What Drives Insect Herbivory Patterns in A Sugar Maple Temperate Forest? Bottom-Up and Top-Down Pressures on Insect Defoliators Within and Between Maple Trees

Mahsa Hakimara

A Thesis
In the Department
of
Biology

Presented in Partial Fulfillment of the Requirements
For the Degree of
Doctor of Philosophy (Biology) at
Concordia University
Montreal, Quebec, Canada

December 2024

CONCORDIA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared

By: Mahsa Hakimara

Entitled: What Drives Insect Herbivory Patterns in A Sugar Maple Temperate Forest? Bottom-Up and Top-Down Pressures on Insect Defoliators Within and Between Maple Trees

and submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (*Biology*)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final examining committee:

Dr. Grant E. Brown Chair
Chair's name

Dr. Michael Andreas Eisenring External Examiner
Examiner's name

Dr. James Grant Examiner
Examiner's name

Dr. Jean-Philippe Lessard Examiner
Examiner's name

Dr. Pedro Peres-Neto Examiner
Examiner's name

Dr. Emma Despland Thesis Supervisor
Supervisor's name

Approved by Dr. Robert B. Weladji
Graduate Program Director

Month/day/year Dr. Pascale Sicotte
Dean's name
Faculty name

Abstract

What Drives Insect Herbivory Patterns in A Sugar Maple temperate Forest? Bottom-Up and Top-Down Pressures on Insect Defoliators Within and Between Maple Trees

Mahsa Hakimara, Ph.D.
Concordia University, 2024

Insect herbivory plays a vital role in forest ecosystems, structuring trophic webs and maintaining biodiversity. While dramatic insect outbreaks often dominate attention, low intensity but persistent background herbivory also contributes to ecological processes. This thesis investigates the ecological drivers of background insect herbivory in sugar maple-dominated forests, focusing on within-tree variations, between-tree diversity, and trophic interactions.

Using the "green world" hypothesis as a framework, this study examines how bottom-up and top-down forces shape herbivore populations. Bottom-up pressures from leaf physical traits varied with vertical stratification and light gradients: sunlit canopy leaves exhibited higher thickness, lower specific leaf area (SLA), and lower water content with less herbivory damage, whereas shaded understory leaves showed lower thickness, higher SLA and greater water content with increased herbivory damage. Paradoxically, feeding bioassays revealed that a local lepidopteran herbivore preferred sun-exposed leaves over shaded leaves and had a better performance on sun-exposed leaves. These findings underscore the complex interplay between leaf traits and herbivore behavior, indicating that physical defenses alone cannot fully explain observed patterns of herbivory.

Top-down forces, including predation and parasitism, were further studied. Predation rates varied across vertical gradients but not between saplings under different light conditions, with arthropods dominating shaded understories and birds in sunlit canopies. While higher bird predation may contribute to reduced herbivory in the sun canopy, limited parasitoids data prevented robust conclusions about vertical variation of natural enemies. These spatially variable top-down pressures on herbivore populations deserves further attention.

Comparisons between sugar maple and black maple (*Acer nigrum*) revealed key differences in leaf traits, like tougher leaves and denser trichomes in black maples, but no difference in herbivore communities. This suggests that despite notable trait differences, insect herbivores did not distinguish between the two tree species, likely reflecting their close evolutionary relationship.

By integrating within-tree and between-tree variations with trophic interactions, this thesis provides a comprehensive view of insect herbivory regulation in sugar maple forests. These results refine our understanding of the green world hypothesis by highlighting how spatial variation in plant traits and predator communities shapes herbivore populations. This insight enhances ecological theory and informs forest management strategies.

Acknowledgements

I would like to express my deepest gratitude to Dr. Emma Despland for welcoming me into her lab and supporting me throughout the past four years, a period filled with challenges and growth. I am also immensely grateful to Liane Nowel, the executive director of the Kenauk Institute, where nearly all the data for this study were collected during the summers of 2020 to 2024. Her incredible support in coordinating fieldwork, managing field assistants, and organizing schedules made this research possible.

Thank you to my lab mates over the years, Anne-Sophie Caron, Pamela Yataco Marquez, Sabina Noor, Éric Dexheimer, Bastien Bellemin-Noël, Rosa Alicia Castillo Salazar, Noa Davidai, Ana De Castro Severo De Oliveira, Spencer Schurman, and Erfan Bari for your insights, encouragement, and companionship. A special thanks to my field assistants, Colette Ethier, Isabel Fournier, Marielle Affognon, Lilah Elder, Mariam Mills, Aimee Fraser, and Jessica Turgeon, for the great memories we made climbing trees and trekking through the forest during fieldwork.

To my beloved husband and son, Mohammad Hossein and Mahdiyar, thank you for your unwavering support through every high and low. To my little sister, Mahtab, friends and communities, both in-person and online, thank you for your companionship, laughter, and the much-needed moments to recharge.

This thesis is dedicated to my parents, Mahmonir and Mohammad Reza, who have always encouraged my dreams and made it possible for me to pursue higher education, a privilege they did not have themselves due to challenging incidents happened in my home country.

A Ph.D. journey is supported by a wide scientific community, and I am grateful to the undergraduate students, Marie-eve Jarry, Geovana Demarchi, Milla Shakleva, Amy Badi, Sarah Lorenzetti, Hedieh Niknam, Alin Buruiana, Anthony Dodson and Vanier College intern, Victoria Yip who assisted in the lab and part of field survey. I also wish to thank my friend and

colleague, Paul Savary, for his invaluable help with statistical analysis. My sincere thanks go to my committee members, Dr. Pedro Peres-Neto and Dr. Jean-Philippe Lessard, for their guidance and feedback throughout my Ph.D.

This research was funded by the Kenauk Institute, the Natural Sciences and Engineering Research Council of Canada (NSERC), and SERG International.

Contribution of Authors

I was the principal investigator for all the research work undertaken in this thesis under the supervision of Dr. Emma Despland.

Chapter 2: This chapter resulted in a manuscript which has been published in international peer-reviewed journals, for which I am the corresponding author and is entitled:” *Vertical stratification of leaf physical traits exerts bottom–up pressures on insect herbivory in a sugar maple temperate forest”*

Chapter 3: This chapter also resulted in a manuscript which has been submitted in international peer-reviewed journals, for which I am the corresponding author and is entitled:” *Impact of Light Intensity on Sugar Maple Leaf Physical Traits and Consequences for Caterpillar Preference and Performance*”

Chapter 4: The experiments for this chapter were conducted by me; in the event that the manuscript is accepted for future publication, the authors will be credited in the following order: Hakimara M, Despland E.

Chapter 5: This part has been submitted as a manuscript in a peer-reviewed journal titled:” *Bottom-up pressures on insect herbivory patterns on two closely related maple trees in a temperate forest in Quebec*”. If the manuscript is accepted for future publication, the authors will be credited in the following order: Hakimara M, Despland E.

Table of contents

List of figures

List of Tables

1 Chapter 1: General Introduction

Background herbivory gradually stresses host plants, weakening their physiological resilience and increasing their susceptibility to subsequent biotic and abiotic challenges (Cánovas et al., 2018; Horsley et al., 2002). For sugar maple (*Acer saccharum*), a species of immense ecological, economic, and cultural value in the temperate forests of eastern North America, such herbivory may worsen its decline, particularly when combined with external stressors like climate change (Duchesne et al., 2003; Horsley et al., 2002).

The decline of sugar maple is concerning not only for its ecological significance as a dominant canopy species and habitat provider but also for its economic importance, particularly in maple syrup production (Horsley et al., 2002). Recurring damage from insect herbivores, including caterpillars and stipplers, poses a threat to sugar maples, especially during vulnerable sapling and early growth stages (Thomas et al., 2010; Ulyshen, 2011). While individual herbivory events might seem minor, their cumulative impact over multiple seasons and years can significantly undermine the tree's ability to withstand other disturbances. Investigating the potential role of background herbivory in weakening sugar maples before they encounter severe stressors is therefore critical for the conservation of this iconic species.

The "green world" hypothesis suggests that insect herbivore populations are regulated by a balance of interactions among plants, herbivores, and their natural enemies, resulting in a vegetated, "green" world (Hairston et al., 1960; Wilkinson & Sherratt, 2016). Two key forces are playing role in this regulation: bottom-up forces, derived from the plant's traits that influence herbivore survival and performance, and top-down forces, imposed by predators and parasitoids. The relative strengths of these forces vary across ecosystems and spatial scales, shaping the composition of herbivore communities and the extent of herbivory (Schoonhoven et al., 2006; Schowalter et al., 1986; Ulyshen, 2011).

In temperate forests, bottom-up forces, specifically plant defenses playing a pivotal role in shaping herbivore behavior and fitness (Coley et al., 1985; Coley & Barone, 1996; Fortin & Mauffette, 2002). Physical defenses, such as leaf thickness, toughness and presence of trichomes, create barriers to herbivory, while chemical defenses, including tannins and phenolics can deter feeding (Agrawal, 2007; Levesque et al., 2002). On the other hand, natural enemies like birds, arthropod predators and parasitoids regulate insect herbivore populations thorough enemy avoidance mechanism (Heinrich, 1993; Mooney et al., 2012; Murphy & Loewy, 2015). These pressures can vary significantly across habitats and seasons, introducing further complexity to herbivory dynamics (Singer & Stireman, 2005; Thomas et al., 2010; Ulyshen, 2011; Zvereva et al., 2020). Understanding the interplay of these forces, particularly in the context of sugar maple forests, is essential to unraveling the mechanisms behind herbivore population regulation and their broader ecological impacts.

1.1 Bottom-up Forces: Physical and Nutritional Traits

The role of bottom-up forces in determining herbivory patterns stems from the host plant's ability to optimize leaf traits based on light conditions. According to the leaf economic spectrum, resource-rich environments drive plants to invest in photosynthesis, while resource-poor conditions encourage developing traits that increase survival and defense (Wright et al., 2004). For sugar maples, light intensity is an important determinant of leaf traits, with sunlit leaves in the upper canopy generally exhibiting greater toughness and thickness but higher nutrient content compared to shaded understory leaves (Fortin and Mauffette 2002).

These spatial gradients in physical traits influence herbivore feeding preferences and performance, as evidenced in several studies (Messier et al., 2017; Morrow et al., 2022; Reich, 2014). For example, enhanced structural defenses in sun-exposed leaves may deter feeding, whereas their nutritional value could still support faster herbivore growth under certain conditions (Eisenring et al., 2021). Shaded leaves, in contrast, are often more palatable but may have reduced nutritional content, creating a trade-off that herbivores must navigate. This variability within trees underscores the importance of understanding how vertical stratification linked with light gradient influence bottom-up pressures. Such a bottom-up force can also vary between closely related host plants, as observed in black maple and sugar maple. Although, these plants have been variously considered a subspecies or a separate species, they have noticeably different leaf physical traits that may affect insect herbivory on these hosts (Gabriel, 1990; Skepner, A. P., 1997).

Further insight into these dynamics comes from behavioral experiments, which reveal how herbivores respond to variations in bottom-up forces. For example, preference-performance studies can reveal whether herbivores favor sunlit or shaded leaves and how these preferences align with growth and survival outcomes (Jaenike, 1978; Lill & Marquis, 2001). Such studies are particularly relevant for understanding how caterpillars of different feeding guilds (e.g., exposed feeders vs. shelter builders) navigate trade-offs between nutrition and physical barriers (Seifert et al., 2020; Vidal & Murphy, 2018).

1.2 Top-down Forces: Predators and Parasitoids

While bottom-up forces shape the nutritional and structural landscape of herbivore habitats, top-down forces act as critical regulator on herbivore populations. Predation by ants, birds, and spiders, as well as parasitism by wasps and flies, can exert strong pressure on herbivores, particularly in structurally diverse forests (Low et al., 2014; Vidal & Murphy, 2018; Zvereva et al., 2020). For sugar maples, these forces vary with vertical gradients, with understory saplings showing higher parasitoid abundance than the canopy (Vance et al., 2007).

Predation dynamics also differ by habitat openness and light availability, influencing natural enemy effectiveness. For instance, sunlit foliage may attract visual predators like birds, whereas shaded understory conditions favor ground-based predators like ants and spiders (Zvereva et al., 2020). Investigating these interactions helps clarify the balance of pressures shaping herbivory patterns in sugar maple forests.

1.3 Structural Diversity: Effects on Herbivory Patterns

Vertical stratification, a hallmark of forest ecosystems, introduces microhabitat variability within trees, particularly between the canopy and understory. In sugar maple forests, this stratification results in a dynamic interplay of light intensity, leaf trait and phenology, and herbivory (Murakami et al., 2005; Thomas et al., 2010; Ulyshen, 2011; Zhong-Tao et al., 2024). Sunlit leaves tend to flush later than shaded leaves, affecting herbivores' ability to synchronize feeding with optimal foliage quality (Murakami et al., 2005). Moreover, young saplings in the understory, with lower level of age-related leaf traits and increased palatability, often experience greater herbivory than mature canopy leaves (Thomas et al., 2010).

Despite significant research on vertical stratification in tropical forests, temperate deciduous forests remain understudied in this context (Brehm, 2007; Ulyshen, 2011). This knowledge gap limits our ability to predict how herbivory patterns differ across vertical gradients and how saplings might face different pressures than canopy foliage. By investigating these dynamics, we can uncover the contributions of vertical stratification to overall herbivore diversity and damage patterns.

1.4 Purpose and Objectives

The interplay between bottom-up and top-down forces in sugar maple forests provides an opportunity to assess their relative importance in herbivore population regulation. By examining how leaf traits and natural enemy pressures vary across spatial gradients, this thesis seeks to determine which factors play a stronger role in shaping herbivory patterns. Understanding these dynamics is not only relevant to ecological theory but also critical for forest management, as herbivory-induced stress could contribute to sugar maple decline.

This thesis investigates the drivers of insect herbivory in sugar maple temperate forests by addressing the roles of bottom-up (in chapter 2, 3 and 5) and top-down (in chapter 4) forces across vertical and horizontal gradients. Through four complementary chapters, it explores:

1. Vertical stratification of leaf traits and herbivory (Chapter 2): Understanding how leaf quality changes with height and its effects on herbivore feeding guilds.
2. Caterpillar preference and performance (Chapter 3): Examining how sunlit and shaded leaves influence caterpillar behavior and growth.
3. Natural enemies and predation dynamics (Chapter 4): Assessing how height and light conditions affect parasitoid and predator activity.
4. Comparative herbivory between sugar and black maples (Chapter 5): Investigating species-specific differences in herbivore communities and leaf defenses.

2 Chapter 2: Vertical stratification of leaf physical traits exerts bottom-up pressures on insect herbivory in a sugar maple temperate forest

The following chapter is based on the published manuscript: Hakimara, M., & Despland, E. (2024). Vertical stratification of leaf physical traits exerts bottom–up pressures on insect herbivory in a sugar maple temperate forest, *Insect conservation and diversity*, early view, **https://doi-org.lib-ezproxy.concordia.ca/10.1111/icad.12777**

2.1 Abstract

Do light vertical gradients in temperate forest structure insect herbivore communities? We tested the hypothesis that the increase in light intensity from understory to forest canopy drives differences in leaf physical traits and budburst phenology that impact insect herbivores and thus play a role in structuring both herbivore communities and the leaf damage they cause. Understanding these interactions is essential for addressing knowledge gaps in the dynamics of temperate deciduous forest ecosystems.

Twelve sugar maple (*Acer saccharum*) sites were monitored in southern Quebec, examining insect herbivore patterns from understory saplings to mature tree shaded and sun canopy (where intensity is highest and canopy cover lowest) over the summers of 2020, 2021, and 2022. Additionally, we recorded leaf physical traits and sun exposure.

