ultimately leaving the chamber clean. In other systems, wasps (*e.g.*, *Eurytoma* sp) colonizing galls exhibit a similar behavior, clearing the gall interior before entering a low-metabolism state in preparation for overwintering (Lee et al., 1995; Leggo and Shorthouse, 2006; Price et al., 1987). Nonparasitized galls open naturally as *Phylloxera* completes its life cycle (Stoetzel, 1985), and the inducer remains likely intertwine with subsequential arthropod activity leading to the accumulation/retention of frass and organic material as they age. Additionally, the higher frequency of Hymenoptera larvae in galls with active, green tissue and the fact that they are sole in the habitat, suggests that these wasps may be adapted to influence gall tissue, potentially preventing or delaying senescence even in the absence of the original gall inducer (as in Mitchell et al., 1984).

While dissections provided resolution on main interactions, DNA metabarcoding revealed a broader view of the arthropod assemblage associated with *Phylloxera*-induced galls in pecan leaves. Over 30 taxa were detected, including diverse insects and arachnids that may serve as inquilines, predators, detritivores, or transient visitors. This molecular approach provided complementary insights into the dissections, capturing transient or cryptic taxa that were not physically observed, and offering a more comprehensive view of gall-associated biodiversity. Indeed, such molecular method enables the examination of multiple organisms across various trophic levels, significantly enhancing the precision and volume of ecological data (Kaartinen et al., 2010; Sow et al., 2020), especially if compared to orthodox methods such as the dissections presented here.

DNA metabarcoding uncovered a Hymenoptera-dominated assemblage, with *E. gigantea* as the most common species detected in whole gall extractions. Other detected families such as

Eupelmidae (*Eupelmus* sp.), Pteromalidae (*Sycophapter* sp.), and Eulophidae (*Aprostocetus* sp.), include taxa known to exploit galls, which suggests a diverse parasitoid/hyperparasitoid guild targeting immature Hymenoptera or other inquilines. Lepidoptera were detected at lower abundances, with *Gretchena bolliana* (Tortricidae), the pecan bud moth, standing out as it may interact with the gall system similarly to other Lepidopteran pests such as the hickory shuckworm (Boethel et al., 1974; Dinkins and Reid, 1988). Coleoptera were primarily represented by Cleridae (checkered beetles), a group known for predation within enclosed microhabitats. Additionally, we found relatively high reads for waxy lacewings (Neuroptera: Coniopterygidae), small predators that can likely utilize these galls for food and shelter.

Among arachnids, mites (Tydeidae) were abundant across both reads from active and senescent galls. Tydeids are frequently found on pecan foliage, clustered at the junction between leaf veins and the midrib (leaf domatia) (Ball, 1982). Hence, they likely engage in herbivory and scavenging both around its structure (externally) and possibly within the gall. Additionally, the detection of the blackmargined aphid (*Monellia caryella*) and its parasitoid (*Aphelinus perpallidus*) could reflect the opportunistic use of these galls by aphids, their predators and/or environmental DNA (eDNA) hanging around the gall environment. For instance, DNA from certain taxa may have been introduced into gall samples through abiotic (*e.g.*, rain-wash; Macher et al., 2023) or biotic processes (*e.g.*, arthropods transporting DNA into galls).

Unexpectedly, *Phylloxera* DNA reads were significantly lower than the above taxa, suggesting DNA degradation over time. While DNA degradation due to UV exposure, humidity, and temperature fluctuations occurs (Harrison et al., 2019), the enclosed nature of galls may partially buffer against these effects. Instead, microbial activity (*e.g.*, fungal growth) or enzymatic breakdown by plant tissue, predators (*e.g.*, *Eurytoma* wasps), or scavengers (*e.g.*, mites) could have contributed to DNA loss. Alternatively, methodological PCR biases (Krehenwinkel et al., 2017) such as primer efficiency could have favored preferential amplification of abundant sequences, potentially “swamping” (Cuff et al., 2023) *Phylloxera* DNA in the extracted samples and should be considered.

The structure of gall communities was strongly influenced by phenology. Early in the season, active and senescent galls supported distinct arthropod assemblages, primarily driven by Hymenoptera and mites. Over time, community composition converged, likely due to species turnover as primary occupants exited and scavengers or opportunists colonized aging structures. We also expected spatial structuring (and distribution of resources) within the canopy to influence arthropod assemblages (Lawton, 1983). Although Hymenoptera were less frequently detected in the lower canopy, vertical stratification did not significantly affect overall community structure or richness. This suggests that while certain interactions are vertically structured, canopy position was not a dominant filter shaping gall-associated arthropod communities. The higher abundance of Hymenoptera in upper strata may reflect differences in foraging behavior, microclimate, or competitive interactions (Rezende et al., 2021; Sitch et al., 1988; Weis’, 1982).

The microhabitat hypothesis suggests that gall-inducing insects benefit from the gall environment, which buffer against external stressors such as desiccation and ultraviolet radiation (Connor and Taverner, 1997; Price et al., 1987; Shorthouse et al., 2005; Stone and Schönrogge, 2003; Takeda et al., 2021). However, chemical exposure altered associated gall community structure by influencing persistence and richness of taxa over time. Hymenoptera presence declined from July to September in all trees, but more so in control trees, suggesting that chemical inputs may have buffered some sources of mortality or stress, facilitating persistence.

Interestingly, species richness was slightly higher in pesticide-treated trees, which may reflect the suppression of arthropod and fungal competitors within and around the gall system.

Taken together, by integrating classical dissection with DNA metabarcoding, we provide insights into the structuring of arthropod communities inhabiting *Phylloxera*-induced galls in pecan trees. We found that gall phenology strongly shaped species presence and turnover, with additional influences from pesticide exposure and, to a lesser extent, vertical canopy position. This study provides the most comprehensive account to date of the arthropod fauna associated with *Phylloxera* spp. galls and advances our understanding of community assembly in hemipteran-induced plant structures. As persistent microhabitats embedded within intensively managed tree crops, these galls offer a tractable and sensitive framework for detecting ecological shifts and monitoring biodiversity in agroecosystems.

Acknowledgments

We thank Kirsten Flinn, Bryce Vaughn, Rachel Ibbetson, Kirby Moncrief, and Dr. Kyle Slusher for technical assistance. The project was supported by the Agriculture and Food Initiative, project award no. 2023-67013-40765, from the U.S. Department of Agriculture's National Institute of Food and Agriculture, a SSARE award no. LS20-340, and a SSARE graduate student award no. GS21-236. This work was supported, in part, by the University of Georgia, and USDA-NIFA Multistate Hatch Project GEO00884-S1073. CHB received additional support from USDA-ARS projects 6606-21220-014–00D and 6042-22000-024-000-D, respectively. The mention of any trademark or proprietary product is for informational purposes only and does not constitute a guarantee, warranty, or endorsement by the U.S. Department of Agriculture or the University of Georgia. Likewise, references to specific commercial agrochemical products used

in this study do not imply endorsement, recommendation, or preference by the authors or their respective institutions over other potentially suitable products.

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Table 3.1 Contents observed from dissected *Phylloxera*-induced leaf galls in the pecan canopy. A total of 1,150 galls were dissected, where 307 had only frass and 138 were empty. Numbers indicate the presence of each arthropod taxon, as well as arthropod detritus (frass) and fungi, represented by the total count of galls containing these items across the canopy strata.

Sample Date	**Canopy Location**	**Number of *Phylloxera* galls containing:**									
		***Phyll*[a]**	**Hym[b]**	**Col[c]**	**Neu[d]**	**Lep[e]**	**Thy[f]**	**Mites**	**Fungi**	**Frass**	**Spider**
July 25th	Upper	4	113 (6)	5	1	0	1	1	3	63	0
	Middle	1	110 (7)	4	2	1	3	1	4	50	0
	Lower	3	81 (3)	7	3	2	4	0	3	78	1
Sep 5th	Upper	4	76 (7)	10	2	3	2	4	15	62	1
	Middle	7	56 (3)	15	1	2	3	5	7	83	0
	Lower	10	40 (1)	10	2	2	0	2	8	91	0
Total		28	476	51	17	10	13	13	40	427	2

[a] Represent the number of galls containing *Phylloxera* individuals. [b] Hymenoptera larva, pupa, and adult (The number in parenthesis represents the number of times a parasitoid larva was found parasitizing the main Hymenoptera larva within those galls). [c] Represent the number of beetle larvae and pupae. [d] Represent the number of Neuropteran larvae and pupae. [e] Represent the number of Lepidoptera larvae. [f] Represent the number of immature and adult Thysanoptera.

Table 3.2 Summary statistics from the best-fit generalized linear mixed-effects models (GLMMs) analyzing the presence or absence of Hymenoptera and frass, the most common contents found in dissections of *Phylloxera*-induced galls.

Sources of variation		*df*	χ^2	*P*-value
Hymenoptera	Date (D)	1	76,52	$<2.20e^{-16}$*
	Stage (S)	1	86.82	$<2.20e^{-16}$*
	Pesticide (P)	1	00.74	0.3912
	Canopy Location (L)	2	18.55	$9.363e-^{05}$*
	D x S	1	28.88	$7.72e^{-08}$*
	D x P	1	4.44	0.0352*
	S x P	1	2.75	0.0975
Frass	Date (D)	1	4.54	0.0331*
	Stage (S)	1	135.98	$<2.20e^{-16}$*
	Canopy Location (L)	2	14.07	0.0009*
	D x S	1	10.87	0.0010*
	D x L	1	7.79	0.0203*

*Significant at $P < 0.05$

Table 3.3 Initial inventory of Hymenoptera found at various life stages within dissected *Phylloxera* galls, identified through DNA sequencing. Molecular identification was performed using either a full-barcode approach (primers targeting a 658 bp COI sequence in the Folmer region) or a metabarcoding approach (mlep/lep primers targeting a 350 bp COI sequence in the Folmer region). Metabarcoding was used to clarify identification for samples that did not yield sufficient reads through the full-barcode approach.

Order	**Family**	**Genus**	**Life stage⸸**	**No. of reads**	**AN§**
		DNA sequencing approach: Full barcode			
Hymenoptera	Bethylidae	-	Adult	-	
	Eurytomidae	*Eurytoma**	6x larvae	-	
	Eupelmidae	*Eupelmus*	Pupa	-	
		Sycophilla	Larva	-	
	Perilampidae	*Perilampus*	Adult	-	
	Pteromalidae		Adult	-	
		DNA sequencing approach: Metabarcoding			
Hymenoptera	Eurytomidae	*Eurytoma**	Egg	23639	-
	Bethylidae	-			-
	Megaspilidae	-	Adult	15024	-

⸸Life stage of the specimen used for DNA analysis; §AN: GenBank Accession Number for specimens submitted to DNA sequencing attempting to recover full barcodes (primers targeting a 658 bp COI sequence). **Eurytoma* sp represents most of the immature larvae found in dissected galls.

Table 3.4 Summary statistics from the best-fit linear mixed-effects model (LME) analyzing richness (S) in the arthropod community detected through DNA sequencing (metabarcoding) of whole galls.

Sources of variation		$df_{\mathrm{num}}/df_{\mathrm{den}}$	F	P-value
Richness (S)	Date (D)	1/135	0.00	0.9991
	Stage (S)	1/135	0.11	0.7457
	Pesticide (P)	1/9	5.54	0.0431*
	D x S	1/135	4.03	0.0466*
	D x P	1/135	1.08	0.3009
	S x P	1/135	3.26	0.0732

*Significant at $P < 0.05$

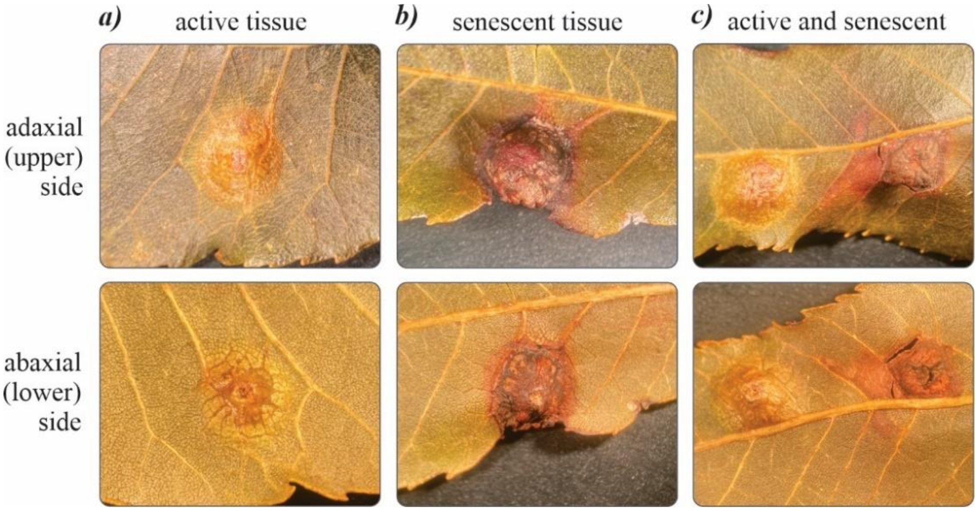

Figure 3.1 Phenotypic variation in *Phylloxera* sp. galls on pecan leaves. Images show the adaxial (upper) and abaxial (lower) leaf surfaces, depicting (***a***) an active gall with green-yellowish tissue, (***b***) a senescent gall with darker, wrinkled tissue, and (***c***) a leaflet with both active and senescent galls.

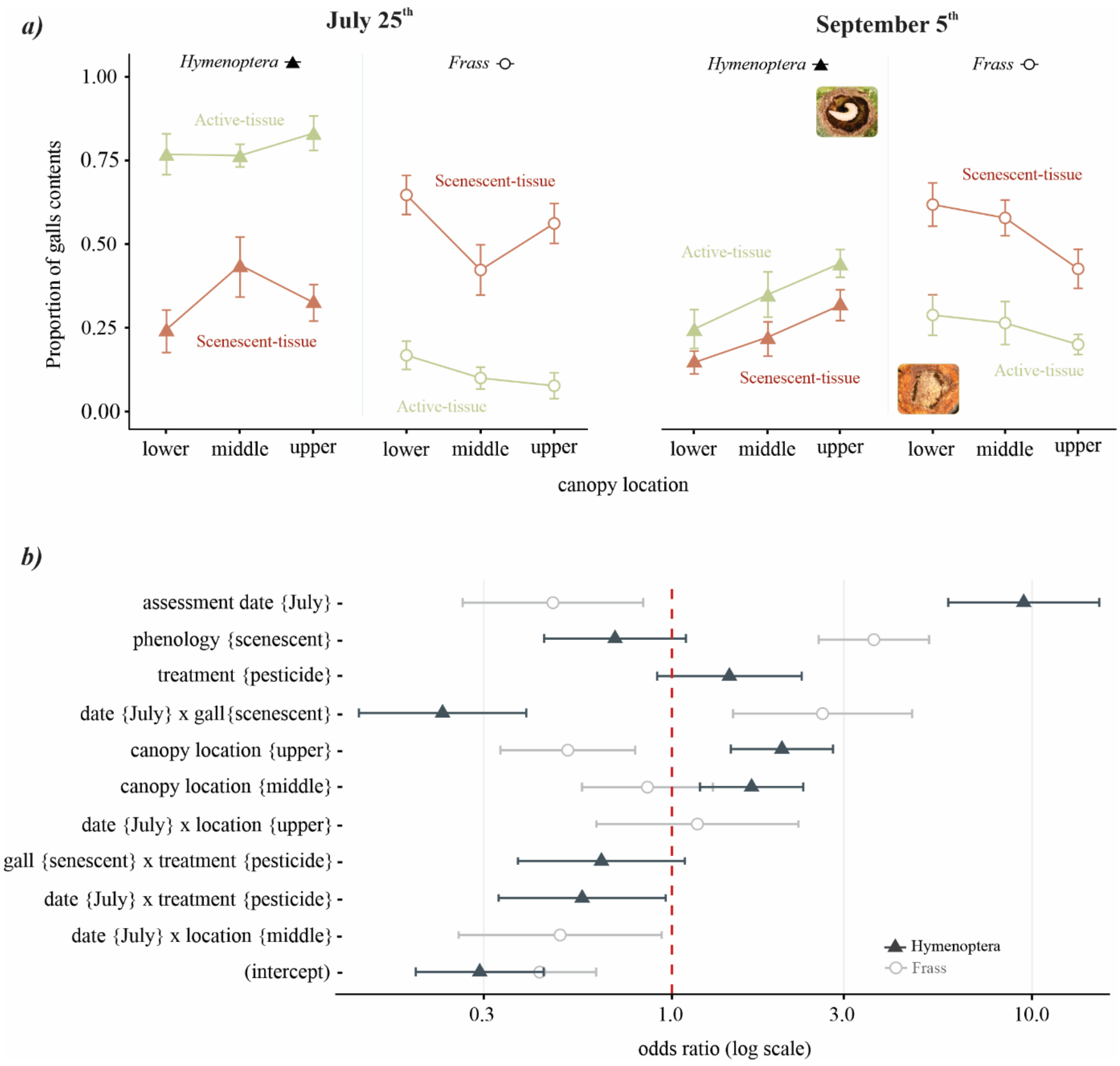

Figure 3.2 Variation in gall contents across canopy strata and sampling dates under different canopy management regimes. **(*a*)** Proportion of galls containing Hymenoptera (triangles) and frass (circles) on July 25th and September 5th, categorized by canopy location and gall developmental stage. Symbols represent mean proportions ± standard error. **(*b*)** Log odds ratios (±95% confidence intervals) plotted on a log scale for the presence of Hymenoptera and frass in galls, derived from the best-ranked generalized linear mixed models. Displayed predictors reflect the variables and interactions retained in the final models for each response.