Our findings revealed that leaf thickness increased along the vertical gradient in 2021, making mature tree leaves in the canopy less favorable to herbivores than understory sapling leaves. Accordingly, we recorded a consistent decrease in insect herbivory damage rates from understory to shaded and to sun canopy in 2020 and 2021, driven by leaf cutters, skeletonizers, stipplers, and leaf miners. These results support our hypothesis that variation in leaf physical traits contributes to the vertical stratification of insect damage. This variation in leaf traits can be linked to light levels or to tree ontogeny. In 2022, the gradient of insect herbivore abundance corroborated the observed damage trends from the previous years. We calculated an average annual herbivory rate of 9.1% of the leaf surface in our study site. Overall, our study highlights the importance of vertical gradients in structuring insect herbivore communities and emphasizes the role of leaf traits in mediating these interactions. In addition, average annual herbivory rate suggests limited evidence supporting a significant contribution of background herbivory to the decline of sugar maple forests.

2.2 Introduction

The pattern of insect herbivore communities varies in relation to heterogeneity in forest structure, linked to differences in bottom-up and top-down forces on herbivorous insects. While predators and parasitoids act as the top-down pressure in controlling herbivore population density, bottom-up forces on insect herbivores arise from plant defensive traits, which can be particularly essential for plant species to maintain themselves in ecosystems in the face of herbivory (Hairston et al., 1960; Schowalter et al., 1986; Singer & Stireman, 2005; Ulyshen, 2011; Wilkinson & Sherratt, 2016). However, much of the variation in plant defensive traits occurs in response to light intensity in line with the leaf economic spectrum, in order to maximize photosynthesis under different conditions (Messier et al., 2017). These physical traits can also influence leaf resistance to herbivores and can therefore spatially structure bottom-up pressures on herbivores along sunlight gradients (Messier et al., 2017; Morrow et al., 2022; Reich, 2014).

The effect of light level on leaf phenology and leaf physical traits is one of the key processes shaping microhabitats in a vertical gradient of deciduous forest trees throughout the season (Murakami et al., 2005; Thomas et al., 2010; Ulyshen, 2011). Sun-exposed leaves in the upper canopy tend to be smaller, drier, and better structurally and chemically defended, resulting in a decline in herbivory damage in upper strata (Murakami et al., 2005; Ulyshen, 2011). Furthermore, saplings in deciduous forests tend to break buds earlier than canopy of mature trees, which is an adaptation to seasonal light availability in the understory. This variation in the phenology of bud burst over the growing season directly impacts leaf quality in each stratum and can increase herbivore damage on saplings (Murakami et al., 2005). Moreover, leaf quality generally declines from spring to summer due to an increase in toughness and tannin content and a decrease in water and nitrogen contents as leaves mature, making spring leaves more favorable for herbivores and emphasizing the importance of budburst phenology (Murakami et al., 2005; Seifert et al., 2021). This spatio-temporal variation in leaf quality, from understory to the canopy level and from spring to summer, can shape herbivore communities and predict patterns of damage they cause .

The vertical stratification effect on insect herbivores has been well-researched in tropical forests and large differences have been recorded in herbivore richness, assemblage and distribution between the canopy and understory (Ashton et al., 2016; Brehm, 2007; Graça et al., 2017; Grimbacher & Stork, 2007; B. Yang et al., 2018). The general pattern in tropical forests seems to show a reduction in insect herbivore defoliation in the sun canopy compared to the shade canopy, and higher herbivory in mature upper canopy than young leaves in understory (Basset, 1991; Lowman, 1992; Zhang et al., 2023). However, knowledge about the spatio-temporal pattern of insect herbivores in deciduous forests in relation to heterogeneity in leaf quality is lacking (Thomas et al., 2010; Ulyshen, 2011; L. H. Yang, 2014) and this knowledge gap limits our understanding of factors shaping biodiversity in temperate forest ecosystems.

A few studies done in deciduous forests show a decline in herbivory damage and richness going up towards the canopy, linking it to the lower leaf quality in upper forest strata (Corff & Marquis, 1999; Murakami et al., 2005; Rowe & Potter, 1996; Seifert et al., 2020; Thomas et al., 2010). However, insects with different feeding guilds do not react to this heterogeneity of resources in the same way. Insect herbivores can be divided into two groups based on their feeding guilds: the shelter builders including miners, rollers and gall makers in the first group are predicted to survive better in harsh condition in the upper canopy, while the exposed feeders composed of cutters, skeletonisers and stippler insects in the second group are expected to prefer the lower canopy (Seifert et al., 2020; Thomas et al., 2010).

We aimed to study this vertical stratified leaf traits effect on insect herbivore community on sugar maples (*Acer saccharum* Marsh) as a crucial species in Eastern Canadian temperate forests, serving as a keystone species and playing a significant role in the local economy and culture (Horsley et al., 2002). Sugar maple is a shade-tolerant tree species that typically grows in late-succession stages, regenerating under a forest canopy. Young sugar maple trees can spend decades in the understory before a gap opens up and allows them to grow to the canopy (Bray, 1956; Godman et al., 1990). This ontogenetic journey from shade to light is crucial for understanding how young saplings grow into mature canopy trees. During this transition, saplings experience different environmental conditions, influencing their vulnerability to herbivore damage. Previous work suggests that leaves of these young trees are more vulnerable to herbivore damage than those in the canopy of mature trees (Thomas et al., 2010).

Since the late 1970s, there has been a significant decline in sugar maple dominated deciduous forests, and insect defoliation has been identified as one of the factors contributing to this decline (Côté & Ouimet, 1996; Duchesne et al., 2003; Horsley et al., 2002; Payette et al., 1996). Outbreaking defoliators can cause crown deterioration, decreased radial growth and even mortality in sugar maples that are also under abiotic stress (Horsley et al., 2002; Johns et al., 2016). However, background herbivory, which results in mild but continuous damage on host plants, can also cause a progressive weakening in trees, contribute to dieback and increase tree susceptibility to the next abiotic or biotic stress (Cánovas et al., 2018; Horsley et al., 2002). To understand the extent to which this background herbivory could contribute to sugar maple decline, we need to first study insect herbivore community on these maple trees.

To this end, our study examines vertical stratification of insect herbivory damage on sugar maples in an eastern Canadian temperate forest, to understand how it varies between different herbivore guilds and through the seasons and testing the role of bottom-up forces linked to plant ontological and phenological traits in shaping this pattern. Based on previous literature, we hypothesize that leaves become less palatable and flush out later along a vertical gradient from understory to the shade and sun canopy strata, leading to lower overall insect herbivory damage and reduced abundance of insect herbivores in the sun canopy. Additionally, we expect to observe variations in damage patterns among herbivores belonging to different feeding guilds.

2.3 Materials and Methods

2.3.1 *Study site and plant species*

We conducted our sampling at the Kenauk Nature property, an old-growth natural forest located in southwestern Quebec, Canada (45°42'N; 74°53'W). The property spans two regions from the Outaouais river valley to the Laurentian Mountains. Based on the provided domains map by the Quebec Ministry of Forests, it is in a maple/bitternut hickory domain of temperate deciduous forest (Quebec Ministry of Forests, 2023). To account for the variation in insect herbivory throughout the growing season, we conducted three sampling sessions at the end of June, the end of July, and the middle of August in 2020, 2021, and 2022. During each session, we sampled the same 12 sites. Aside from two sites where the distance between trees was 18 meters, the rest were over 40 meters apart, with a maximum distance of 9 kilometers between some sites. Tree species composition was relatively uniform in all sites. These sites were selected based on the similarity in tree size and safety considerations for climbing. Each site comprised one mature sugar maple tree and one sapling. We collected samples from the branches on both the sunlit and shaded canopy strata of the mature trees, as well as from the branches of saplings in the understory. Canopy branches were sampled at a height of 10-13 meters above ground on mature trees averaging around 16 meters in height, and the understory branches were defined as those on surrounding sugar maple saplings ranging from 1-3 meters in height. To differentiate branches in the shaded canopy from those in the sun canopy, we measured light intensity on the targeted branches, to make sure the average amount of light shows a difference between shaded and sunlit branches. No serious outbreaks of herbivorous insects were observed throughout the study duration. For visual clarity and to present all sampling events and their respective timings in one place, an infographic has been included as Figure 2.1.

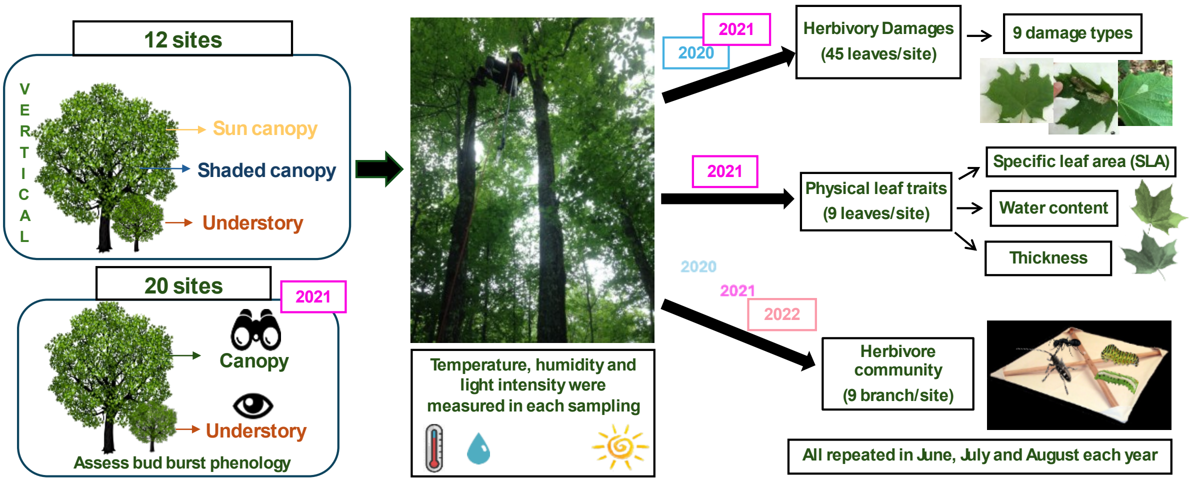


Figure 2.1 Infographic detailing the data collection methods used in the study from 2020 to 2022 in Kenauk nature. This graphic provides an overview of the experimental setup, timeline, data collection techniques, and key variables measured. Insect herbivory data collected in 2020 and 2021 are depicted with dashed-line borders on the arrow, indicating that they were excluded from the analysis due to a low number of samples.

2.3.2 *Assessment of insect herbivore damage*

Leaf herbivory damage is recognized as a good measure of the structure of insect herbivore communities and their interactions with host plants (Schowalter, 2006; Schowalter et al., 1986). Among the methods of assessing leaf herbivory damage, visual assessments are accurate enough for comparative analysis (M. T. J. Johnson et al., 2016; Landsberg, 1989). In this study, herbivory damage was visually quantified as the percentage of leaf area damaged by different insect feeding guilds (Houston et al., 1990; M. T. J. Johnson et al., 2016; Thomas et al., 2010; Wint, 1983). We averaged the damage scores for 15 fully expanded leaves per stratum per site in both 2020 and 2021. Nine insect herbivory damage types identified on sugar maples (Thomas et al., 2010) were assessed, including leaf rollers, leaf skeletonisers, leaf cutters, leaf stippling, spindle galls, erineum gall, ocellate gall, maple leaf-cutter miner and maple leaf-blotch miner (Figure 2.2). Trained interns conducted assessments using the guidebook by Houston et al. (1990), which features clear images of insect herbivores induced damage on sugar maple leaves (Houston et al., 1990). During assessments, each leaf was visually divided into four parts, and the percentage of observed damage per part was estimated. The sum of these percentages yielded the total percentage of a particular damage type on the leaf. To ensure consistency in assessment methods, subsequent interns received supervision from the previous year's intern, and the first 20-30 leaves were assessed together until their estimations reached the closest possible approximation. To capture both spatial and temporal effects on herbivory damage, we randomly collected 15 leaves per stratum per site at three sampling sessions (June, July, and August). In total 1620 leaves were collected (12 sites*3 strata*15 leaves*3 sampling dates)(Schowalter et al., 1986; Turcotte et al., 2014; Zehnder et al., 2009). For understory, leaves were collected by hand from accessible branches on the ground. Canopy leaves were accessed using single rope technique and climbing gear and were cut with a two-meter pole pruner; shaded leaves were collected from mid-center canopy branches under near-complete canopy cover receiving less light, while sunlit leaves were sampled from higher and more marginal canopy branches with less than 50% canopy cover to ensure greater sun exposure. Due to the limitations of single rope techniques, sampling was often confined to the inner section of the tree crown (Basset, 1991; Maguire et al., 2014; Thomas et al., 2010). Environmental factors such as light intensity, temperature, and humidity were recorded at each sampled branch during the three sampling sessions to confirm microhabitats differences between branches in sun-exposed canopies, shaded canopies, and understory. Light intensity was measured using a Reed light meter model R1930, while temperature and humidity were recorded using a Reed temperature and humidity monitor model R6000.

2.3.3 *Measurement of leaf physical traits and phenology of leaf bud burst*

In 2021, we evaluated the effect of microhabitat heterogeneity on leaf quality by collecting 36 undamaged, fully expanded leaves per stratum per sampling date (total of 108 leaves per stratum=3 leaves* 12 sites* 3 sampling dates). Sampling size was determined based on previous studies that measured leaf traits in a vertical gradient of deciduous forests (Corff & Marquis, 1999; Fortin & Mauffette, 2002; Murakami et al., 2005; Zvereva et al., 2020). The leaf sampling was synchronized with the herbivore damage sampling. Fresh leaves were weighed on-site, and their thickness was measured using a digital caliper. Subsequently, the leaves were pressed, labeled, transported to the lab, and dried in an oven for 72 hours. Dried leaves were then weighed again, and all leaves were scanned to measure leaf area using ImageJ software. All efforts were made to ensure that dried leaves remain intact and free from any folding or breaking to maintain measurement accuracy. This allowed us to determine water content and specific leaf area (SLA) for all sampled leaves, facilitating the comparison of leaf traits variation across the three strata.

In 2022, we conducted a comparative study on the phenology of leaf-flush between the understory (sugar maple saplings) and the canopy (mature sugar maples trees). On May 10, 2022, we recorded the bud burst ranking by visually inspecting 10 buds on a single sugar maple sapling with the naked eye, and 10 buds on the canopy of mature trees adjacent to the selected sapling using binoculars. The bud burst ranking was assessed on a scale of 0 to 30; with 0 representing small buds, 10 indicating swollen buds, 20 indicating small leaves, and 30 indicating fully expanded leaves (Hannerz, 1999). This recording process was repeated at 20 sites which were distinct from the 12 sites used for herbivory damage and leaf trait measurements but were located in close proximity to those sampled sites and exhibit similar tree species composition. Each site was spaced at least 10 meters apart and included one mature sugar maple with a height of 10-15 meters to assess canopy level bud burst timing and one sugar maple sapling with a height of 1-3 meters representing the understory.

2.3.4 *Insect herbivore species identification*

To establish a link between the observed herbivory damage types to the actual insect herbivore community on sugar maple trees, we employed the beat sheeting method to collect insects from all three strata at the same 12 sugar maple sites used for herbivory damage assessments. Using the beat sheeting method, three branches from each stratum were struck with a one-meter length stick, while an 85 cm* 85 cm sheet with a detachable jar at the center was positioned beneath the branch. All organisms that dislodged onto the sheet were carefully collected in the jar. Subsequently, the jars containing samples from the canopy strata were lowered to the ground and filled with 70% ethanol to prevent predation on the herbivores within the container. Upon retrieval, the samples were sorted into morphotypes and identified to the lowest possible taxonomic level in the lab. Each sample was meticulously labeled, pinned, and deposited at the Lyman Entomological Museum in St-Anne-de-Bellevue, QC, Canada. Although sampling was conducted during the summer of 2020, 2021 and 2022, repeated three times over the sampling season (June, July and August), technical issues resulted in the collection of fewer than 20 individual insect herbivores in the first two years, which were subsequently excluded from the analysis. Therefore, only data from 2022 was considered for analysis.