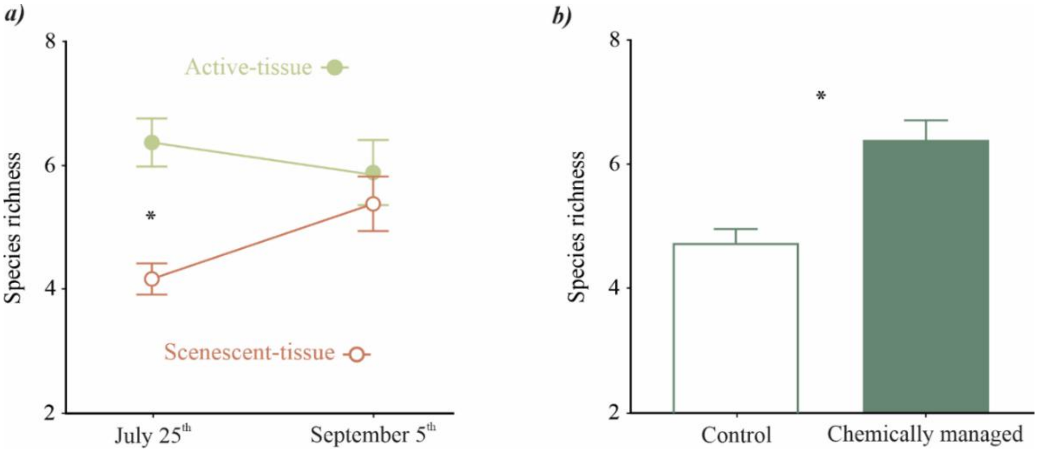

Figure 3.3 Species richness of arthropod communities associated with *Phylloxera* sp. galls, assessed using DNA metabarcoding. **(*a*)** Richness across two sampling dates (July 25th and September 5th), shown separately for galls with active (green, filled circles) and senescent (brown, open circles) tissue. **(*b*)** Richness compared between control and chemically managed trees. Symbols and histograms represent means ± standard error. Asterisks indicate statistically significant differences.

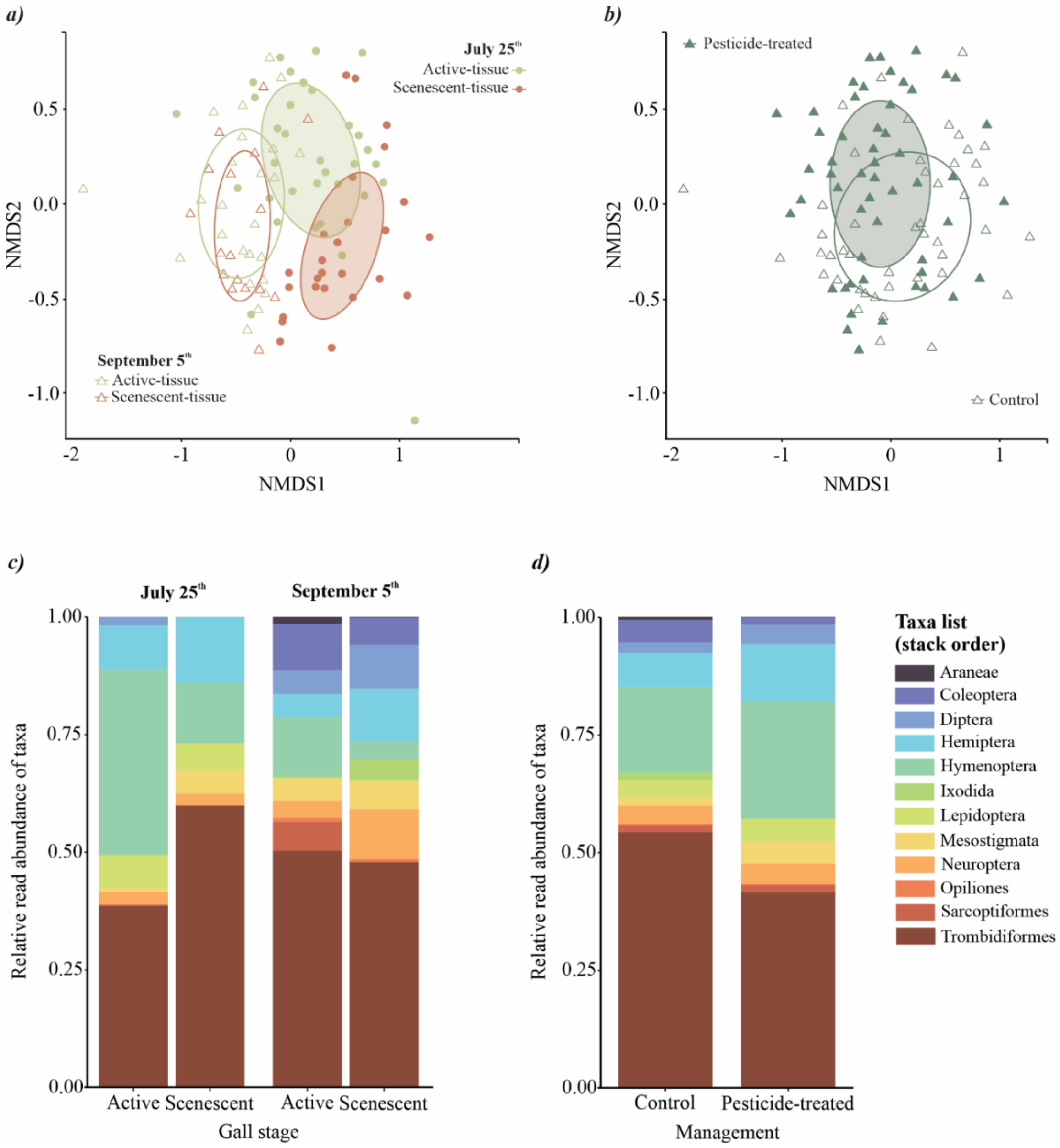

Figure 3.4 Variation in arthropod communities across canopy strata and sampling dates under different canopy management regimes, revealed by DNA metabarcoding. (Upper panels) NMDS plots illustrating dissimilarities in arthropod community composition. Symbols represent individual samples, and ellipses indicate 95% confidence intervals for **(*a*)** the interaction between sampling date and gall developmental stage and **(*b*)** the effect of canopy management (chemically managed vs. unmanaged). (Lower panels) Stacked bar charts showing the relative read abundance of arthropod taxa, grouped by order, for **(*c*)** the interaction between sampling date and gall developmental stage and **(*d*)** the effect of canopy management on these communities

Supplementary Table 3.1 Chemical management (fungicide and insecticide) records during both seasons of the experiment. Pesticides were sprayed using air blast sprayers. The schedule was based on standard recommendations for the state of Georgia (Wells, 2021)

Date	**Pesticide**[†]	**Type**[‡]	**Active ingredient**	**Rate (L/ha)**
April 12	Kphite	Fungicide	Phosphorous Acid	4.70
May 11	Propiconizole	Fungicide	Propiconizole	0.58
May 24	Absolute	Fungicide	Tebuconazole/Trifloxystrobin	0.51
June 07	Kphite	Fungicide	Phosphorous Acid	4.70
June 17	Intrepid Edge	Insecticide	Methoxyfenozide/Spinetoram	0.29
June 27	Elast	Fungicide	Dodine	3.65
July 18	SuperTin	Fungicide	Triphenyltin hydroxide	0.88
July 25[Ω]	First assessment of galls			
Aug 03	Elast	Fungicide	Dodine	3.65
Aug 04	Intrepid Edge	Insecticide	Methoxyfenozide/Spinetoram	0.29
	Closer	Insecticide	Sulfoxaflor	0.11
Aug 15	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	1.21
Aug 26	Closer	Insecticide	Sulfoxaflor	0.11
	Carbaryl	Insecticide	Carbaryl	4.68
	ProGibb	Insecticide	Gibberellic acid	0.37
Sep 05[Ω]	Second assessment of galls			

[†] Trade name of products utilized in treated canopies. [‡] Pesticide category. [Ω] Dates sampling was carried out.

Supplementary Table 3.2 Model selection results using the MuMIn package (dredge function), assessing how multiple factors influence the presence or absence of (I) Hymenoptera and (II) frass in galls, as well as (III) the Shannon diversity of taxa detected in sequencing reads and (IV) the richness of these taxa. The table presents the candidate models, including their Akaike Information Criterion (AIC) values, the number of model parameters (k), and ΔAIC values.

	Terms in the model[a]	AIC value	k	ΔAIC
	IV. Presence of Hymenoptera			
1	~ date + location + stage + treatment + date:stage + location:stage + stage:treatment	476.2	10	0.00
2	~ date + location + stage + treatment + date:stage + location:stage	476.6	9	0.37
3	~ date + location + stage + treatment + date:stage + location:stage + location:treatment + stage:treatment	477.1	12	0.90
4	~ date + location + stage + treatment + date:stage + location:stage + location:treatment	477.5	11	1.25
5	~ date + location + stage + treatment + date:location + date:stage + location:stage + stage:treatment	477.6	12	1.34
6	~ date + location + stage + treatment + date:location + date:stage + location:stage	477.8	11	1.61
	V. Presence of Insect Frass			
1	~ date + location + stage + date:stage + stage:treatment	457.2	9	0.00
2	~ date + location + stage + treatment + date:stage + location:stage + location:treatment	458.2	11	0.98
3	~ date + location + stage + treatment + date:stage + location:stage	458.5	10	1.32
4	~ date + location + stage + treatment + date:stage + location:stage + stage:treatment	459.0	11	1.79
5	~ date + location + stage + treatment + date:stage + location:stage + location:treatment + stage:treatment	459.3	12	2.15
6	~ date + location + stage + date:stage	460.4	7	3.23
	VI. Diversity (H) of recovered reads			
2	~ 1	224.3	3	0
3	~ stage	228.0	4	3.66
4	~ location	228.4	4	4.09
5	~ date	229.6	4	5.25
6	~ stage + location	232.1	5	7.81
	VII. Richness (S) of recovered reads			
1	~ date + stage + treatment + date:stage + date:treatment + stage:treatment	704.3	9	0
2	~ date + stage + treatment + date:stage + stage:treatment	704.6	8	0.24
3	~ stage + treatment + stage:treatment	706.0	6	1.70
4	~ date + location + stage + treatment + date:stage + date:treatment + stage:treatment	706.5	13	2.18
5	~ date + location + stage + treatment + date:stage + date:treatment	706.5	12	2.18

Supplementary Table 3.3 Summary of DNA metabarcoding for whole galls using mlep/lep primers for a 350bp target COI sequence within the Folmer region. The table presents the total number of samples containing reads and the mean ± standard error of reads per sample for each taxon within active and senescent galls (represented in >5% of galls analyzed; n ≥ 7 samples).

Taxa		**No. of galls**[a]	**Mean (± SE) reads per sample**	
			Active galls[b]	**Senescent galls**[c]
Insecta: Hemiptera				
Miridae	*Tuxedo elongatus*	57	210 (±40)	235 (±85)
	sp.	16	1,862 (±993)	0 (±0)
Aphididae	*Monellia* sp.	10	33 (±22)	0.2 (±0.2)
Insecta: Hymenoptera				
Eurytomidae	*Eurytoma gigantea*	40	443 (±132)	3 (±3)
	Eurytoma sp. (1)	30	461 (±150)	3 (±3)
	Eurytoma sp. (2)	20	145 (±87)	1 (±1)
Eupelmidae	*Eupelmus utahensis*	31	212 (±101)	36 (±26)
	Eupelmus sp	28	139 (±63)	2 (±1)
	Eupelmus tibicinis	18	64 (±32)	0.2 (±0.2)
	Eupelmus opacus	16	70 (±33)	11 (±9)
	Eupelmus orientalis	11	18 (±8)	0.4 (±0.4)
	Eupelmus logicalvus	10	15 (±7)	0.4 (±0.4)
	Eupelmus kieferi	9	6 (±3)	0 (±0)
	Eupelmus vuilleti	9	19 (±10)	0 (±0)
Eulophidae	sp.	22	151 (±80)	11 (±8)
	Aprostocetus sp.	21	93 (±44)	16 (±12)
	Aprostocetus citrinus	19	89 (±43)	15 (±11)
Pteromalidae	*Sycoscapter* sp.	18	111 (±53)	12 (±9)
Aphelinidae	*Aphelinus* sp.	15	119 (±73)	1 (±1)
Insecta: Neuroptera				
Coniopterigydae	sp.	27	484 (±458)	131 (±59)
Insecta: Coleoptera				
Cleridae	*Isohydnocera curtipenis*	22	1,801 (±1,029)	60 (±52)
Scarabeidae	*Oryctes elegans*	13	7 (±3)	10 (±6)
Cerambycidae	*Anomophysis spniosa*	9	8 (±4)	13 (±10)
Insecta: Lepidoptera				
Erebidae	*Apantensis phyllira*	31	122 (±40)	3 (± 1)
Depranidae	*Thyatira batis*	20	23 (±8)	1 (±1)
Nymphalidae	*Vanessa atalanta*	16	10 (±6)	0.3 (±0.3)
Noctuidae	*Mythimna impura*	12	6 (±2)	0 (±0)
Tortricidae	*Gretchena bolliana*	9	144 (±125)	1 (±1)
Pyralidae	*Carnarsia* sp.	7	10 (±6)	2 (±2)
Insecta: Diptera				
Dolichopodidae	*Condylostylus* sp.	35	49 (±13)	44 (±31)
Sciomyzidae	*Dictya* sp.	21	98 (±35)	44 (±27)
Asilidae	*Neolophonotus pellitus*	11	2 (±1)	167 (±113)
Culicidae	*Aedes* sp.	9	1 (±1)	2 (±1)
Arachnida: Trombidiformes				
Tydeidae	sp.	91	8,997 (±3419)	2,642 (±1162)
Tarsonemidae	sp.	28	68 (±34)	60 (± 43)

Diptilomiopidae	*Diptilomiopus assamica*	10	5 (±2)	0 (±0)
Eriophyidae	*Eriophyes* sp	7	10 (±5)	1 (±1)
Penthaleidae	sp	7	2 (±1)	217 (±214)
Triophtydeidae	*Triophtydeus* sp.	7	20 (±12)	4 (±3)
Arachnida: Mesostigmata				
Macrochelidae	*Macrocheles merdarius*	36	58 (±16)	53 (±37)
Phytoseiidae	sp.	17	68 (±38)	29 (±18)
Achipteriidae	*Anachipteria* sp.	15	73 (±43)	17 (±13)
Arachnida: Ixodida				
Ixodidae	*Ixodes texanus*	13	50 (±23)	216 (± 153)
Arachnida: Sarcoptiformes				
Suctobelbidae	*Suctobelbella* sp.	13	11 (±4)	2 (±2)
Tectocepheidae	sp.	7	5 (±3)	2 (±2)
Arachnida: Araneae				
Lycosidae	*Melocosa fumosa*	12	9 (±4)	4 (±3)
Arachnida: Opiliones				
Triaenonychidae	*Nuncia nigriflava*	12	11 (±5)	16 (±11)
Total[d]:		146	1,427,929	241,313

[a]Total number of samples testing positive for each taxon. [b]Active galls, with tissue exhibiting shades of green and yellow. [c]Senescent galls, exhibiting tissue in shades of brown and red. [d]Total number of galls, followed by the total number of reads captured in each gall type across all samples. Refer to Figure 1 for further details on gall development stages and the classification criteria for "active" and "senescent" galls used in this study.

Supplementary Table 3.4 Model comparison for community analysis of DNA sequences recovered from whole galls. The table summarizes the various models evaluated for their effectiveness in explaining dissimilarities among insect communities, presenting their respective Akaike Information Criterion (AIC) values, the number of parameters (k), and ΔAIC values. The highest-ranked models indicate the best performance in capturing the observed variations in community structure.

Rank	**Terms in the model**[a]	**AIC value**	**k**	**ΔAIC**
1	~ **pesticide** + **date*gall stage**	-116.48	5	0
2	~ **pesticide** + **date** + **gall stage**	-116.44	4	0.04
3	~ date*pesticide + **date*gall stage**	-116.27	6	0.21
4	~ pesticide + location + date*gall stage	-114.49	7	1.99
5	~ **pesticide** + location + **date** + **gall stage**	-114.43	6	2.05
6	~ date + pesticide + location + **date*gall stage**	-114.26	8	2.22
7	~ date*pesticide + date*location + **date*gall stage**	-112.74	10	3.74

[a] Terms highlighted in bold font were statistically significant in the presented models.

Supplementary Figure 3.1 The selected photographs illustrate examples of interactors found within galls, serving as an initial inventory of gall-associated species. The images depict various specimens, including **(A)** an adult and larva of the primary hymenopteran (Eurytomidae), along with a parasitized larva of the same species. Additionally, the panels feature **(B)** a variety of pupae discovered within the galls, belonging to the families Eurytomidae and Eupelmidae. **(C)** Eggs laid within the inner walls of the galls. Adult wasps from the families **(D)** Eurytomidae, Bethylidae, **(E)** Pteromalidae, Perilampidae, and Megaspilidae.

CHAPTER 4

TEMPORAL AND SPATIAL ACTIVITY OF AUCHENORRHYNCHA COMMUNITY RESPOND TO CANOPY MANAGEMENT IN A TALL TREE AGROECOSYSTEM[4]

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[4] Toledo, P.F.S. & Schmidt J.M. To be submitted to Pest Management Science.

Abstract

BACKGROUND: Understanding herbivore activity patterns in perennial agroecosystems is essential for sustainably managing their populations across seasons. In pecan orchards, Auchenorrhyncha species can cause direct feeding damage and potentially transmit pathogens, yet their community structure and ecological dynamics remain poorly understood. We examined seasonal (June-September) and within-canopy (vertical) activity of Auchenorrhyncha in a mature pecan orchard over two growing seasons and assessed their response to two common management interventions: hedge pruning and calendar-based insecticide and fungicide applications.

RESULTS: We collected 5,882 Auchenorrhyncha from pecan canopies, representing seven families. The assemblage was overwhelmingly dominated by Cicadellidae, with over 95% of individuals belonging to the subfamily Typhlocybinae. Abundance increased from June to September, and the lower canopy consistently harbored fewer insects, emphasizing spatial structuring. Pesticide applications reduced Auchenorrhyncha abundance, although effects varied across sampling dates and canopy strata. Hedge pruning significantly increased abundance, likely due to altered canopy resources and microclimate.

CONCLUSION: These findings offer insights into the spatial and temporal activity of Auchenorrhyncha in pecan orchards and how farming practices, including chemical management and pruning, may shape their populations. This foundation may guide improved monitoring and management strategies in pecans and other tree agroecosystems.

Keywords: *Carya illinoinesis*, hedge-pruning, orchard pest management, tree canopy ecology, Cicadellidae

Introduction

Understanding the structure and activity patterns of arthropod herbivore communities in agricultural systems is crucial for developing ecology-based strategies that can sustainably manage their populations across seasons[1,2]. However, as research has historically prioritized economically relevant species, knowledge of the diversity and distribution of commonly occurring herbivore communities remains limited for many agroecosystems.