2.4 Data analysis

All the statistical analyses were completed using the R program and RStudio version 2024.04.1 Graphical representations of the overall and separate damage types, leaf traits, and insect herbivore abundance were all produced using the ggplot2 package in R (Wickham, 2016).

2.4.1 *Insect herbivory damage*

The recorded percentages for nine insect herbivory damage types were summed to obtain the total herbivory damage for each leaf. This total damage was then averaged for all 15 leaves providing an estimate of total herbivory damage on each stratum per sampling month. The total and individual percentage damage data were used as response variables for both years. No correlation was observed between damage types in the exploratory data analysis. Given the presence of response variables with percentage values and some zero-inflated damage types, as well as the inclusion of site as random factor, we opted to use generalised mixed models with glmmTMB package (Brooks et al., 2017). The first model with beta distribution included the effects of forest strata (sun canopy, shaded canopy, understory), the time of sampling (late June, late July, and mid-August), and their interaction on insect herbivore damage. Since no interaction was detected, we simplified the model by excluding the interaction between tree strata and sampling time. The total damage types, leaf cutters, leaf skeletonisers and leaf stipplers all followed beta distributions, and so we utilised the beta family in our model. However, the less common damage types such as leaf rollers, gall makers and leaf miners included more than 50 % zero values, fitted in a model with the zero-inflated beta family. As no significant trend was shown over tree strata in individual gall maker and individual leaf miner damage types, these were pooled over similar feeding guilds. Therefore, the sum of the percentage of gall damage (spindle gall, erineum gall and ocellate gall) and the sum of the percentage of leaf miners (maple leaf-cutter and maple leaf-blotch miner) were used in the final statistical analysis using the zero-inflated beta family in glmmTMB model. All model assumptions were evaluated by DHARMa package (Hartig, 2022). Separate models were built for damage types in each sampling year.

2.4.2 *Leaf physical traits and phenology of leaf bud burst*

To observe the trend in leaf traits in 2021 data set, including specific leaf area, thickness, and water content across the vertical strata of sugar maple trees over the sampling season, we used a linear mixed model with lme 4 package including tree stratum and sampling month as fixed factors and site as a random factor (Bates et al., 2015).

Since the leaf trait data were collected at the same time and the same sites with herbivory damage data in 2021, it enabled us to perform redundancy analysis (RDA) to model our damage type response variables as a function of predictor variables including leaf traits and tree strata. RDA results are shown in a correlation triplot with leaf traits as quantitative explanatory variable shown by arrows, strata as qualitative explanatory variables demonstrated by circles, and our damage trait response variables shown by lines (Zuur et al., 2007).

Bud burst phenology of understory and canopy leaves were recorded in 2022 but from different sites from the other leaf traits, therefore it was not included in the RDA analysis. Instead, to test the difference between bud burst phenology of those two strata, we conducted a Welch's t-test for unequal variances (Welch, 1938).

2.4.3 *Insect herbivore community*

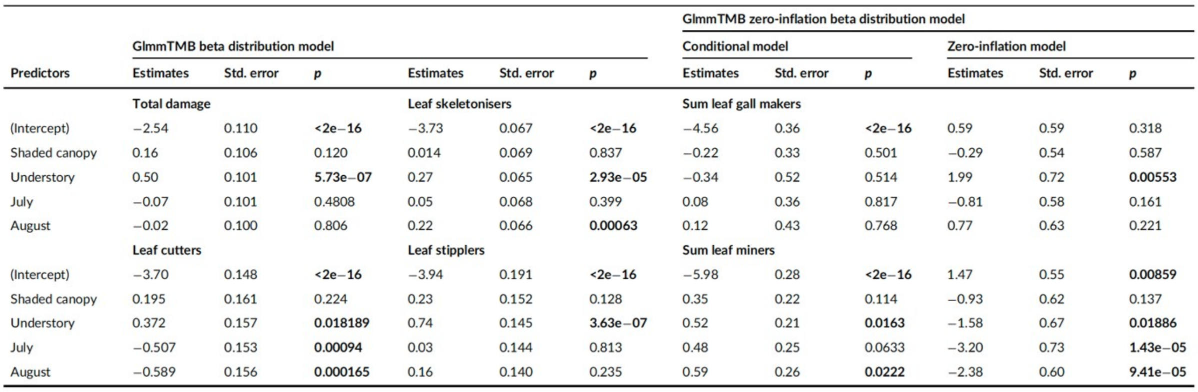
The variation in overall abundance of insect herbivores and abundance of various feeding guilds was assessed across a vertical gradient and over the growing season using generalised linear mixed model regression with glmmTMB package, with site as a random factor (Brooks et al., 2017). Due to low abundance of leaf skeletonisers and rollers, they were not included in statistical analysis. Total abundance of insect herbivores, leaf cutters and leaf stipplers were fit in the model with gaussian family. Models were simplified when no significant interaction between forest level and sampling date was observed. All model assumptions were evaluated by DHARMa package (Hartig, 2022).

2.5 Results

2.5.1 *Insect herbivore damage*

In 2020, there was a significant increase in overall insect herbivory damage on leaves from sun canopy toward the shade canopy and understory (z = 5.00, P<0.001) (Figure 2.2a and b; Table 2.1; Figure S 2.1). While sampling periods and interaction between strata and sampling date did not significantly affect total herbivory damage on leaves, certain individual damage types exhibited notable patterns. Specifically, damage from leaf skeletonisers (z =3.43, P<0.001) and miners (z = 2.28, P=0.022) significantly increased toward the end of the season, whereas leaf cutter damage (z =-4.17, P<0.001) showed the opposite pattern (Figure 2.2b; Table 2.1; Figure S 2.1). Regarding vertical stratification, damage caused by leaf cutters (z =2.36, P=0.018), leaf skeletonisers (z =4.21, P<0.001), leaf stipplers (z = 5.08, P<0.001), and leaf miners (z =2.40, P=0.016) were significantly higher in the understory compared to the sun canopy. However, gall makers showed a reverse pattern, with significantly higher probability of showing zero values for damage in the understory (z =-2.77, P=0.005) than the canopy (Figure 2.2a; Table 2.1; Figure S 2.1).

Table 2.1 Generalized linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) and sampling period on insect herbivore damage of 12 sugar maple trees at Kenauk nature in 2020.



							GlmmTMB zero-inflation beta distribution model					
	GlmmTMB beta distribution model						Conditional model			Zero-inflation model		
Predictors	Estimates	Std. error	*p*	Estimates	Std. error	*p*	Estimates	Std. error	*p*	Estimates	Std. error	*p*
	Total damage			**Leaf skeletonisers**			**Sum leaf gall makers**					
(Intercept)	−2.54	0.110	**<2e−16**	−3.73	0.067	**<2e−16**	−4.56	0.36	**<2e−16**	0.59	0.59	0.318
Shaded canopy	0.16	0.106	0.120	0.014	0.069	0.837	−0.22	0.33	0.501	−0.29	0.54	0.587
Understory	0.50	0.101	**5.73e−07**	0.27	0.065	**2.93e−05**	−0.34	0.52	0.514	1.99	0.72	**0.00553**
July	−0.07	0.101	0.4808	0.05	0.068	0.399	0.08	0.36	0.817	−0.81	0.58	0.161
August	−0.02	0.100	0.806	0.22	0.066	**0.00063**	0.12	0.43	0.768	0.77	0.63	0.221
	Leaf cutters			**Leaf stipplers**			**Sum leaf miners**					
(Intercept)	−3.70	0.148	**<2e−16**	−3.94	0.191	**<2e−16**	−5.98	0.28	**<2e−16**	1.47	0.55	**0.00859**
Shaded canopy	0.195	0.161	0.224	0.23	0.152	0.128	0.35	0.22	0.114	−0.93	0.62	0.137
Understory	0.372	0.157	**0.018189**	0.74	0.145	**3.63e−07**	0.52	0.21	**0.0163**	−1.58	0.67	**0.01886**
July	−0.507	0.153	**0.00094**	0.03	0.144	0.813	0.48	0.25	0.0633	−3.20	0.73	**1.43e−05**
August	−0.589	0.156	**0.000165**	0.16	0.140	0.235	0.59	0.26	**0.0222**	−2.38	0.60	**9.41e−05**

Note: The table distinguishes between models with a beta distribution and zero-inflated beta distribution. The results include estimates, standard errors, and p-values for different predictors. Intercept is sun canopy strata. Leaf cutter damage, leaf skeletonisers and leaf stipplers, as well as total damage with beta distribution fit in the model: **GlmmTMB (leaf damage ~ tree strata + sampling date + (1|site), family = beta_family ((link="logit"), data).** The sum of leaf gall maker and the sum of leaf miner damage with zero inflated beta distribution fit in model: **GlmmTMB (leaf damage ~ tree strata + sampling date + (1|site), ziformula=~ tree strata + sampling date + (1|site), family = beta_family(), data).** The conditional model results account for the predictors' effects on leaf damage considering only the non-zero proportions. The zero-inflated model results account for how the predictors affect the probability of having zero damage. Leaf roller damage showed no significant change over strata and Sampling time so the results for that are not included here. Significant effects are indicated in bold.

In 2021, total damage did not show any significant changes across a vertical gradient of sugar maple trees. However, herbivory damage significantly increased towards the third sampling period (z =3.42, P<0.001), with no interaction between strata and sampling period (Figure 2.2c and d; Table 2.2; Figure S 2.2). Damage caused by leaf stipplers (z =6.87, P<0.001), leaf miners (z =2.93, P=0.00331) and leaf rollers (z= 7.71, P<0.001) increased towards the last sampling date, with leaf rollers having significantly higher probability of showing zeros values in the second sampling period (z=-2.11, P=0.03489), while leaf skeletonisers caused more damage in the early season (z =-2.33, P=0.019) (Figure 2.2d; Table 2.2; Figure S 2.2). Significant vertical stratification was observed in damage caused by leaf cutters (z =2.53, P=0.011), leaf skeletonisers (z =2.06, P=0.039) and rollers (z= 3.94, P<0.001) with higher values in the understory compared to the canopy level (Figure 2.2c; Table 2.2; Figure S 2.2). Box plots for total herbivory and individual damage types for both sampling years are included in the supporting information.

Table 2.2 Generalized linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) and sampling period on insect herbivore damage of 12 sugar maple trees at Kenauk nature in 2021.

							GlmmTMB zero-inflation beta distribution model					
	GlmmTMB beta distribution model						Conditional model			Zero-inflation model		
Predictors	Estimates	Std. error	*p*	Estimates	Std. error	*p*	Estimates	Std. error	*p*	Estimates	Std. error	*p*
	Total damage			Leaf skeletonisers			Leaf rollers					
(Intercept)	−2.45	0.108	**<2e−16**	−4.011	0.116	**<2e−16**	−8.32	0.66	**<2e−16**	2.93	0.86	**0.00067**
Shaded canopy	−0.037	0.097	0.69	0.010	0.109	0.924	−1.56	0.61	**0.0105**	1.865e−08	0.77	1.00
Understory	0.140	0.094	0.13	0.217	0.105	**0.039**	1.71	0.43	**8.12e−05**	−0.27	0.74	0.710
July	−0.008	0.098	0.92	−0.195	0.105	0.064	1.49	0.53	**0.00496**	−1.73	0.82	**0.03489**
August	0.307	0.093	**0.00105**	−0.254	0.108	**0.0196**	3.93	0.50	**1.23e−14**	−1.834e−07	1.02	1.00
	Leaf cutters			Leaf stipplers			Sum leaf miners					
(Intercept)	−3.69	0.16	**<2e−16**	−3.90	0.157	**<2e−16**	−6.07	0.31	**<2e−16**	2.07	0.67	**0.00224**
Shaded canopy	−0.021	0.13	0.869	−0.04	0.113	0.6833	−0.17	0.19	0.350	−0.81	0.75	0.279
Understory	0.314	0.12	**0.0113**	0.13	0.110	0.2397	0.11	0.17	0.533	−1.37	0.78	0.079
July	−0.018	0.12	0.8841	0.022	0.123	0.628	0.50	0.30	0.09878	−3.28	0.67	**1.38e−06**
August	−0.014	0.12	0.9056	0.78	0.114	**6.26e−12**	0.87	0.29	**0.00331**	−5.04	1.13	**7.99e−06**

Note: The table distinguishes between models with a beta distribution and zero-inflated beta distribution. The results include estimates, standard errors, and p-values for different predictors. Intercept is sun canopy strata. Leaf cutter damage, leaf skeletonisers and leaf stippleres, as well as total damage with beta distribution fit in the model: **GlmmTMB (leaf damage ~ tree strata + sampling date + (1|site), family = beta_family ((link="logit"), data).** The leaf rollers and the sum of leaf miner damage with zero-inflated beta distribution fit in model: **GlmmTMB (leaf damage ~ tree strata + sampling date + (1|site), ziformula=~ tree strata + sampling date + (1|site), family = beta_family(), data).** The conditional model results account for the predictors' effects on leaf damage considering only the non-zero proportions. The zero-inflated model results account for how the predictors affect the probability of having zero damage. Sum of leaf gall maker showed no significant change over strata and sampling timeso the results for that are not included here.Significant effects are indicated in bold.

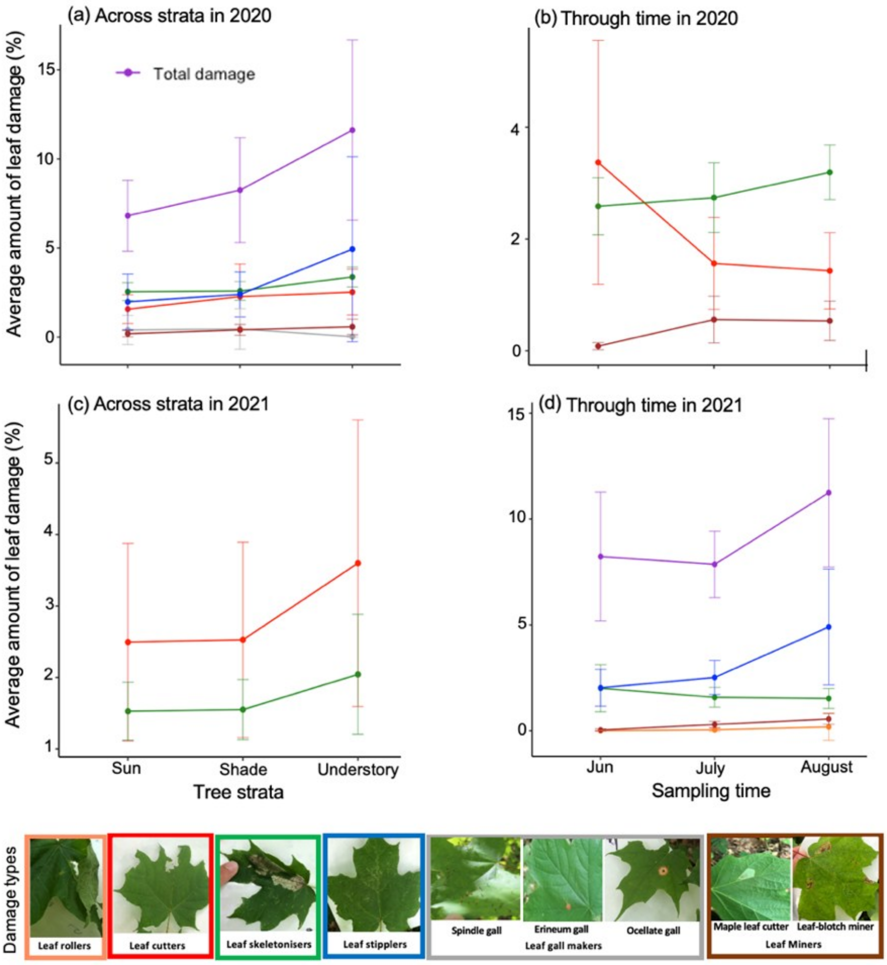


Figure 2.2 Line plots showing the variation in percentage of insect herbivory damage on leaf surface over three strata (sun leaves, shaded leaves, and understory) of sugar maple trees and through the sampling season (June, July and August) in 2020 and 2021(n= 180 leaves per stratum per sampling date = 1620). Only the damage types that significantly change through time and over vertical gradient are presented in these graphs. Error bars represent standard deviation (SD). a) Insect herbivory damage over three vertical strata of sugar maple trees in 2020, b) Insect herbivory damage through the sampling season in 2020, c) Insect herbivory damage over three vertical strata of sugar maple trees in 2021 and d) Insect herbivory damage through the sampling season in 2021.