Herbivore community structure and spatiotemporal activity within agroecosystems are influenced by a complex interplay between natural environmental conditions and farming management practices[3–6]. This complexity is particularly evident in tall tree perennial systems, such as pecan [*Carya illinoinensis* (Wangenh.) K. Koch] orchards, where vertically structured canopies support arthropod populations exposed to alternating periods of minimal intervention (winter dormancy) and intensive management (spring through late summer)[7,8].

In mature pecan orchards, both plant structural features (e.g., canopy height) and management interventions influence herbivore community dynamics. Tall canopies create spatial structuring, and herbivore activity can vary across the lower and upper canopy strata[9–13]. Recently, we found that reshaping the pecan canopy through mechanical hedge pruning can impact arthropod abundance[14] . However, pruning's impact on the spatial activity of arthropods within the pecan canopy remains largely understudied. In addition, routine chemical applications targeting persistent pests and diseases in these orchards[8] can affect both target and non-target arthropod communities through direct toxicity or altered ecological interactions[15]. These drivers influence habitat structure, resource availability, and species interactions, often producing system-specific responses. For instance, due to tree height, chemical applications in pecans using air-blast sprayers often provide uneven canopy coverage, leaving the upper canopies more vulnerable[16,17]. Consequently, herbivores that predominantly inhabit the upper canopy may experience reduced chemical exposure. Nevertheless, how these structural and management factors combine to shape commonly occurring herbivore communities, such as true hoppers (Hemiptera: Auchenorrhyncha), within pecan canopies remains poorly understood.

In pecan orchards, sap-sucking Auchenorrhyncha, including leafhoppers, planthoppers, and treehoppers, are abundant and can occasionally cause direct feeding damage. For instance, the pecan spittlebug (*Clastoptera achatina* Germar, 1839), a known pest that feeds on nut clusters, can negatively impact production[18]. Additionally, *C. achatina*, along with other Auchenorrhyncha species such as *Homalodisca insolita* Walker, 1851 and *Homalodisca vitripennis* Germar, 1821 serve as vectors of *Xylella fastidiosa*, the causal agent of pecan bacterial leaf scorch[19]. This disease can severely reduce pecan yield in susceptible cultivars (e.g., 'Cape Fear'), has no economically viable treatment, and may necessitate tree removal to prevent its spread[20–23]. Managing the disease is particularly challenging due to the complex interactions between *X. fastidiosa*, Auchenorrhyncha vectors[24], and pecan trees. Although our study does not evaluate vector status or pathogen spread, understanding the ecological distribution and management sensitivity of this herbivore group is an important step toward improving pest and disease management in pecan systems.

Therefore, this study investigates the effects of hedge pruning and chemical management (calendar-based insecticide and fungicide applications) on the spatial and temporal activity of Auchenorrhyncha in a Southeastern U.S. pecan orchard over two growing seasons. Our primary objectives were to: (1) assess the diversity, abundance, and distribution of Auchenorrhyncha across canopy strata during seasonal periods (June-September), and (2) understand how these parameters respond to hedge pruning, chemical management, and their interactions. Ultimately, our findings will provide insights into the taxa, temporal dynamics and intra-canopy distribution of Auchenorrhyncha in pecan, enhancing our understanding of how orchard management shapes the activity of arthropod communities in tall tree systems.

Material and Methods

Study site, design and management approaches

The study was conducted in an experimental pecan orchard (32°39'55.1"N, 83°43'45.7"W) at the Southern Fruit and Tree Nut Research Station, USDA-ARS, in Byron, Georgia. The orchard consisted of 40-year-old 'Pawnee' cultivar trees, approximately 17 meters tall, spaced 12 meters apart within rows and 24 meters between rows. The areas between rows were regularly mowed, while the strips beneath the tree rows were kept vegetation-free using a 6-meter-wide herbicide strip. No irrigation was applied aside from natural rainfall. The orchard contained roughly 110 trees, and from these, 12 groups (each consisting of three consecutive trees in a row) were randomly selected, totaling 36 trees for the study. Six of these groups were pruned, with three receiving routine pest and disease management, and the other three left untreated. The remaining six groups were not pruned, following the same pest and disease management division. Thus, the study followed a 2x2 factorial design, with three replicates (three groups of three trees) for each treatment combination: pruned and sprayed, pruned and unsprayed, unpruned and sprayed, and unpruned and unsprayed. Sampling was conducted from the middle tree in each group, while the other two trees acted as buffers. Pruning was carried out manually using chainsaws, mimicking mechanical hedge pruning at a height of approximately 9.5 meters. Hedge-pruning was done on the north side of the trees in the winter of 2020 and on the south side in the winter of 2021. Chemical management (pesticide spray) was performed using air-blast sprayers following Georgia standard recommendations and records are displayed in detail in Table 4.1.

Estimating the abundance of Auchenorrhyncha in pecan canopies

Auchenorrhyncha specimens were collected using a suction sampler (reverse leaf blower; STIHL BG 86, Andreas Stihl AG & Co. KG, Waiblingen, Germany) operated for 30 seconds while being gently moved around pecan branches. Sampling was conducted from a telescopic crawler boom lift (JLG 660SJC, JLG Industries, Inc., McConnellsburg, PA, USA) to access different canopy heights. Each tree was sampled at three canopy strata: upper, middle, and lower canopy. Within each stratum, two vacuum samples were taken, one from the north side and one from the south side, resulting in six total samples per tree. Counts from the two sides (north and south) within each stratum were summed for analysis. Lower canopy samples were collected from the lowest accessible branches, middle canopy samples were taken at approximately 9.5 meters above ground, corresponding to the height of hedge-pruning, and upper canopy samples were obtained from the highest branches. Following collection, samples were transported to the laboratory and stored at -20°C until processing. In the laboratory, all Auchenorrhyncha specimens were counted and identified to at least to subfamily, but most to species level. Photographs of adult Auchenorrhyncha specimens were taken using a Jenoptik GRYPHAX® NAOS camera (Jenoptik Optical Systems GmbH, Jena, Germany) mounted on a stereomicroscope.

Statistical Analysis

The abundance of Auchenorrhyncha was analyzed by fitting the natural log-transformed values (log(Auchenorrhyncha + 1)) to a linear mixed-effects model{package: nlme}[25]. Fixed effects included hedge-pruning, assessment date, canopy location, and chemical management (pesticide treatment), while year was a random effect to account for variation across years. Model selection was conducted using the MuMIn package, which systematically evaluates all possible combinations of predictor variables and interactions derived from a global model to identify the most parsimonious model, balancing explanatory power and complexity. The dredge function was used to evaluate and rank all possible models based on Akaike's Information Criterion corrected for small sample sizes (AICc). The top-ranked model, which included hedge-pruning, canopy location, pesticide treatment, date, and two interaction terms (location:treatment and date:treatment), was identified as the best-fitting model (Table 4.3). Post hoc pairwise comparisons were performed using the {package: emmeans} with Tukey's adjustment for multiple comparisons. These comparisons were used to clarify main effects: Auchenorrhyncha abundance between canopy locations and across dates, and interaction effects: chemical management effects within each date (pesticide × date) and within each canopy location (pesticide × location). All statistical analyses were performed in R version 4.4.0 "Puppy Cup" (R Core Team, 2024), with figures generated in R. Figure icons, labels, and colors were added using CorelDRAW Graphics Suite 2021.

Results

Diversity and taxonomic composition of Auchenorrhyncha in pecan canopies

We sampled a total of 5,882 Auchenorrhyncha individuals from pecan canopies during the 2021 and 2022 seasons. The assemblage was dominated by the family Cicadellidae, which accounted for over 95% of all individuals across both years. In addition to Cicadellidae, six other families contributed to the overall taxonomic diversity, though they were represented in much lower numbers: Clastopteridae (n = 63), Acanaloniidae (n = 64), Flatidae (n = 22), Issidae (n = 5), Membracidae (n = 3), Tropiduchidae (n = 2). Due to its dominance, Cicadellidae was further resolved into major subgroups. Species of the subfamily Typhlocybinae comprised ~97% of all Auchenorrhyncha collected across both years, while representatives of the subfamily Cicadellinae, specifically the tribes Proconiini (n = 15) and Cicadellini (n= 8) were rare. A complete list of the 17 adult taxa identified across all families is provided in Table 4.3, with representative images shown in Figure 4.1.

Auchenorrhyncha activity under different canopy management regimes

Auchenorrhyncha abundance varied with sampling date, canopy position, chemical management, and hedging, with significant interactions indicating that the effects of pesticide treatment depended on both sampling date and canopy height (Table 4.3). Seasonal trends showed an increase in abundance from June to September (Figure 1*a*), with significant increases in July (t = 2.13, $P = 0.0337$), August ($t = 5.61$, $P < 0.0001$), and September ($t = 7.57$, $P < 0.0001$) compared to June. Abundance also differed across canopy layers, with the lower canopy supporting fewer individuals than both the middle ($t = 6.04$, $P < 0.0001$) and upper canopy ($t = 5.66$, $P < 0.0001$; Figure 2*a*). No significant difference in abundance was detected between the middle and upper strata (*t* = 0.38, *P* = 0.9256). A pesticide × date interaction showed that pesticide effects were time-dependent. No significant difference in abundance was observed between treated and untreated trees in June (*t* = 1.38, *P* = 0.1693) or July (*t* = -1.27, *P* = 0.2041), but in August and September, abundance was significantly lower in treated trees (*t* = -3.95, *P* = 0.0001; *t* = -3.76, *P* = 0.0002; Figure 1*b*). A pesticide × canopy position interaction indicated that pesticide effects varied within the canopy strata. Abundance was reduced in the lower (*t* = -3.80, *P* = 0.0002) and middle canopy (*t* = -3.52, *P* = 0.0005), but not in the upper canopy (*t* = -0.55, *P* = 0.5805; Figure 4.2*c*). In contrast, hedging effects were consistent, with significantly higher Auchenorrhyncha abundance in hedge-pruned canopies across all strata and time points (Table 4.3; Figure 4.2d), and no interactions with other factors.

Discussion

By assessing Auchenorrhyncha activity in tall pecan canopies, our study demonstrates that these insect populations exhibit distinct seasonal and vertical distribution patterns, significantly shaped by orchard management practices. Although this group ecologically[26,27] and economically important, with potential as indicators of habitat disturbances[28,29], Auchenorrhyncha responses to management strategies in pecans and other tree crops remain largely unexplored (but see[30,31]). Here, we demonstrate that management-induced disturbances interact with system structural features, such as canopy height, resulting in differential spatiotemporal responses within Auchenorrhyncha communities.

Auchenorrhyncha abundance increased from June through September, likely driven by seasonal environmental conditions[32] such as rising temperatures and increased plant sap flow during nut development[33], which may have enhanced resources and/or feeding opportunities.

Although our data did not extend into the winter months, the continued buildup in late summer and early fall suggest the capacity of Auchenorrhyncha to sustain high densities into the late season. Additionally, vertical stratification played a significant role in shaping their distribution, with higher abundances recorded in the middle and upper canopy strata. This pattern may result from microclimatic advantages[27,34], including greater sunlight exposure and/or potentially reduced predation pressure at higher canopy levels [35–37]. These trends underscore the importance of considering canopy height and stratification when designing sampling protocols or insect management tactics, as failing to monitor upper canopy strata may underestimate herbivore presence in this scenario.

We detected seven Auchenorrhyncha families in pecan canopies, with Cicadellidae dominating the assemblage and accounting for over 95% of all individuals collected. This pattern is consistent with Auchenorrhyncha assemblages in many agroecosystems[38], including pecans[39], where cicadellids frequently dominate Auchenorrhyncha community composition. In our study, the subfamily Typhlocybinae represented nearly all Cicadellidae individuals, reflecting the dominance of this mesophyll-feeding taxon in the pecan canopy. Other families were present but contributed minimally to overall abundance, highlighting relatively low taxonomic evenness despite the presence of multiple families. Among the 17 taxa recorded, only two have been experimentally confirmed as vectors of *X. fastidiosa* in pecan[19]. The vector potential of many other Auchenorrhyncha species remains uncharacterized. Nevertheless, vector pressure in the studied orchard appeared very low, despite *Xylella*'s endemic status in Southeastern U.S. pecan orchards[23].

Chemical management (calendar-based fungicides and insecticide applications) is widely practiced in commercial pecan orchards, with growers following recommendations from the pecan spray guide [8]. While these treatments primarily target persistent pests and pathogens, they can also affect non-target insect communities[40]. Here we show that chemical management in the canopy reduced Auchenorrhyncha abundance, particularly in August and September, when insecticide applications intensified. These late-season sprays often target nut-feeding pests such as pecan weevil and stink bugs and typically involve broad-spectrum insecticides (bifenthrin) that can suppress non-target taxa. Although an insecticide application (methoxyfenozide) was applied in early June (targeting caterpillars), early-season applications consisted primarily of fungicides. The absence of significant effects on Auchenorrhyncha during June and July suggests that these early inputs exerted limited influence on canopy-dwelling Auchenorrhyncha populations. Interestingly, the impact of pesticides on Auchenorrhyncha was not uniform across canopy strata, with lower and middle canopies experiencing significant abundance declines, whereas the upper canopy remained unaffected. The lack of effects in the upper canopy may reflect uneven spray coverage by air-blast sprayers, which diminishes as canopy height increases (>10m) in pecan systems [16]. This suggests that incomplete spray coverage in tall trees may allow populations in the upper canopy layers to persist. Such dynamics may present challenges for managing Auchenorrhyncha populations, emphasizing the need to optimize pest management strategies that account for canopy height and potential spray coverage limitations.

Hedge pruning in pecans can significantly influence arthropod abundance in the canopy, including Hemipteran insects such as aphids, that apparently benefit from pruning effects [14]. In this study, hedge pruning consistently increased Auchenorrhyncha abundance compared to non-pruned trees. This effect may be partially explained by changes in canopy architecture that create more favorable microhabitats or increase resource availability through altered plant growth and sap flow[33]. If further confirmed, these findings suggest that hedge pruning may elevate the risk of Auchenorrhyncha population surges in the years immediately following pruning. While pruning increased Auchenorrhyncha abundance, its implications for *Xylella fastidiosa* transmission remain uncertain and warrant further investigation. Given that mechanical pruning wounds could facilitate *X. fastidiosa* transmission through contaminated equipment [22], it is reasonable to believe that the elevated Auchenorrhyncha abundance resulting from hedge pruning may increase the risk of post-pruning *X. fastidiosa* transmission in orchards planted with susceptible cultivars.

Conclusion

This study provides valuable insights into the diversity, seasonal activity and vertical distribution of Auchenorrhyncha populations in pecan canopies in the Southeastern U.S. Our findings demonstrate that populations can grow from June through late summer, with a predominant distribution in the middle and upper canopy strata. Additionally, farming interventions influenced Auchenorrhyncha abundance and distribution, underscoring that farming interventions, whether pruning, spraying, or habitat simplification, influence arthropod communities in orchard systems. As such, pest management must account for vertical distribution, and other structural traits specific to each crop. Notably, the population surge following pruning and the uneven effects of chemical management across canopy strata highlight potential challenges in managing both insect vectors and disease. Although crop damage or pathogen transmission were not the focus of this work, characterizing the composition, distribution, and management sensitivity of Auchenorrhyncha communities may inform future pest and vector management efforts. These findings contribute to a broader understanding of herbivore dynamics in perennial tree systems, emphasizing the complexity of farming-induced stressors in shaping arthropod communities.

Acknowledgments

We thank Kirsten Flinn, Bryce Vaughn, Rachel Ibbetson, Kirby Moncrief, and Dr. Kyle Slusher for the technical assistance. The project was funded by SSARE Project LS20-340. This work was supported, in part, by the University of Georgia, and USDA-NIFA Multistate Hatch Project GEO00884-S1073. CHB received additional support from USDA-ARS projects 6606-21220-014–00D and 6042-22000-024-000-D, respectively. The mention of any trademark or proprietary product is for informational purposes only and does not constitute a guarantee, warranty, or endorsement by the U.S. Department of Agriculture or the University of Georgia. Likewise, references to specific commercial agrochemical products used in this study do not imply endorsement, recommendation, or preference by the authors or their respective institutions over other potentially suitable products.

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Table 4.1 Chemical management (fungicide and insecticide) records during both seasons of the experiment. Pesticides were sprayed using air blast sprayers. The schedule was based on standard recommendations for the state of Georgia.

	Date	Pesticide[†]	Type[‡]	Active ingredient	Rate (L/ha)
2021 Season	May 04	Propiconizole	Fungicide	Propiconizole	0.58
	May 24	DynaPhite	Fungicide	Phosphorous Acid	4.68
	May 26	Reliant	Fungicide	Phosphorous Acid	4.68
	June 02	Intrepid	Insecticide	Methoxyfenozide	0.29
	June 08	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	1.00
	June 15	Intrepid	Insecticide	Methoxyfenozide	0.29
	June 23	Absolute	Fungicide	Tebuconazole/Trifloxystrobin	0.51
	July 09	Elast	Fungicide	Dodine	0.51
	July 29	SuperTin	Fungicide	Triphenyltin hydroxide	0.51
	Aug 06	Intrepid	Insecticide	Methoxyfenozide	0.29
		Closer	Insecticide	Sulfoxaflor	0.11
	Aug 12	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	1.00
	Aug 18	Closer	Insecticide	Sulfoxaflor	0.11
		Carbaryl	Insecticide	Carbaryl	4.68
		ProGibb	Insecticide	Gibberellic acid	0.37
	Aug 25	Elast	Fungicide	Dodine	0.51
	Sep 02	Brigade	Insecticide	Bifenthrin	0.37
		Closer	Insecticide	Sulfoxaflor	0.11
		ProGibb LV Plus	Insecticide	Gibberellic acid	0.37
2022 Season	May 11	Propiconizole	Fungicide	Propiconizole	0.58
	May 24	Absolute	Fungicide	Tebuconazole/Trifloxystrobin	0.51
	June 07	Kphite	Fungicide	Phosphorous Acid	4.70
	June 17	Intrepid Edge	Insecticide	Methoxyfenozide/Spinetoram	0.29
	June 27	Elast	Fungicide	Dodine	3.65
	July 18	SuperTin	Fungicide	Triphenyltin hydroxide	0.88
	Aug 03	Elast	Fungicide	Dodine	3.65
	Aug 04	Intrepid Edge	Insecticide	Methoxyfenozide/Spinetoram	0.29
		Closer	Insecticide	Sulfoxaflor	0.11
	Aug 15	Miravis Top	Fungicide	Pydiflumetofen/Difenoconazole	1.21
	Aug 26	Closer	Insecticide	Sulfoxaflor	0.11
		Carbaryl	Insecticide	Carbaryl	4.68
		ProGibb	Insecticide	Gibberellic acid	0.37
	Sep 07	Brigade	Insecticide	Bifenthrin	0.37
		Closer	Insecticide	Sulfoxaflor	0.11
		ProGibb LV Plus	Insecticide	Gibberellic acid	0.37

[†] Trade name of product used. [‡] Pesticide category.