Amongst the three environmental factors measured for three strata of sugar maple trees in 2021, including temperature, humidity, and light intensity, only light intensity showed a statistically significant difference between three strata (df=96, t =-9.63, P<0.001) with average value of 1120± SE 191 lux in the understory, 1363± SE 118 lux in the shaded canopy and 3187± SE 238 lux in the sun canopy for all three sampling sessions.

2.5.2 *Insect herbivory damage - leaf traits correlation*

Results showed a consistent seasonal pattern of change in leaf physical traits: SLA, water content, and thickness all decreased over the course of the season. This trend was consistent across all tree strata and was not affected by interactions between strata and sampling time (Figure 2.3; Table S 2.1). Additionally, we found significant vertical variation in leaf traits with lower leaf thickness (df=96, t =-7.33, P<0.001), higher SLA (df=96, t = 15.51, P<0.001) and higher water content (df=96, t =6.23, P<0.001) observed in the understory compared to the canopy level (Figure 2.3; Table S 2.1). Detailed result table from the linear mixed model analysis of the effects of strata and time on leaf traits is provided in supporting information.

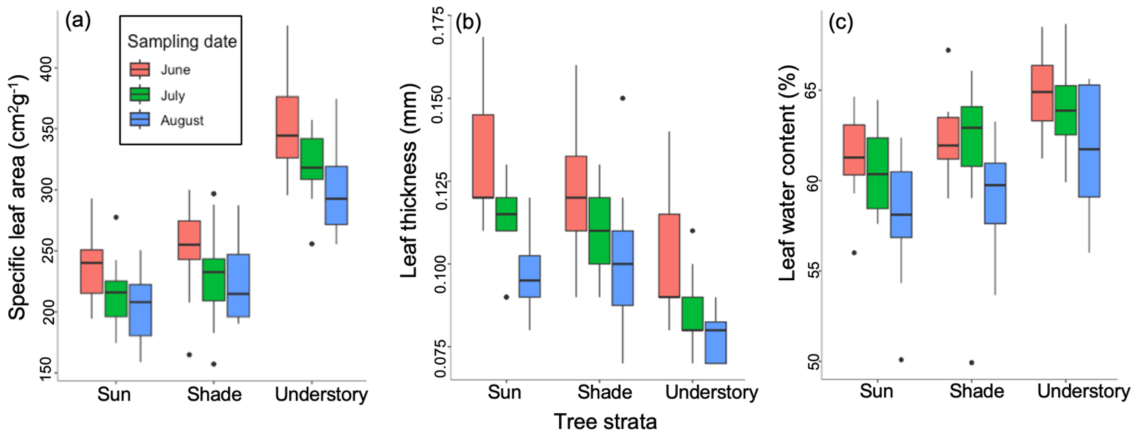


Figure 2.3 Boxplots showing variation of three measured leaf physical traits over three strata (sun leaves, shaded leaves and understory) of 12 sugar maple trees and through sampling season (June, July and August) in 2021 (n= 36 leaves per

stratum per sampling date) a) SLA on three strata over the sampling dates, b) Leaf thickness on three strata over the sampling dates and c) Leaf water content on three strata over the sampling dates.

The Welch's test results of bud burst phenology recorded on sugar maple saplings and the canopy of mature trees indicate a statistically significant difference between the two groups (df=23.74, t=-7.96, P<0.001). On average, sapling exhibited a leaf flush ranking of 15.13± SE 0.40 (indicating the observation of very small leaves), while the canopy of mature sugar maples had an average ranking of 11.71± SE 0.14 (indicating the presence of swollen buds).

The redundancy analysis (RDA) between herbivore damage and leaf traits demonstrated that the first two axes of the ordination explained a total of 16% of the variation in the data. Axis one explained 13.79%of the variation, primarily driven by positive correlation with SLA and water content, and a negative correlation with leaf thickness. Similarly, axis 2 explained 2.17% of the variation, with positive correlations with SLA and water content, and a negative correlation with leaf thickness (Figure 2.4; Table 2.3).

Table 2.3 Redundancy analysis (RDA) results showing the relation between leaf physical traits (thickness, water content, SLA) and insect herbivore damage on tree strata of 12 sugar maple sites at Kenauk Institute in 2021.

	RDA1	**RDA2**
Variance explained by each constrained axis		
Eigenvalue	0.00561	0.0008825
Proportion Explained	0.13789	0.0216913
Cumulative Proportion	0.13789	0.1595785
Explanatory variables score		
Thickness	-0.3229	-0.8563
Water content	0.5721	0.4755
SLA	0.8214	0.5690
Scores for Insect herbivore damage		
Leaf Roller	3.871e-02	1.086e-01
Leaf Cutter	1.220e-01	-6.432e-02
Leaf Skeletoniser	6.137e-02	9.452e-02
Leaf Stippling	7.523e-02	-2.913e-02
Gall Makers	-3.666e-01	-2.351e-03
Leaf Miners	6.586e-02	-1.259e-02
Total Damage	-2.859e-05	-5.434e-05

Note: Canonical axis values show axis contribution to the variance. Constraining variables scores and insect herbivore damage scores indicate how strongly each predictor variables and each damage types are associated with two axes. Damage types with higher scores along a particular axis contribute more to the variation explained by that axis and have strong correlations with the explanatory variables represented by that axis compared to species with lower scores (Model: **rda(formula =leaf damage ~ Thickness + Water content+ SLA, data**).

Analysis of the RDA plot and leaf damage scores (Figure 2.4; Table 2.3) revealed the damage caused by cutters, stipplers, skeletonisers, miners and rollers contributed to the variation explained by axis one in descending order. This suggests that their variation is positively correlated with SLA and leaf water content, and negatively correlated with leaf thickness. Conversely, damage caused by gall makers exhibited a strong negative contribution to axis one, indicating that its variation is negatively associated with SLA and water content, and positively associated with leaf thickness. The Permutation test for RDA under the reduced model indicated that the RDA model approached statistical significance (df= 3, F= 2.043, P=0.052) suggesting that the explanatory variables included in the model collectively contribute to explaining the variation in the response variables.

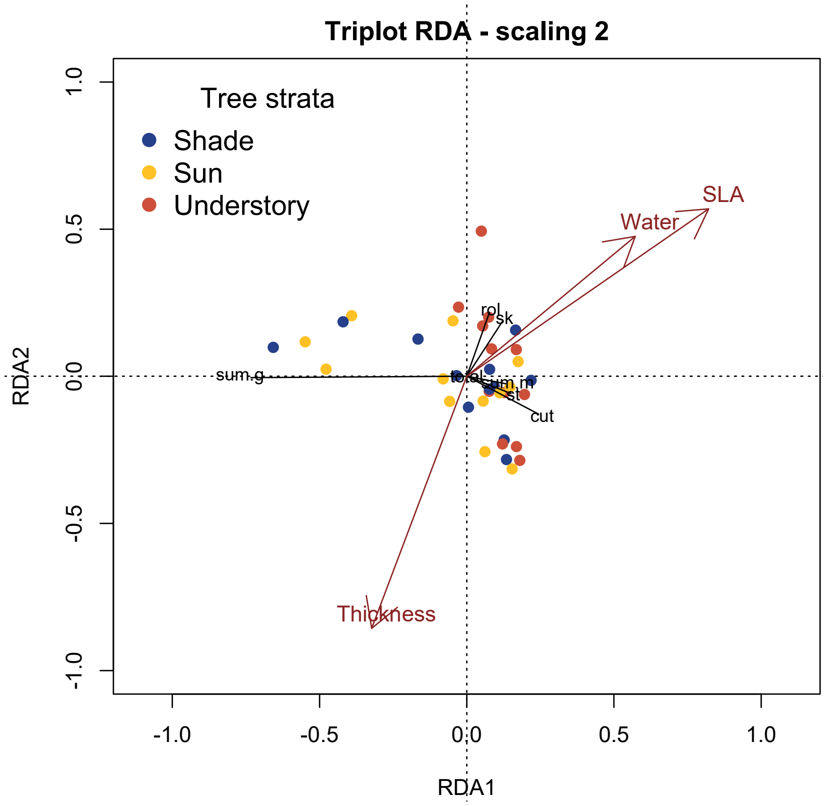


Figure 2.4 Scaling 2 redundancy analysis (RDA) correlation triplot showing the relation between quantitative explanatory variable (sugar maple SLA, leaf water content and leaf thickness demonstrated by brown arrows), the tree strata as the qualitative explanatory variable indicated by colored circles (yellow circles= sun canopy, blue circles= shaded canopy and red circles= understory), and the six different damage types caused by insect herbivores and total herbivory damage on leaves shown by black lines (rol=Leaf roller, cut=Leaf cutter, sk=leaf skeletoniser, st=leaf stippler, sum.m=all leaf miners, sum.g=all

gall makers and total=total damage). Angles between damage type lines and between leaf trait arrows represent correlation between damage and between leaf traits. Longer arrows mean variable has stronger effect on the variation in the community matrix. Leaf trait arrows pointing in opposite directions of another leaf trait arrow or damage line have a negative relationship with the representative leaf trait and damage type. Arrows and line pointing in the same direction have a positive relationship.

2.5.3 *Insect herbivore community response*

In this study, we collected a total of 604 arthropod individuals across all three strata of sugar maple trees in 2022. Out of these individuals, only 203 were identified as insect herbivores, while the rest consisted primarily of predatory spiders and mites, predatory hemipterans, as well as detrivore Dipterans and Psocopterans, a few fungivore Collembola, and hymenopteran parasitoids. Non-herbivore samples were identified only to the order level. Analysis of the data revealed that the abundance of leaf stippling was slightly higher in understory with no significant difference (Figure 2.5). Model results further indicated that the abundance of leaf cutters ($z = 3.78$, $P<0.001$) and total insect herbivores ($z = 2.474$, $P<0.001$) were significantly higher in the understory. Moreover, the total abundance of insect herbivores exhibits a significant decrease towards the last sampling date, primarily driven by a decline in the abundance of leaf cutters throughout the sampling season (Figure 2.5; Table S 2.2). Detailed general mixed model results of herbivore abundance over time and between strata (Table S 2.2), as well as a complete list of collected insect herbivore species and their abundance values, are presented in supporting information (Table S 2.3).

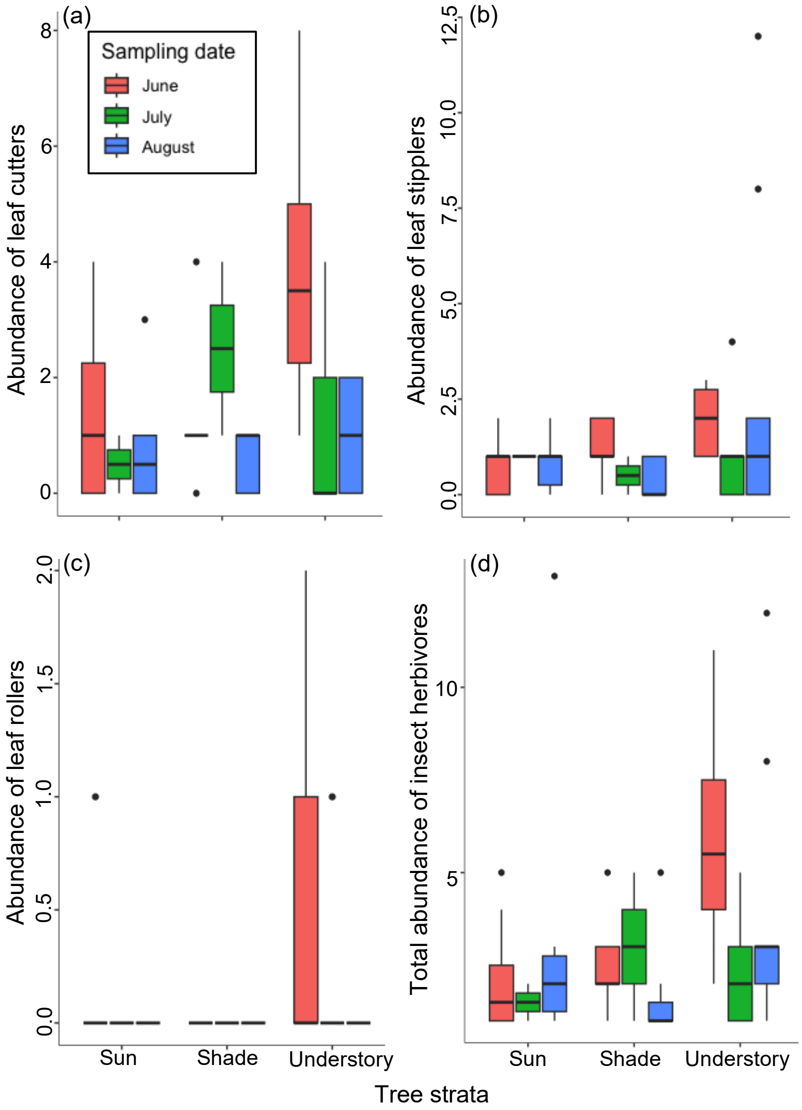


Figure 2.5 Box plots showing the variation of abundance of leaf cutter, leaf stippling, leaf roller and total insect herbivores collected on three strata (understory, shaded canopy, and sun canopy) of 12 sugar maple sites through the sampling season (June, July and August) at Kenauk nature in 2022. a) Variation in abundance of leaf cutter insect herbivores, b) Variation in the abundance of leaf stippling insect herbivores and c) Variation in the abundance of leaf roller insect herbivores d) Variation in the total abundance of insect herbivores.

2.6 Discussion

The results of our generalised mixed effect and RDA models suggest that vertical stratification in herbivore damage is related to variation in morphological traits of sun and shaded canopy on mature trees and understory leaves on sapling. Previous work on sugar maples has shown that sun canopy leaves have stronger physical defenses such as increased toughness and thickness, compared to the more shaded leaves in understory (Fortin and Mauffette 2002). In our study, consistently lower SLA and water content and thicker leaves in sun canopy over the sampling season support the idea that sun leaves have stronger physical defense and that these traits are linked to the lower damage rates observed on sun leaves compared to understory leaves. Low SLA make leaves thicker (Agrawal & Fishbein, 2006) and less palatable to herbivores (Wright et al., 2004). Leaves with lower SLA are suited to upper canopy conditions due to their compact structure, which helps them withstand strong winds and optimizes photosynthesis in high-light environments (Eisenring et al., 2021). However, our result indicates no significant differences in leaf traits and leaf damage between sun and shaded strata on mature three, while clear differences in leaf traits and herbivory damage were observed between saplings in understory and sun leaves on mature trees. This suggests that ontogeny effect may be stronger than light intensity effect on insect herbivory.

The timing of budburst can also influence herbivore damage on leaves in different strata. As expected, bud break in our study occurred earlier in sugar maple saplings than in tree canopies. The timing of bud burst, which depends on weather conditions and light intensity (Seiwa, 1998), has the potential to influence the period when foliage is most nutritionally beneficial for insect herbivores and to contribute to changes in herbivore assemblages (Fortin & Mauffette, 2002). This variation in the phenology of bud burst could lead to higher herbivory in the understory very early in the season, as these are the only leaves available (Murakami et al., 2005), but our sampling did not begin early enough in the season to record any such effect.

Within-species variation in herbivore damage has often been linked to plant defensive strategies, arguing that trees should invest more heavily in defending the most valuable leaves (Eisenring et al., 2021; Fortin & Mauffette, 2002): by this argument, mature trees should defend sun-leaves more than shade leaves (Dudt & Shure, 1994; Eisenring et al., 2021). Our results suggest that a vertical gradient in leaf defense can arise from leaf physical traits in response to photosynthesis, whereby the most valuable photosynthetically active leaves also inherently have highest physical defense without any investment in defense per se. However, these leaves also often have higher nutritional value: in sugar maple, sun leaves have higher sugar and protein content than shade leaves (Fortin & Mauffette, 2002; Levesque et al., 2002; Panzuto et al., 2001). Theory suggests that chemical defenses should be invested mostly in these high value leaves (Dudt & Shure, 1994; Eisenring et al., 2021). However, this is not what is observed in all related literature: in sugar maple, sun leaves contain lower concentrations of phenolics than shade leaves (Fortin & Mauffette, 2002; Levesque et al., 2002). This study showed that physical traits could protect these leaves without additional chemical defense.

Additionally, the vertical gradient implies differences in tree ontogeny, with young trees in understory and only mature trees in the upper canopy (Thomas et al., 2010). Optimal defense theory suggests that saplings with only few leaves should invest more in their defense than many-leaved mature trees (Boege & Marquis, 2006); however, this does not seem to be generally supported (Barton & Koricheva, 2010) and has not been observed in sugar maple (Thomas et al., 2010). Thomas et al. (2010) proposed that the decline in leaf palatability as trees age stems from a combination of selective pressures and physiological effects linked to increased tree size and reproductive investment. Consequently, leaves of mature trees are less susceptible to herbivory damage than the leaves of young saplings. Sugar maple saplings show a survivalist strategy by exhibiting a high root-to-shoot ratio and ceasing shoot growth early in the season (Gaucher et al., 2005); in this context, early budburst phenology is essential to accumulate carbohydrates, but leaf herbivory is perhaps less costly since photosynthetic activity is mostly finished by the time leaf damage occurs.

Other factors that could also contribute to higher leaf protection in sun leaves include direct abiotic effects of high sunlight on insects (e.g. overheating and desiccation) or higher top-down pressure from natural enemies (Zvereva et al., 2020). Habitat heterogeneity linked to vertical stratification affects the interactions between parasitoids and predators and their phytophagous hosts (Sigut et al., 2018; Stireman et al., 2012; Zvereva et al., 2020). Although parasitism and predation rates on herbivores in a vertical gradient in deciduous forests is not well-researched (Sigut et al., 2018; Zvereva et al., 2020), a few studies suggested that stratification exist in both parasitoid and predator communities. We aimed to evaluate this top-down force in our study site as a future project.

Regarding the temporal changes in insect herbivory damage, studies have shown that sugar maple leaf quality changes significantly from spring to summer with an increase in leaf toughness and tannin content and decrease in water and nitrogen contents (Fortin & Mauffette, 2002; Hunter & Lechowicz, 1992; Schultz et al., 1982). This pattern was confirmed in our study with spring leaves having higher SLA and water content. These variations in leaf quality will influence insect herbivores in diverse ways, notably due to differences in their feeding guilds and life histories (Eisenring et al., 2021; Murakami et al., 2005). Spring leaves are preferred by many leaf cutters (Murakami et al., 2005; Zehnder et al., 2009) and we observed higher cutter damage earlier in the season (Figure 2.2). Similarly, leaf roller damage is expected to be higher in the early season, which is not consistent with the result we had in 2021. This type of damage is primarily attributed to *Sparganothis* (Tortricidae) species (Houston et al., 1990; Thomas et al., 2010). We encountered six individuals of these species in our early season collections, but none later. These insects create nest and feed within them until pupating by the end of June (Hébert et al., 2017; Murakami et al., 2005; Thomas et al., 2010). By contrast, leaf skeletoniser damage, as was observed in 2020, increased through August due to the emergence of the maple trumpet skeletoniser larvae (*Castastega aceriella*) at the end of June and its increasing leaf consumption until pupation in September (Hébert et al., 2017; Thomas et al., 2010). These larvae were not yet active during the first sampling done in June. Similarly, leaf miner damage from species like *Paraclemensia acerifoliella*, which produces pale spots on sugar maple leaves, also increased until the end of summer before pupation (Hébert et al., 2017).

Our results overall supported the hypotheses: damage was generally highest in the understory. In 2020, total damage was higher in the understory and shaded canopy leaves, and most damage types, including leaf cutters and skeletonisers showed the same pattern. In 2021, similar patterns were seen in fewer damage types. There was also a strong seasonal pattern of increase in total damage from spring to summer. Looking at the monthly overall average temperature of 2020 and 2021 reported from the Kenauk weather station, it is shown that 2020 had a warmer July with an average of 21.75° C than July 2021 with 18.85° C. This may have contributed to differences in vertical stratification in herbivory patterns in the two years.

Based on a dataset of the percentage of leaf herbivory damage on many vascular plant species (Turcotte et al., 2014), the recorded annual rate of standing insect herbivory damage on sugar maple leaves in natural forests in the US and Canada was 8.4% (N= 6 studies). To record insect herbivory damage in these studies, leaf samples ranged from 30 to 630, and excluded damage caused by gall makers (Turcotte et al., 2014). Another study by Thomas et al. (2010) on sugar maple reported a total insect herbivory damage of 11.5% on leaves and 7.7% insect herbivory damage excluding gall maker damage. This assessment was based on the examination of 1800 leaves exclusively from the canopy level (Thomas et al., 2010). In our study, we observed an average annual insect herbivore damage rate of 9.1% ± SE 0.35 (excluding gall maker damage, the rate was 8.1% ± SE 0.33). Our assessment involved a total of 1620 leaves, collected from both the canopy (1080 leaves) and understory (540 leaves) of sugar maple trees. The recorded annual herbivory damage rate in this study is consistent with the overall rate reported in Turcotte et al.'s dataset, despite the much smaller sample size in those studies. When examining canopy level insect herbivory only, our damage rate of 8.6% is slightly lower than that reported by Thomas et al. (2010).

An annual rate of 9.1% of background herbivory on sugar maples provides limited evidence to support a significant contribution of this type of herbivory to the decline of sugar maple forests.

However, the higher levels of herbivory damage on saplings in the understory (10.5%) could impact growth of these very small trees that already face challenging conditions of low light levels and high competition. In this context, even moderate levels of herbivory could contribute to decreasing sugar maple regeneration (Henry et al., 2021). However, previous research does not support an important role for background herbivory on the growth of sugar maple saplings (Henry et al., 2021; Strong et al., 2000).

Finally, sugar maple supports considerable invertebrate diversity (Maguire et al., 2014; Turgeon, 2019) and the diversity of insect herbivores collected from the understory, including various groups of insects, indicates the significance of this stratum in providing a habitat for herbivorous insects and preserving biodiversity. These insects play a crucial role as key trophic levels affecting forest ecosystem functions and production. While some of these insect herbivores may be pests, high insect diversity tends to decrease the severity of pest outbreaks and to improve forest resilience (Mina et al., 2022; Stemmelen et al., 2022).

2.7 Conclusion

Overall, this study shows that vertical stratification in maple trees affects herbivory patterns, with the most damage in the understory, followed by shaded, then sun leaves in the canopy. This pattern varies among different herbivore feeding guilds: exposed feeders such as skeletonisers and cutters were more abundant in the understory, while gall makers as shelter builders were more present in the sun canopy. Leaf physical traits varied as expected according to the leaf economics spectrum showing greater thickness, lower SLA, lower water content and later phenology with more sun exposure; they were also strong predictors of herbivore damage. These results suggest that a defensive gradient arises from leaf functional traits in response to photosynthesis generating stronger physical defenses in the most photosynthetically valuable sun leaves. Ontogenetic changes as trees grow from saplings in the understory to mature canopy trees, including earlier phenology in the understory than in the canopy, can also contribute to vertical gradients in leaf physical traits. We also show a temporal gradient in leaf physical traits as leaves become thicker and tougher during the growing season, which is linked to a shift in the insect herbivore community. Leaf functional traits in response to light gradients and tree age can thus contribute to structuring patterns of herbivory across vertical microhabitats in a temperate forest.

2.8 Supplementary material

Table S 2.1 Linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) and sampling period on leaf traits of 12 sugar maple trees at Kenauk nature in 2021.

	Leaf thickness			**Leaf water content**			**Specific leaf area**		
Predictors	*Estimates*	*Std. Error*	*p*	*Estimates*	*Std. Error*	*p*	*Estimates*	*Std. Error*	*p*
(Intercept)	0.128	0.0036	**< 2e-16**	61.25	0.679	**< 2e-16**	238.6	7.671	**< 2e-16**
Shaded canopy	-0.003	0.0035	0.388	1.094	0.577	0.0612	16.53	6.80	0.016
Understory	-0.025	0.0035	**7.10e-11**	3.600	0.577	**1.23e-08**	105.5	6.80	**< 2e-16**
July	-0.015	0.0035	**1.94e-05**	-0.740	0.577	0.203	-24.61	6.80	**0.000476**
August	-0.026	0.0035	**3.33e-11**	-3.117	0.577	**4.92e-07**	-36.03	6.80	**7.52e-07**

Note: Leaf thickness, leaf water content and leaf SLA fit in the model: **lmer (leaf trait ~ tree strata + sampling date + (1|site), data).**

Table S 2.2 Generalized linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) and sampling period on insect herbivore abundance on 12 sugar maple trees at Kenauk nature in 2022.

	GlmmTMB gaussian distribution model								
	Total herbivores			**Cutter herbivores**			**Stippling herbivores**		
Predictors	*Estimates*	*Std. Error*	*p*	*Estimates*	*Std. Error*	*p*	*Estimates*	*Std. Error*	*p*
(Intercept)	3.39	0.74	**4.54e-06**	1.62	0.44	**0.000279**	0.93	0.49	0.061
Shaded canopy	-0.47	0.94	0.616	0.24	0.52	0.636	-0.13	0.63	0.935
Understory	1.99	0.80	**0.01335**	1.72	0.45	**0.000154**	0.93	0.54	0.084
July	-2.89	0.90	**0.00131**	-2.00	0.49	**5.49e-05**	-0.72	0.60	0.228
August	-0.99	0.76	0.190	-1.43	0.42	**0.000814**	0.05	0.51	0.909

Note: Total abundance of insect herbivores and leaf cutters fit in the model: **GlmmTMB (total abundance ~ tree strata + sampling date + (1|site), family = gaussian, data)**. No significant change in skeletoniser and roller insects were detected so they are not included in the table. No gall maker and leaf miners were collected.

Table S 2.3 Abundance of Insect herbivore species collected from three strata (sun leaves, shaded leaves, and understory) of 12 sugar maple trees through the sampling season at Kenauk Institute in 2022.

Date	Tree strata	Order/ family	species	feeding guild	Life stage	Method	Abundance
June	Sun	Coleoptera/ Curculionidae	*Otiorhyncus singularis*	cutter	Adult	beat sheet	5
June	Sun	Coleoptera/ Curculionidae	*Phyllobius oblongus*	cutter	Adult	beat sheet	6
June	Sun	Hemiptera/ Aphididae	*Not.Identified*	stippling	Adult	beat sheet	2
June	Sun	Hemiptera/ Miridae	*Not.Identified*	stippling	Nymph	beat sheet	4
June	Sun	Lepidoptera/ Tortricidae	*Sparganothis sp.*	roller	Caterpillar	beat sheet	1
June	Shade	Coleoptera/ Curculionidae	*Otiorhyncus singularis*	cutter	Adult	beat sheet	4
June	Shade	Coleoptera/ Curculionidae	*Phyllobius oblongus*	cutter	Adult	beat sheet	2
June	Shade	Coleoptera/ Elateridae	*Limonius basilaris*	cutter	Adult	beat sheet	1
June	Shade	Hemiptera/ Aphididae	*Not.Identified*	stippling	Adult	beat sheet	2
June	Shade	Hemiptera/ Cicadellidae	*Not.Identified*	stippling	Adult	beat sheet	1
June	Shade	Hemiptera/ Miridae	*Not.Identified*	stippling	Nymph	beat sheet	1
June	Shade	Hemiptera/ Not.identified	*Not.Identified*	stippling	Nymph	beat sheet	2
June	Understory	Coleoptera/ Curculionidae	*Otiorhyncus singularis*	cutter	Adult	by hand	6
June	Understory	Coleoptera/ Curculionidae	*Phyllobius oblongus*	cutter	Adult	beat sheet	4
June	Understory	Coleoptera/ Curculionidae	*Phyllobius oblongus*	cutter	Adult	by hand	2
June	Understory	Coleoptera/ Curculionidae	*Polydrusus sparsus*	cutter	Adult	beat sheet	11
June	Understory	Coleoptera/ Curculionidae	*Polydrusus sparsus*	cutter	Adult	by hand	4
June	Understory	Coleoptera/ Curculionidae	*Pseudanthonomus validus*	cutter	Adult	beat sheet	2
June	Understory	Coleoptera/ Melandryidae	*Spilotus quadripustulatus*	cutter	Adult	beat sheet	1
June	Understory	Coleoptera/ Scarabaeidae	*Phyllophaga sp.*	*cutter*	Adult	by hand	1
June	Understory	Hemiptera/ Aphididae	*Not.Identified*	stippling	Adult	beat sheet/ by hand	6
June	Understory	Hemiptera/ Miridae	*Not.Identified*	stippling	Adult	beat sheet/ by hand	2
June	Understory	Hemiptera/ Miridae	*Not.Identified*	stippling	Nymph	beat sheet/ by hand	8
June	Understory	Hemiptera/ Psyllidae	*Not.Identified*	stippling	Adult	beat sheet/ by hand	3
June	Understory	Lepidoptera/ Erebidae	*Lymantia dispar*	cutter	Caterpillar	beat sheet	1
June	Understory	Lepidoptera/ Geometridae	*Lambdina sp.*	cutter	Adult	beat sheet	1
June	Understory	Lepidoptera/ Geometridae	*Not.Identified*	cutter	Caterpillar	beat sheet	5
June	Understory	Lepidoptera	*Not.Identified*	cutter	Adult	beat sheet	1
June	Understory	Lepidoptera/ Noctuidae	*Not.Identified*	cutter	Caterpillar	beat sheet	4
June	Understory	Lepidoptera/ Tortricidae	*Not.Identified*	roller	Adult	by hand	2
June	Understory	Lepidoptera/ Tortricidae	*Sparganothis sp.*	roller	Adult	by hand	3
June	Understory	Lepidoptera/ Tortricidae	*Sparganothis sp.*	roller	Caterpillar	beat sheet	2
June	Understory	Thysanoptera/ Thripidae	*Not.Identified*	stippling	Adult	by hand	1
July	Sun	Hemiptera/ Aphididae	*Not.Identified*	stippling	Adult	beat sheet	2
July	Sun	Hemiptera/ Miridae	*Not.Identified*	stippling	Adult	beat sheet	1
July	Sun	Lepidoptera/ Noctuidae	*Morrisonia confusa*	cutter	Caterpillar	beat sheet	1