Table 4.2 Table ranks the best-fitting linear mixed-effects models used to assess the influence of various factors on the abundance of Auchenorrhyncha within pecan canopies. Models are ranked by their AIC values, with lower values indicating better model fit. ΔAIC represents the difference in AIC between each model and the best-fitting model (Rank 1). The models include main effects and interaction terms, reflecting spatial, temporal, and management-related influences on Auchenorrhyncha abundance. The parameter count (k) denotes the number of estimated parameters, providing a measure of model complexity.

Rank	**Terms in the model**	**AIC**	**k**	**ΔAIC**
1	~ hedging + location + date + treatment + location:treatment + date:treatment	648.3	15	0
2	~ hedging + location + date + treatment + date:treatment	650.3	21	2.03
3	~ hedging + location + date + treatment + location:treatment	652.2	16	3.84
4	~ hedging + location + date + treatment	652.4	18	4.05
5	~ hedging + location + treatment	652.5	13	4.16
6	~ location + date + treatment + location:treatment	654.3	22	6.02

Table 4.3 Taxonomic diversity of Auchenorrhyncha collected from pecan canopies. Taxa are organized hierarchically by family, subfamily, and species. Only adult individuals identified to species or subfamily level are included.

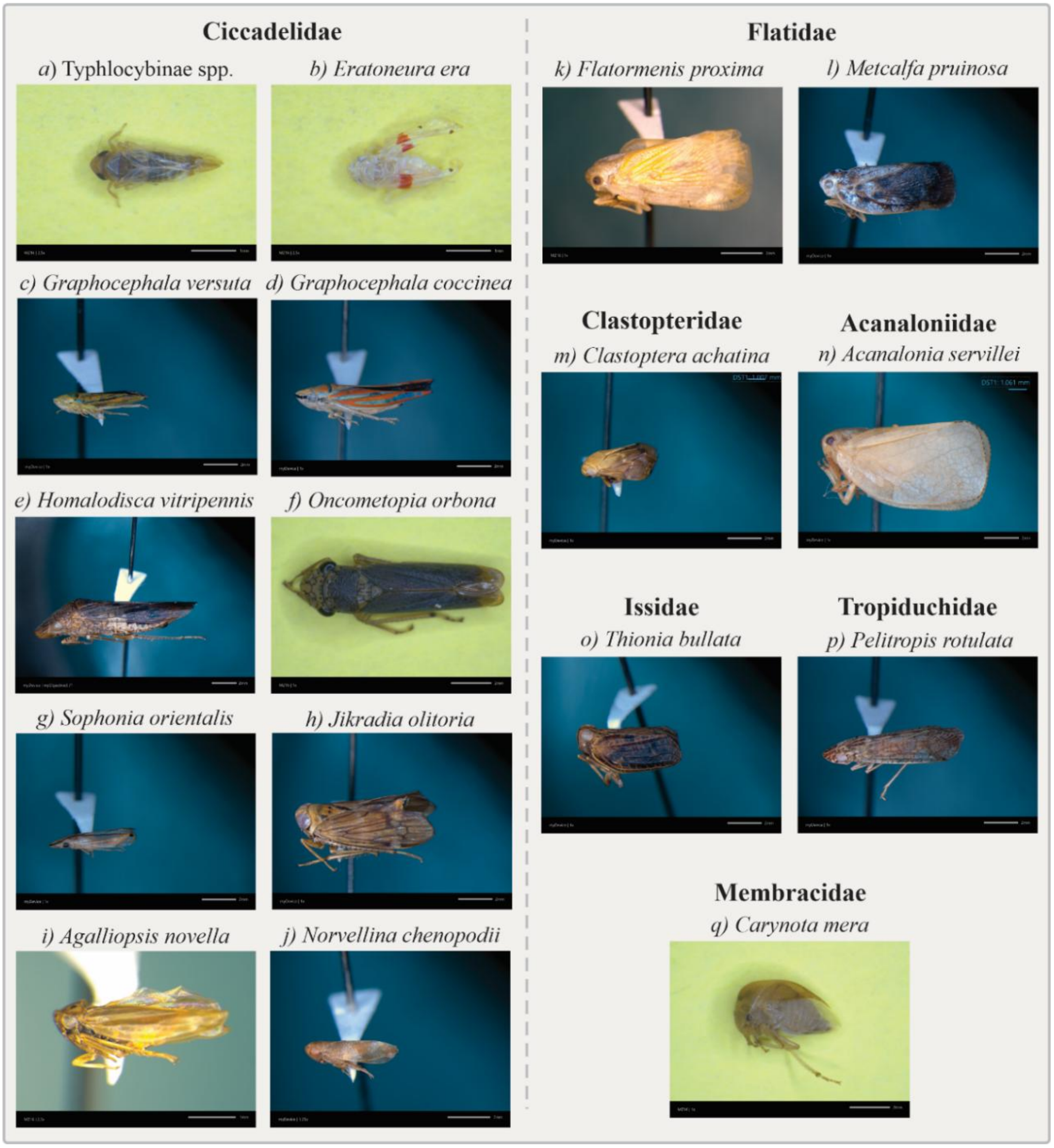
Family	Subfamily	Species	Figure
Cicadellidae	Typhlocybinae	Typhlocybinae spp.	Fig. 2*a*
		Eratoneura era (McAtee)	Fig. 2*b*
	Cicadellinae	*Graphocephala versuta* (Say)	Fig. 2*c*
		Graphocephala coccinea (Forster)	Fig. 2*d*
		Homalodisca vitripennis (Germar)	Fig. 2*e*
		Oncometopia orbona (Fabricius)	Fig. 2*f*
	Iassinae	*Sophonia orientalis* (Matsumura)	Fig. 2*g*
		Jikradia olitoria (Say)	Fig. 2*h*
	Agalliinae	*Agalliopsis novella* (Say)	Fig. 2*i*
	Deltocephalinae	*Norvellina chenopodii* (Osborn)	Fig. 2*j*
Flatidae	-	*Metcalfa pruinose* (Say)	Fig. 2*k*
	-	*Flatormenis proxima* (Walker)	Fig. 2*l*
Clastopteridae	-	*Clastoptera achatina* Germar	Fig. 2*m*
Acanaloniidae	-	*Acanalonia servillei* Spinola	Fig. 2*n*
Issidae	-	*Thionia bullata* (Say)	Fig. 2*o*
Tropiduchidae	-	*Pelitropis rotulata* (Van Duzee)	Fig. 2*p*
Membracidae	-	*Carynota mera* (Say)	Fig. 2*q*

Figure references (Fig. 2A–2Q) correspond to representative images presented in Figure 2.

Table 4.4 Summary statistics from the best-fitting linear mixed effect model (LME) used to analyze the effects of date, pruning, pesticide applications and canopy location on abundance of Auchenorrhyncha.

Sources of variation		df_{num}/df_{den}	***F***	***P***
Auchenorrhyncha	Assessment date (D)	3/365	**23.15**	**<.0001***
	Pesticides (P)	1/9	**18.66**	**0.0003***
	Hedge-pruning	1/9	**36.03**	**<.0001***
	Canopy Location (L)	2/265	**22.87**	**<.0001***
	D x P	3/265	**6.00**	**0.0006***
	L x P	2/265	**5.95**	**0.0030***

*Significant at $P < 0.05$ (highlighted in bold font)



Ciccadelidae

a) Typhlocybinae spp.

b) *Eratoneura era*

c) *Graphocephala versuta*

d) *Graphocephala coccinea*

e) *Homalodisca vitripennis*

f) *Oncometopia orbona*

g) *Sophonia orientalis*

h) *Jikradia olitoria*

i) *Agalliopsis novella*

j) *Norvellina chenopodii*

Flatidae

k) *Flatormenis proxima*

l) *Metcalfa pruinosa*

Clastopteridae

m) *Clastoptera achatina*

Acanaloniidae

n) *Acanalonia servillei*

Issidae

o) *Thionia bullata*

Tropiduchidae

p) *Pelitropis rotulata*

Membracidae

q) *Carynota mera*

Figure 4.1 Representative adult Auchenorrhyncha species collected from pecan (*Carya illinoinensis*) canopies. Taxa are displayed in panels (*a* – *q*), grouped by family, following the taxa presented in Table 3. All specimens were photographed under stereomicroscopy, with scale bars (mm) included to provide size reference.