July	Shade	Dermaptera/ Not.Identified	*Not.Identified*	cutter	Adult	beat sheet	1
July	Shade	Hemiptera/ Not.Identified	*Not.Identified*	stippling	Nymph	beat sheet	2
July	Understory	Coleoptera/ Aderidae	*Elonus basalis*	cutter	Adult	beat sheet	1
July	Understory	Coleoptera/ Curculionidae	*Polydrosus sparsus*	cutter	Adult	beat sheet	2
July	Understory	Coleoptera/ Curculionidae	*Pseudanthonomus validus*	cutter	Adult	beat sheet	2
July	Understory	Hemiptera/ Aphididae	*Not.Identified*	stippling	Adult	beat sheet	3
July	Understory	Hemiptera/ Cicadellidae	*Not.Identified*	stippling	Nymph	beat sheet	1
July	Understory	Hemiptera/ Not.Identified	*Not.Identified*	stippling	Adult	beat sheet	1
July	Understory	Hemiptera/ Not.Identified	*Not.Identified*	stippling	Nymph	beat sheet	1
July	Understory	Lepidoptera/ Geometridae	*Alsophila pometaria*	cutter	Caterpillar	beat sheet	2
July	Understory	Lepidoptera/ Geometridae	*Hypagyrtis unipunctat*	cutter	Adult	beat sheet	1
July	Understory	Lepidoptera/ Geometridae	*Melanolophia canadaria*	cutter	Caterpillar	beat sheet	1
July	Understory	Lepidoptera/ Lymantriidae	*Lymantria dispar*	cutter	Caterpillar	beat sheet	1
July	Understory	Lepidoptera/ Tortricidae	*Cenopis pettitana*	roller	Adult	by hand	1
August	Sun	Hemiptera/ Cicadellidae	*Not.Identified*	stippling	Adult	beat sheet	1
August	Sun	Hemiptera/ Cicadellidae	*Not.Identified*	stippling	Nymph	beat sheet	1
August	Sun	Hemiptera/ Cicadellidae	*Typhlocyba pomaria*	stippling	Adult	beat sheet	1
August	Sun	Hemiptera/ Psyllidae	*Not.Identified*	stippling	Adult	beat sheet	2
August	Sun	Lepidoptera/ Lymantriidae	*Orgyia leucostigma*	cutter	Caterpillar	beat sheet	1
August	Sun	Lepidoptera/ Not.Identified	*Not.Identified*	cutter	Caterpillar	beat sheet	1
August	Sun	Lepidoptera/ Noctuidae	*Acronicta americana*	cutter	Caterpillar	beat sheet	1
August	Sun	Lepidoptera/ Tortricidae	*Epinotia aceriella*	skeletoniser	Adult	beat sheet	13
August	Shade	Hemiptera/ Cicadellidae	*Not.Identified*	stippling	Nymph	beat sheet	2
August	Shade	Hemiptera/ Miridae	*Not.Identified*	stippling	Nymph	beat sheet	1
August	Shade	Lepidoptera/ Geometridae	*Alsophila pometaria*	cutter	Caterpillar	beat sheet	1
August	Shade	Lepidoptera/ Geometridae	*Hypagyrtis unipunctata*	cutter	Caterpillar	beat sheet	1
August	Shade	Lepidoptera/ Geometridae	*Not.Identified*	cutter	Caterpillar	beat sheet	2
August	Shade	Lepidoptera/ Lymantriidae	*Orgyia leucostigma*	cutter	Caterpillar	beat sheet	2
August	Shade	Lepidoptera/ Tortricidae	*Epinotia aceriella*	skeletoniser	Adult	beat sheet	5
August	Understory	Coleoptera/ Curculionidae	*Pseudanthonomus validus*	cutter	Adult	beat sheet	4
August	Understory	Hemiptera/ Aphididae	*Not.Identified*	stippling	Adult	beat sheet	2
August	Understory	Hemiptera/ Cicadellidae	*Not.Identified*	stippling	Adult	beat sheet	2
August	Understory	Hemiptera/ Cicadellidae	*Not.Identified*	stippling	Nymph	beat sheet	1
August	Understory	Hemiptera/ Cicadellidae	*Typhlocyba pomaria*	stippling	Adult	beat sheet	19
August	Understory	Hemiptera/ Cicadellidae	*Typhlocyba pomaria*	stippling	Nymph	beat sheet	1
August	Understory	Hemiptera/ Miridae	*Not.Identified*	stippling	Adult	beat sheet	1
August	Understory	Lepidoptera/ Geometridae	*Alsophila pometaria*	cutter	Caterpillar	beat sheet	1

August	Understory	Lepidoptera/ Geometridae	*Ennomos subsignaria*	cutter	Caterpillar	beat sheet	1
August	Understory	Lepidoptera/ Geometridae	*Not.Identified*	cutter	Caterpillar	beat sheet	4
August	Understory	Lepidoptera/ Geometridae	*Plagodis pulveraria*	cutter	Adult	by hand	1
August	Understory	Lepidoptera/ Geometridae	*Plagodis serinaria*	cutter	Adult	by hand	1
August	Understory	Lepidoptera/ Lymantriidae	*Orgyia leucostigma*	cutter	Caterpillar	beat sheet	3
August	Understory	Lepidoptera/ Noctuidae	*Morrisonia confusa*	cutter	Caterpillar	beat sheet	1
August	Understory	Lepidoptera/ Noctuidae	*Not.Identified*	cutter	Caterpillar	beat sheet	1

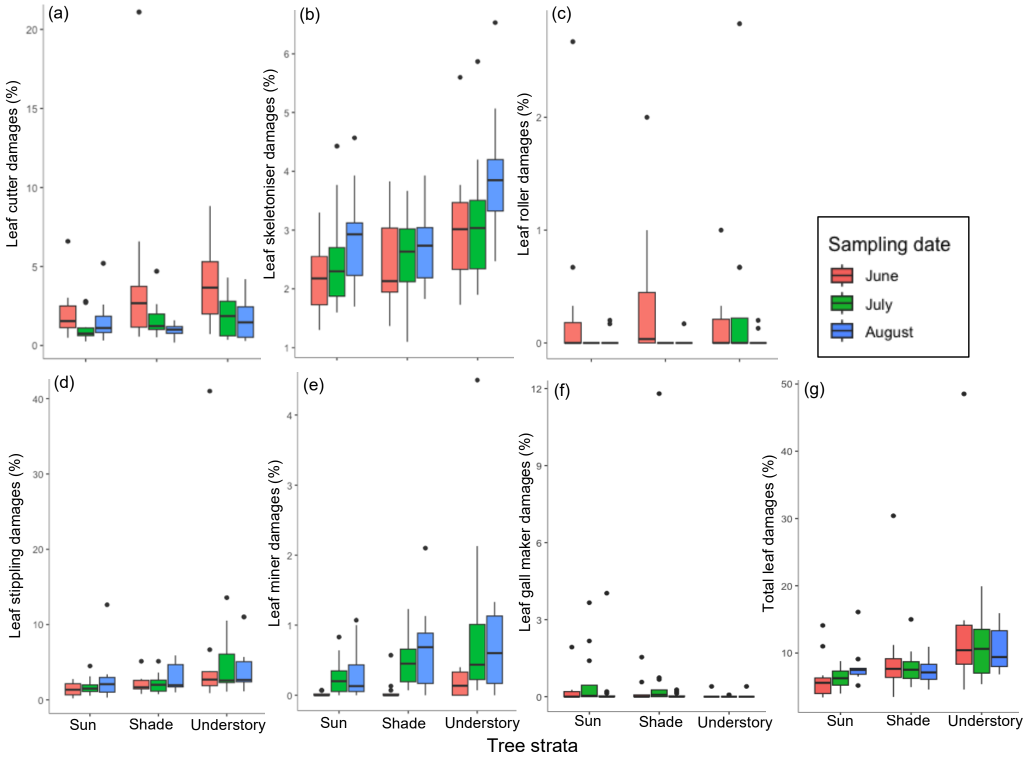


Figure S 2.1 Box plots showing the variation of average percentage of leaf surface damaged by different insect herbivores between three strata (sun leaves, shaded leaves and understory) and through the sampling season on 12 sugar maple trees at Kenauk Institute in 2020 a) average of leaf cutters damage b) average of leaf skeletonisers damage c) average of leaf rollers damage d) average of leaf stippling damage e) average of leaf miners f) average of leaf gall makers and g) average of total insect herbivore damage.

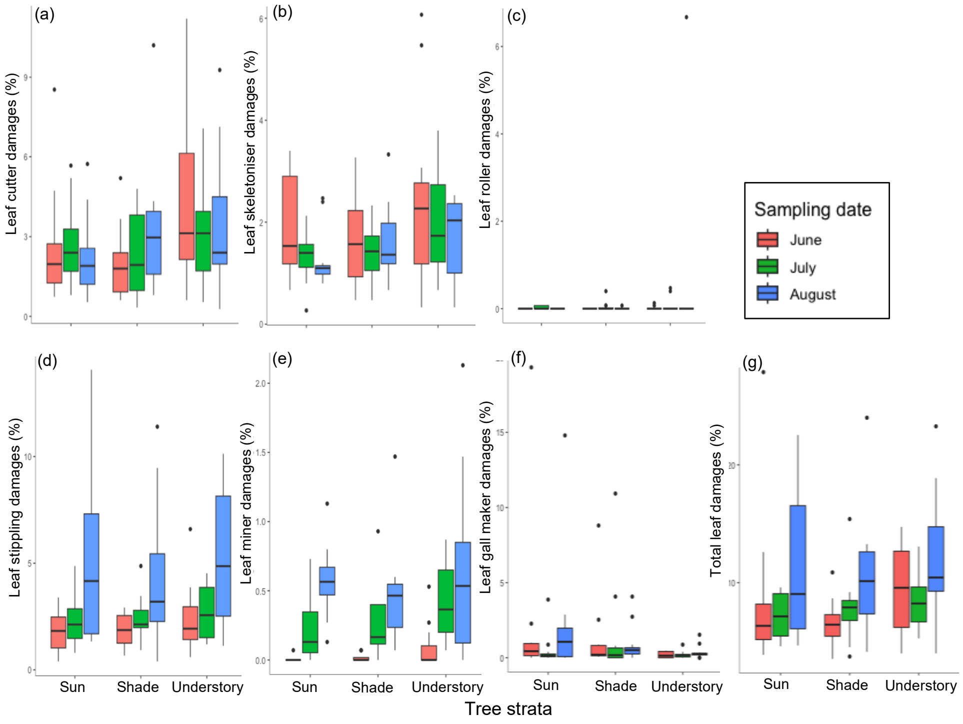


Figure S 2.2 Box plots showing the variation of average percentage of leaf surface damaged by different insect herbivores between three strata (sun leaves, shaded leaves and understory) and through the sampling season on 12 sugar maple trees at Kenauk Institute in 2021 a) average of leaf cutters damage b) average of leaf skeletonisers damage c) average of leaf rollers damage d) average of leaf stippling damage e) average of leaf miners f) average of leaf gall makers and g) average of total insect herbivore damage.

3 Chapter 3: Impact of Light Intensity on Sugar Maple Leaf Physical Traits and Consequences for Caterpillar Preference and Performance

The following chapter is based on the submitted manuscript: Hakimara, M., & Despland, E. (2024). Impact of Light Intensity on Sugar Maple Leaf Physical Traits and Consequences for Caterpillar Preference and Performance, *Entomologia Experimentalis et Applicata.*

3.1 Abstract

This study investigates the impact of light intensity on the physical defenses of sugar maple (*Acer saccharum*) saplings and how these defenses affect interactions with insect herbivores. The study conducted at the Kenauk Nature Reserve in Quebec involved 12 sites representing both shaded and sunlit environments. Leaf traits, including thickness, specific leaf area (SLA), water content, and toughness, were measured from 216 leaves collected in 2021 and 40 in 2022. To explore herbivore interactions, we used two Erebid caterpillar species, the invasive spongy moth (*Lymantria dispar)* and the native white-marked tussock moth (*Orgyia leucostigma*), in controlled bioassays that assessed caterpillar feeding preference and performance.

The results revealed that sunlit leaves were significantly thicker and had lower SLA compared to shaded leaves across both years. While water content was lower in sunlit leaves in 2021, it showed no significant difference in 2022. Leaf toughness, measured only in 2022, did not significantly differ between sunlit and shaded leaves. Caterpillar feeding preference tests indicated that white-marked tussock moth caterpillars consumed more sunlit leaves, showing a significant preference for them. However, spongy moth caterpillars did not exhibit a significant preference. In terms of performance, white-marked tussock moth caterpillars had a higher survival rate and larger pupal mass on sunlit saplings in 2021. However, no significant differences were observed in laboratory feeding initiation assays with first-instar

caterpillars in 2022.

These findings suggest that trees' investment in sun leaves to maximize photosynthesis is not necessarily followed by increased defense against herbivory. This research contributes to our understanding of how environmental factors like light can shape plant defense strategies and impact insect herbivore dynamics in temperate forests.

3.2 Introduction

Intraspecific differences in host plant quality can influence both the preference and performance of herbivorous insects (Gonzáles & Gianoli, 2003). Intra-specific variability, particularly in foliar physical defenses (Choong, 1996; Coley et al., 1985; Murakami & Wada, 1997), is often shaped by the heterogeneous light environments typical of forest ecosystems, leading to significant differences between sunlit and shaded leaves. These leaf quality gradients can influence the distribution and behavior of insect defoliators within forest ecosystems (Gripenberg et al., 2010; Mayhew, 1997; Thompson, 1988).

Leaf traits, driven by the leaf economic spectrum, are optimized for different light conditions and can directly impact herbivore preference and performance (Messier et al., 2017; Morrow et al., 2022; Reich, 2014). Sunlit leaves show higher nitrogen content, which is related to stronger photosynthetic activity (Ellsworth & Reich, 1992). They also tend to display lower water content and tougher cuticle and epidermis to avoid desiccation. Leaves in high-light environments typically exhibit lower specific leaf area (SLA) and increased thickness, making them less palatable despite their higher nutrient levels (Agrawal & Fishbein, 2006; Eisenring et al., 2021; Wright et al., 2004). The theory also predicts that plants invest more to defend these valuable leaves (Angulo-Sandoval & Aide, 2000; Fortin & Mauffette, 2002; Lill & Marquis, 2001). This balance between nutritional value and physical defenses is crucial in determining herbivore feeding behavior between sun and shade leaves.

This study examines the effects of sun exposure on sugar maple (*Acer saccharum*) leaf physical traits and the feeding preference and performance of two common generalist Erebid caterpillar species: the early-season-feeding exotic spongy moth (*Lymantria dispar*) and the late-season-feeding native, white-marked tussock moth (*Orgyia leucostigma*). Sugar maple is a tree of high cultural, economic, and ecological importance, yet has been declining in deciduous forests of Eastern Canada since the late 1970s, in part driven by insect defoliation (Côté & Ouimet, 1996; Duchesne et al., 2003; Horsley et al., 2002; Payette et al., 1996). Building on recent findings that showed higher herbivore damage on shaded understory sugar maple leaves compared to sunlit canopy leaves (Hakimara & Despland, 2024), this study delves further by examining specific leaf traits that vary with light exposure and by assessing responses from both invasive early-season species and a native late-season species. This dual approach highlights the complex effects of light availability on herbivory dynamics, an essential consideration given sugar maple's shade tolerance and its role in late-succession forest stages, where young trees often remain in shaded environments for many years (Bray, 1956; Godman et al., 1990). Understanding these dynamics is crucial for developing effective management and conservation strategies to support sugar maple populations as they encounter changes in light availability due to forest structure shifts.

The preference-performance hypothesis (PPH) posits that herbivores will choose hosts that optimize their growth and survival (Jaenike, 1978), yet empirical studies show mixed results (Awmack & Leather, 2002; Eisenring et al., 2021; Foss & Rieske, 2003; Leyva et al., 2000; Lill & Marquis, 2001) highlighting the value of testing both behavioral preference and the performance outcome of feeding on different foliage types. In the context of light availability, studies suggest that sunlight gradients influence leaf physical defenses and nutrient composition, affecting herbivore preference and performance in complex ways (Messier et al., 2017; Morrow et al., 2022; Reich, 2014). For sugar maples, sun leaves are generally richer in sugars and proteins, and theory suggests that physical and chemical defenses should be invested mainly in these high-value leaves (Dudt & Shure, 1994; Eisenring et al., 2021). However, previous work suggests that these leaves have reduced concentrations of defensive phenolics compared to shade leaves, making physical barriers a more significant factor in herbivore deterrence (Fortin & Mauffette, 2002; Levesque et al., 2002; Panzuto et al., 2001). In a choice situation, caterpillars might be expected to prefer shaded leaves if they are more palatable with fewer physical defenses. However, caterpillars might perform better on sun leaves in a no-choice situation due to higher nutritional values.

We predict that sun-exposed leaves will be thicker, have lower SLA, higher toughness, and lower water content. We examine how these physical traits influence the preference and performance of two confamilial locally common caterpillar species that feed at different times of year on either developing or mature foliage.

3.3 Materials and method

3.3.1 *Study site and Plant species*

This study was conducted at the Kenauk Nature property, an old-growth natural forest spanning two regions from the Outaouais River valley to the Laurentian Mountains in southwestern Quebec, Canada (45°42'N; 74°53'W) in 2021 and 2022. In the first year, we selected 12 sites in this maple/bitternut hickory domain of temperate deciduous forest (Quebec Ministry of Forests, 2023), with each site over 10 meters apart. Tree species composition was relatively uniform across all sites, and site selection was made based on the similarity in tree size (1–2 m height) (Guerra et al., 2010) and ease of access to the branches. Each site comprised one healthy sugar maple sapling (*Acer saccharum*, Sapindaceae) in the shade under almost full canopy cover and one healthy sugar maple sapling in the sun with less canopy cover. Temperature, humidity, and light intensity were measured ten times at 4-

day intervals at different times of the day from late May to early July and three more times in August at 10-day intervals during the bioassays in 2021. Canopy openness was measured once at each site in late August of the same year. We ensured the average light intensity and canopy openness value showed meaningful differences between shaded and sunlit trees per site but not between sites. For visual clarity and to present all experiments and their respective timings in one place, an infographic has been included as Figure 3.1.

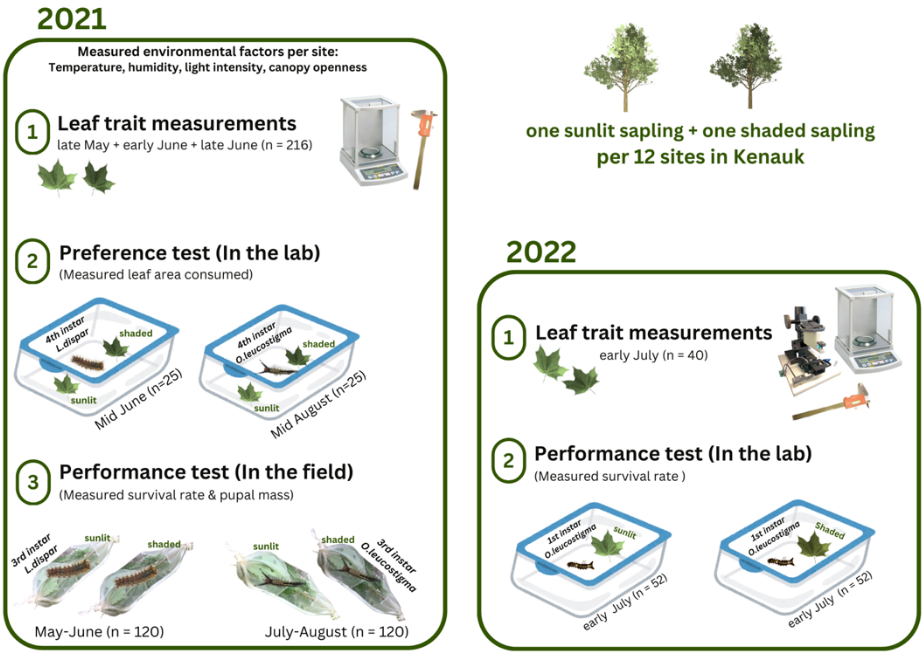


Figure 3.1 Infographic detailing the data collections used in the study in 2021 and 2022 in Kenauk nature. This graphic overview the experimental setup, timeline, data collection techniques, and key variables measured.

3.3.2 *Measurements of leaf physical traits*

In 2021, leaf traits were measured three times to evaluate the effect of light intensity on leaf defensive traits. Once before installing cages for the spongy moth performance test in late May, once in early June before conducting the spongy moth preference test in the lab, and lastly before the performance bioassay and preference test with tussock moth in late June. Considering the completion of leaf growth, which leads to expected stability in leaf traits once leaves are mature, no further measurements were taken after this date. On each sampling date, three undamaged average-sized leaves per sapling were collected per site (216 leaves (3 leaves* 2 saplings* 12 sites) * 3 sampling dates). Leaves were weighed, and their thickness was measured using a digital caliper when they were fresh. Subsequently, the leaves were pressed, labeled, and dried in an oven for 72 hours. Then, we weighed dried leaves again, and all leaves were scanned to measure the leaf area using ImageJ software. All efforts were made to ensure that dried leaves remained intact and free from any folding or breaking to maintain measurement accuracy. These procedures allowed us to determine water content and specific leaf area (SLA) for all sampled leaves. In early July of 2022, we used 10 of the 12 sites from the previous year to collect 20 pairs of leaves (two sunlit and two shaded leaves per site) for leaf trait measurements. This year, we added leaf toughness measurement using a digital penetrometer burrowed from Janice Cooke's laboratory at the University of Alberta, Canada.

3.3.3 *Insect defoliators*

To explore potential differences in leaf palatability and susceptibility to insect herbivores between sugar maple saplings in shade and sun, we focused on insect leaf cutters, which are primarily lepidopteran caterpillars in sugar maple forests (Thomas et al., 2010). As a representative of these lepidopterans, we selected two common leaf-eating caterpillars in our study site, both generalists from the Erebidae family, to minimize the phylogenetic differences and because they are feasible to rear in the lab. The selection of these two species also provides insight into varying ecological pressures: the spongy moth caterpillar (*Lymantria dispar*), an invasive outbreaking pest (Boukouvala et al., 2022), and the white-marked tussock moth (*Orgyia leucostigma*), a native, non-outbreaking species (Hebert et al., 2017). The spongy moth is a key model for invasion biology, with well-documented biology, and has spread in Eastern North America (Grayson et al., 2015). Including both early-season and late-season species allows us to capture seasonal variation in herbivory, enhancing our ability to understand how invasive and native herbivores respond to sun versus shade treatments across different growing periods. For the spongy moth, egg masses were collected from Angrignon Park in Montreal, QC, CA, in late January 2021, and tussock moth egg masses were obtained in diapause from Insect Production and Quarantine Laboratories (IPQL) in Ontario, CA. Both species were allowed to hatch and were reared in our lab before being placed in the field. All were fed on an artificial Bell diet until they finished the first instar. Before the experiment, they were fed on non-experimental red oak (*Quercus rubra*) and Norway maple (*Acer platanoides*) leaves.

3.3.4 *Caterpillars' feeding Preference*

Laboratory study: We conducted a preference test using two caterpillar species in their later instars on sugar maple sapling leaves from shaded and sunny branches in 2021. The preference experiment was performed on June 17 with 4th instar spongy moth caterpillars (early season caterpillar) and on August 12 with 4th instar tussock moth caterpillars (late season caterpillar). From all the previously mentioned 12 sites, 25 undamaged sunlit sugar maple leaves and 25 shaded sugar maple leaves were randomly harvested. Each was stored in a 2.0 ml microcentrifuge tube with a hole punched into the lid and filled with water. Leaf petioles were fixed and sealed in tubes using parafilm to avoid water loss. Subsequently, leaves were sorted by size, scanned, and their area measured using Image J. One sunlit and one shaded sugar maple leaf of similar size were placed on opposite ends of each of the 25 plastic containers (19cm L * 13cm W), with a similar leaf area in each container. A 4th instar caterpillar was then introduced to each container, positioned equidistantly between the two maple leaves to allow free choice. After 48 hours, the caterpillars were removed. Then, we measured the consumed leaf area by rescanning the leaves and calculating the area that was gone, which was used as the measure for host preference (DiTommaso & Losey, 2003; Fortin et al., 1997; Foss & Rieske, 2003; Morrow et al., 2022). This process was repeated later in the season using white-marked tussock moth caterpillars.

3.3.5 *Caterpillars' performance bioassay*

Field study: To assess the performance of the caterpillars on sugar maple leaves, we installed one sleeve cage on a sunlit sapling and one on a shaded sugar maple sapling in all 12 sites in 2021. Then, ten caterpillars in their late 3rd instar were placed in each cage (10 caterpillars * 12 sites = 120 caterpillars per treatment) and left there for approximately three weeks. The cages were checked regularly to collect pupae and count the remaining caterpillars until they all pupated or died. We then weighed all pupae to compare the performance of caterpillars on shaded and sun branches. This performance test was conducted twice, once in the early season (cages and caterpillars set on May 30 and collected on June 30) with spongy moth and once in the late season with white-marked tussock moth (cages and caterpillars set on July 30 and collected on August 31), to coordinate with their natural life cycles.

Laboratory study: In 2022, we conducted an experiment with early instar tussock moth larvae to determine if they would be more affected by the differences detected in leaf physical defenses between the two maple saplings, considering that mortality in lepidopteran first instar larvae is reported to be highest compared to the later stages (Zalucki et al., 2002). We were not able to get spongy moth larvae in 2022. The same protocol as the previous year was followed to obtain tussock moth egg masses and collect and store sugar maple leaves from sunlit and shaded trees in early July in Kenauk. Egg masses were hatched and reared on Bell diet for a few days till their late first instar stage of life. For this bioassay, a total of 104 caterpillars at their first instar larval stage were moved to the plastic containers (19cm L * 13cm W) and were supplied with fresh either sunlit or shaded maple leaves (26 containers * 2 first instar caterpillars per container * 2 treatments). Due to our focus on the evaluation of the 1st instar leaf cutter's ability to establish feeding of young insect herbivores, caterpillars were observed after 48, and their survival rate was recorded at this time point (DiTommaso & Losey, 2003; Murakami & Wada, 1997; Zalucki et al., 2001).

3.4 Statistical analysis

All statistical analyses were conducted using R and RStudio (version 2024.04.1+748). Visualizations were generated using the ggplot2 package in R (Wickham, 2016).

Leaf traits: To examine differences in leaf traits such as specific leaf area, thickness, toughness, and water content between sunlit and shaded saplings, we used the average value measured from three leaves per sapling. Three repeated leaf trait measurements over the season were averaged to be used in the model for the 2021 dataset. Having continuous, normally distributed measurements, we employed a linear mixed model with sunny and shaded treatments as fixed factors and site as a random factor for 2021 and 2022 datasets in separate models using the lme4 package (Bates et al., 2015). All model assumptions were evaluated by using the DHARMa package (Hartig, 2022).

Preference test: To assess the effect of light intensity on fourth instar larval preference after 48 hours, we designed an experiment where each caterpillar was fed in a separate container with one shaded and one sunlit sugar maple leaf. The consumed leaf area served as the response variable. Since the data from each light condition represent paired observations from the same caterpillars, a paired t-test was employed. This statistical test accounts for the natural variability between individual caterpillars and provides a precise evaluation of the effect of light intensity on leaf area consumption by comparing the consumption of the two leaf types by the same individual. Test assumptions, including normality and equal variance, were evaluated using Shapiro-Wilk’s test and Levene's test for each caterpillar species separately.

Performance bioassays: In the 2021 cage experiment, the proportion of surviving caterpillars per cage was calculated as the survival rate. This percentage data with a beta distribution correction fits a specialized betareg model package (Cribari-Neto & Zeileis, 2010). Since the DHARMa package does not work for models with beta distribution, we used a Q-Q residual plot to evaluate our model assumptions. Pupal mass data, a normally distributed continuous response variable with cages having possible effect as a random factor, was analyzed using a linear mixed-effects model (LMM) with lmer from the lme4 package. In 2022, having two caterpillars per container made the survival data a better fit as a binomial distribution to be analyzed by glm with a binomial family.

Due to a high incidence of spongy moth mortality caused by Nucleopolyhedrovirus (NPV), a common viral disease affecting insect larvae, only four individuals survived. As a result, their performance data was excluded from the analysis. However, all samples reared to the 4th instar in the lab for the preference test remained healthy and were monitored post-test until pupation and emergence to ensure no infection influenced the test results.

Finally, the correlations between caterpillars' preference-performance measures and leaf traits, relationships between leaf trait variables, and differences between sun and shaded saplings were analyzed using separate Principal component analysis (PCA) for each experiment. For 2021 tussock moth performance data, the average of three leaf trait measurements on May 20, June 10, and June 24, along with survival rate and pupal mass, were used for PCA. We used early May leaf trait data for spongy moth and late June leaf trait data for tussock moth PCA analysis for the preference experiments. Since the number of average leaf traits measured was less than the number of leaves used for the preference test, missing data for PCA were handled using multiple imputations (van Ginkel, 2023). We standardized all leaf trait variables and caterpillar preference-performance metrics to ensure equal contribution to the analysis. We explicitly scaled each variable to have a mean of zero and a standard deviation of one. Performance from the 2022 bioassay on early instar larvae was not included in any PCA due to its lack of significant difference between treatments.

3.5 Results

The measured temperature and humidity showed similar values between sunny and shaded treatments, with an average temperature of 24.93 ± 0.190 °C (mean ± SE) and 24.87 ± 0.192

°C and average humidity of 49.04 ± 0.667% and 48.83 ± 0.668% respectively for sunlit saplings and shaded ones across all sites in 2021. As expected, light intensity and canopy openness measured in the same year were significantly different between the two habitats. Light levels averaged 3596 ± 156 Lux with 24.2 ± 4.39% canopy openness for sunny saplings compared to 1569 ± 127 Lux with 5.40 ± 0.891% canopy openness for shaded trees.

3.5.1 *Leaf traits*

Analysis on 216 leaves in 2021 and 40 in 2022 showed consistently higher thickness (2021: $t = 4.73$, d.f. = 22, $P < 0.001$; 2022: $t = 2.43$, d.f. = 38, $P = 0.024$) in sunlit leaves than shaded leaves. SLA was significantly lower in sunlit leaves compared to shaded leaves in both years (2021: $t = -9.20$, d.f. = 22, $P < 0.001$; 2022: $t = -4.06$, d.f. = 38, $P < 0.001$). In 2021, the average leaf area was $80.4 \pm 2.8cm^2$ for shaded sugar maple leaves and $65.2 \pm 2.02cm^2$ for sunlit sugar maple leaves. Similarly, in 2022, the average leaf area was $73.2 \pm 7.2cm^2$ for shaded sugar maple leaves and $67.9 \pm 5.01cm^2$ for sunlit sugar maple leaves. Water content (2021: $t = -2.08$, d.f. = 22, $P = 0.048$) was also lower in sun-exposed leaves in 2021 but had no significant difference in the second year. The variation patterns in SLA, thickness, and water content observed in the average amounts across the three sampling dates in 2021 exhibited a consistent pattern throughout (Table S 3.1; Figure 3.2). Leaf toughness was only measured in 2022 and showed no significant difference between sunlit leaves and shaded ones, and its statistical results are presented in Table S 3.1.