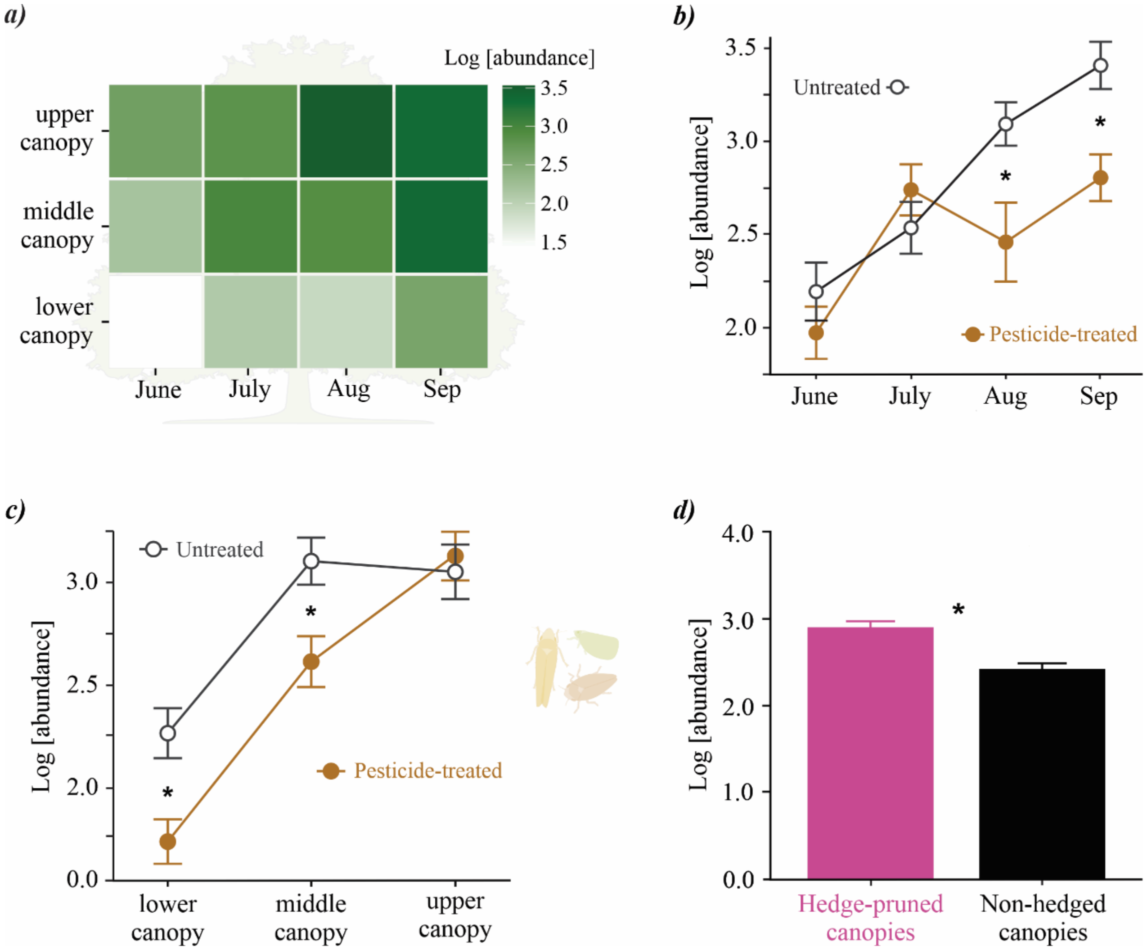
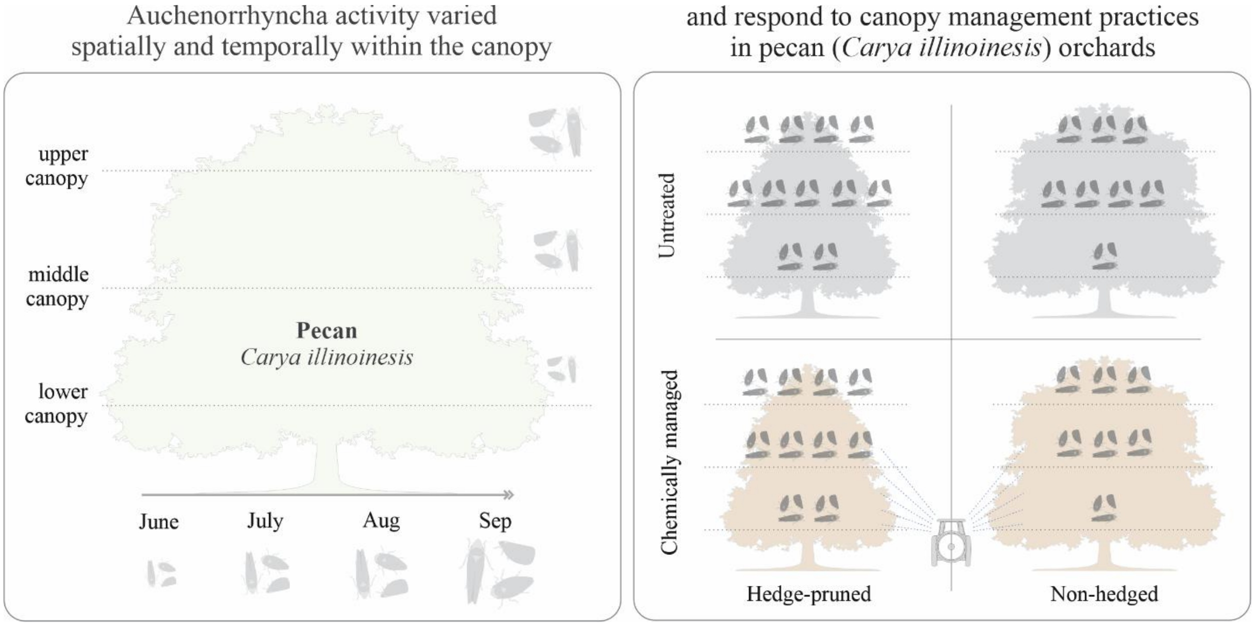


Figure 4.2 ***(a)*** Heat map showing the abundance of Auchenorrhyncha across the lower, middle, and upper canopy strata from June to September, highlighting temporal and vertical distribution patterns. Darker shades indicate higher abundance levels. ***(b)*** Seasonal variation (June to September) and ***(c)*** canopy strata patterns (lower, middle, upper) of Auchenorrhyncha abundance (mean ± 1 SEM) under pesticide-treated (brown line, filled circles) or untreated conditions (black line, open circles). ***(d)*** Histograms representing mean Auchenorrhyncha abundance (± 1 SEM) on hedge-pruned (pink) and non-hedged (black) pecan canopies. Asterisks indicate statistical differences.

Graphical Abstract

Auchenorrhyncha abundance increased through late summer, with lower densities in the lower canopy. Hedge pruning increased abundance, while chemical management effects varied, highlighting the complex influence of orchard practices on herbivore communities.



SUMMARY AND CONCLUDING REMARKS

In this thesis, I used the pecan system as a model to explore ecological questions about arthropod communities in agroecosystems. By integrating observational and molecular tools such as DNA sequencing, I examined how common farming practices influence the spatiotemporal structuring of arthropod communities and natural enemy-prey interactions.

This work brings attention to the fact that farming choices generate group-specific and context-dependent ecological responses in arthropod populations. As demonstrated in Chapter 1, canopy structural alterations via pruning in a commercial orchard led to different outcomes for pest and natural enemy populations. While pruning suppressed infestation from some pests, it favored increased pressures from others, including the yellow aphid complex species, confirmed in Chapter 2. These findings highlight that arthropod responses to habitat restructuring cannot be generalized, emphasizing the need to further understanding of the mechanisms underlying such effects. Building upon these findings, Chapter 2 revealed that farming practices also restructure species interactions, affecting predation and parasitism within the canopy. Hedge pruning and pesticide applications reshaped the ecological context in which interactions took place. These results reinforce that biological control outcomes are emergent properties shaped by the interaction of habitat filtering, temporal dynamics, and community structure.

In Chapter 3, we expanded the scope of this work by tracking *Phylloxera*-induced galls as a spatially and temporally restricted microhabitat where species interactions and ecological filters could be studied. Galls offer a natural microcosm in which community structuring processes unfold within a confined space and defined phenological window, providing an opportunity to investigate how environmental stress shapes community assembly under managed conditions. By integrating classical dissections with DNA metabarcoding, we characterized a rich arthropod assemblage structured by gall phenology, seasonality, and by pesticide-induced stress. This work contributes novel insights into hemipteran-induced gall systems, a gall-inducer group whose associated arthropod communities have remained largely undocumented compared to the more extensively studied dipteran and hymenopteran galls. These findings advance our understanding of *Phylloxera* gall ecology and suggest that these galls are tractable microcosms for studying community responses.

Finally, in Chapter 4, I demonstrated how farming practices interact with the plant structure and influence herbivore dynamics. Chemical control suppressed Auchenorrhyncha populations unevenly across canopy layers, likely due to limitations in spray coverage in tall tree systems. Hedge pruning promoted Auchenorrhyncha abundance, regardless of pesticides, season or canopy position. These findings contribute to a broader ecological framework for anticipating how management shapes herbivore dynamics and support the development of spatially informed strategies for pest management in pecans.

Taken together, arthropod populations and community responses to environmental stress are complex, can be group-specific, and are mediated by habitat features. This work contributes to ecological frameworks on habitat filtering and disturbance-driven community assembly and offers applied insights for more ecologically informed management strategies in pecans and other perennial tree systems.

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