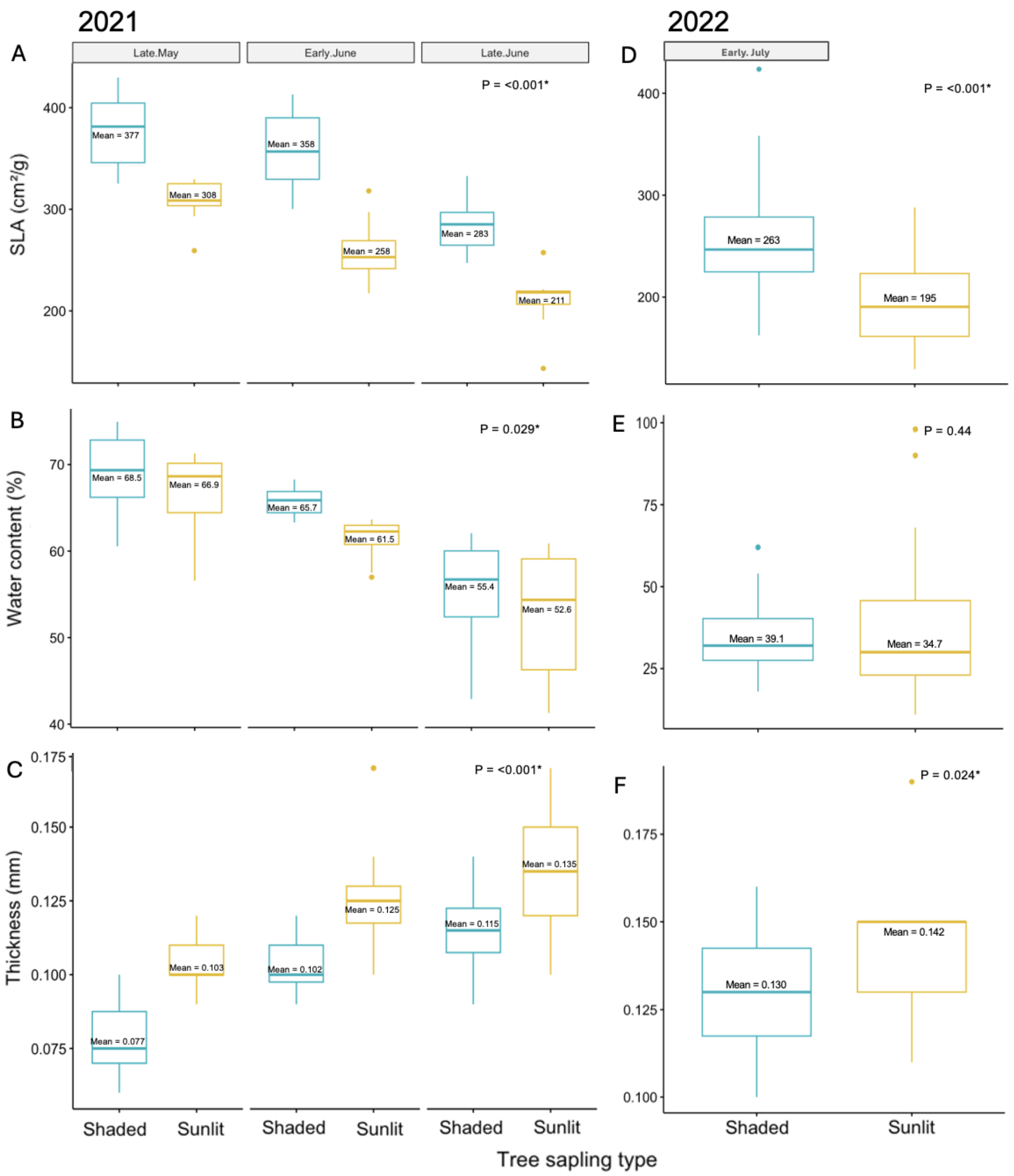
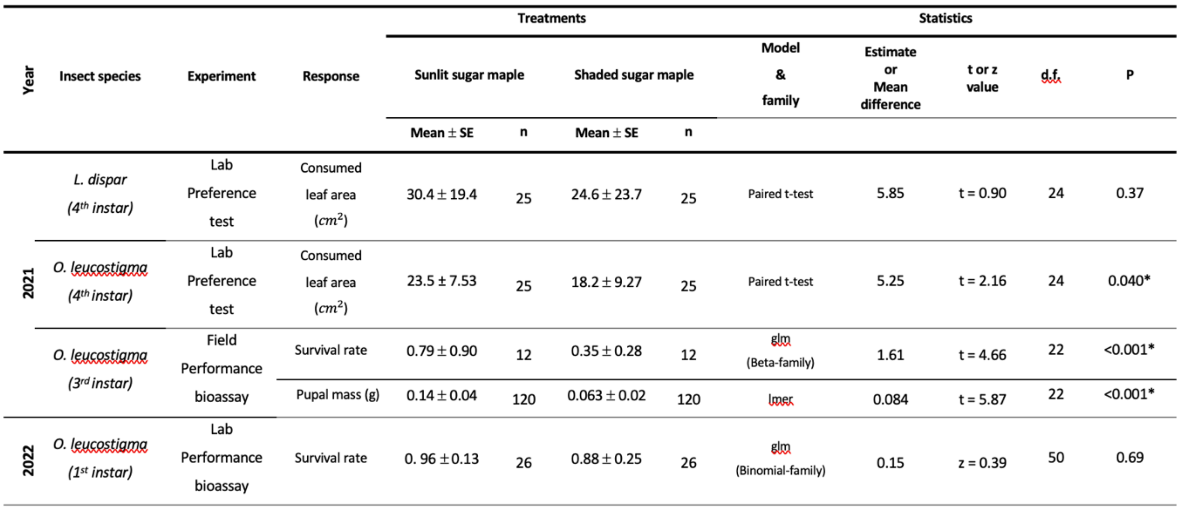


Figure 3.2 Boxplots showing variation in three measured leaf physical traits of two sugar maple saplings (shaded and sunlit) across 12 sites. Right column (2021): Measurements were taken in early May, early June, and late June (n = 72 per sampling date) for (A) Specific leaf area (cm²/g), (B) Leaf thickness (mm), (C) Leaf water content (%). Left column (2022): Measurements were taken in early July (n = 20 leaves per sapling type) for (D) Specific leaf area (cm²/g), (E) Leaf thickness (mm), and (F) Leaf water content (%). The central line inside each box represents the median (50th percentile). The lower and upper edges of the box represent the first quartile (Q1, 25th percentile) and the third quartile (Q3, 75th percentile). Whiskers extend to the smallest and largest values within 1.5 times the interquartile range (IQR) from Q1 and Q3, respectively. Points outside the whiskers are considered outliers. Mean values are presented inside the box plots, and P-values are shown above each plot.

3.5.2 *Caterpillars feeding Preference*

The results of the feeding preference tests revealed distinct differences in the consumption patterns of two lepidopteran species during their early 4th instar stage. Both species displayed a tendency to consume more leaf area from sunlit leaves compared to shaded ones. Notably, this preference was statistically significant for the tussock moth (t = 2.16, d.f. = 24, P = 0.040), indicating a strong preference for sunlit leaves. In contrast, the spongy moth did not show a significant preference (t = 0.90, d.f. = 24, P = 0.37) (Table 3.1; Figure S 3.1).

Table 3.1 Mean ± *SE and the results of statistical analysis for preference and performance responses of spongy moth (Lymantria dispar) and white-marked tussock moth (Orgyia leucostigma) on sunlit versus shaded sugar maple saplings in 2021 and 2022.*



				Treatments				Statistics				
Year	**Insect species**	**Experiment**	**Response**	**Sunlit sugar maple**		**Shaded sugar maple**		**Model & family**	**Estimate or Mean difference**	**t or z value**	**d.f.**	**P**
				Mean ± SE	**n**	**Mean ± SE**	**n**					
	L. dispar (4th instar)	Lab Preference test	Consumed leaf area (cm^2)	30.4 ± 19.4	25	24.6 ± 23.7	25	Paired t-test	5.85	t = 0.90	24	0.37
2021	*O. leucostigma (4th instar)*	Lab Preference test	Consumed leaf area (cm^2)	23.5 ± 7.53	25	18.2 ± 9.27	25	Paired t-test	5.25	t = 2.16	24	0.040*
	O. leucostigma (3rd instar)	Field Performance bioassay	Survival rate	0.79 ± 0.90	12	0.35 ± 0.28	12	glm (Beta-family)	1.61	t = 4.66	22	<0.001*
			Pupal mass (g)	0.14 ± 0.04	120	0.063 ± 0.02	120	lmer	0.084	t = 5.87	22	<0.001*
2022	*O. leucostigma (1st instar)*	Lab Performance bioassay	Survival rate	0. 96 ±0.13	26	0.88 ± 0.25	26	glm (Binomial-family)	0.15	z = 0.39	50	0.69

Note: Response variables and analyses used to compare their mean include consumed leaf area (cm²) for the lab preference test analyzed by paired t-test. Survival rate (3rd instar caterpillars surviving to pupation) fit in the model: g**lm (survival ~ treatment, family = beta_family (link = "logit"), data)**. and pupal mass (g) per cage in the field performance test in 2021fit in the model: **lmer (pupal mass ~ treatment + (1|cage), data)**. In addition to the survival rate (first instar caterpillars surviving after 48 hours) in the lab performance test in 2022 fit in the model: g**lm (survival ~ treatment, family = binomial (link = "logit"), data).** *L.dispar* performance test failed due to virus infection; therefore, means and model results are not included. Significant results are shown by * = P < 0.05.

In the spongy moth preference test, the PCA analysis accounted for 80% of the variance with the first two components. Here, leaf consumption (-4%), SLA (-59%), and water content (-57%) negatively correlated with PC1, while thickness (57%) was positively correlated. Remarkably, all measured traits except for water content (-11% negative correlation) showed positive correlations with PC2 (leaf consumption: 97%, SLA: 13%, thickness: 11%) (Table S 3.2; Figure 3.3A).

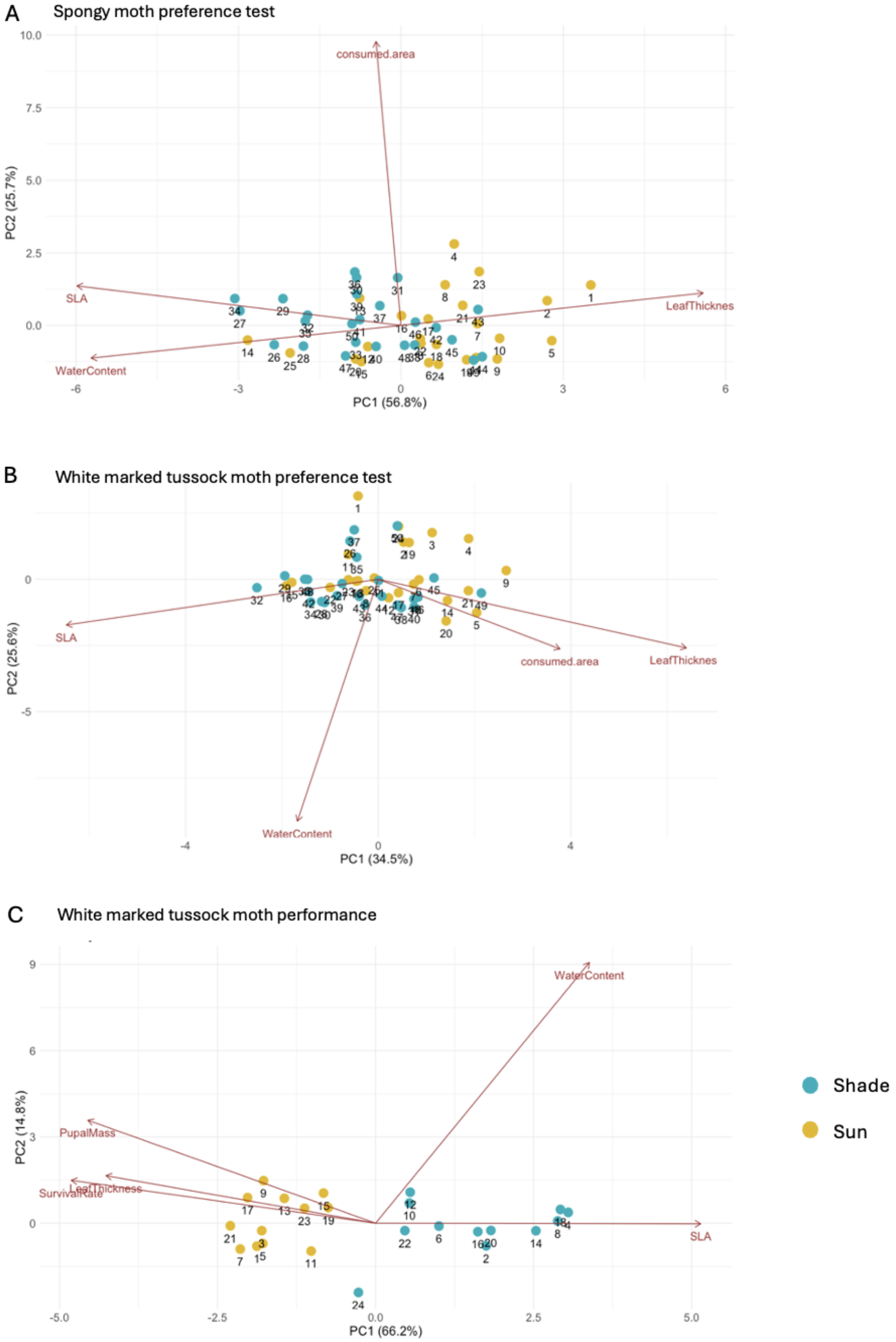


Figure 3.3 Biplots showing the preference and performance responses of spongy moths (Lymantria dispar) and white-marked tussock moth caterpillars (Orgyia leucostigma) to shaded versus sunlit sugar maple sapling leaves (shaded leaves indicated by blue circles and sunlit leaves by yellow circles), in relation to three leaf physical traits: specific leaf area (SLA), water content, and thickness measured for each experiment. (A) leaf area consumed by spongy moth caterpillars in the preference test, (B) leaf area consumed by white marked tussock moth caterpillars in the preference test, and (C) caterpillar survival rate and pupal mass as two measures of performance for tussock moth (L. dispar performance test was excluded due to virus infection).

For the tussock moth, PCA results highlighted that the first two principal components explained 60% of the variation. Leaf consumption (37%) and thickness (64%) positively correlated with PC1, whereas specific leaf area (SLA) (-64%) and water content (-16%) showed a negative correlation. However, all these traits exhibited negative correlations with PC2 (leaf consumption: -26%, SLA: -17%, water content: -91%, thickness -25%) (Table S 3.2; Figure 3.3B).

3.5.3 *Caterpillars Performance bioassay*

In 2021, from 240 third instar tussock moth caterpillars, 138 individuals survived, of which 95 were in cages installed on sunny branches, and the rest (43 caterpillars) were in shaded cages. The tussock moth survival rate from 3rd instar to pupa was significantly higher in cages on sunlit sugar maples (t = 4.66, d.f. = 22, P = <0.001) than in shade. Regarding the mean value of pupal mass, it showed a significant difference (t = 5.87, d.f. = 22, P = <0.001) between two treatments with larger pupal mass recorded for caterpillars reared in sunlit cages (Table S 3.2; Figure S 3.2)

The PCA results show a clear separation between shaded and sun leaves based on leaf traits and performance variables. It indicates a positive correlation of SLA and water content with PC1 (SLA: 51% & water content: 33%) and water content with also PC2 (water content: 90%), particularly in the direction of shaded leaves. In contrast, leaf thickness and survival rate show an opposite trend, correlating negatively with PC1 (thickness: -42% & survival: -48%) but positively with PC2 (thickness: 16% & survival: 14%). Pupal mass follows a similar trend to leaf thickness and survival rate, aligning on the negative side of PC1 (-45%) but exhibiting a positive correlation with PC2 (35%). These findings suggest that while SLA and water content are more associated with shaded leaves, leaf thickness, survival rate, and pupal mass are linked to sunlit leaves (Table S 3.2; Figure 3.3C)

The 2022 performance bioassay with 1st instar tussock moth larvae showed a slightly higher survival rate in the first 48 h on sun leaves. However, this was not statistically significant (z = 0.39, P = 0.69) (Table 3.1).

3.6 Discussion

Our results confirm the expected differences in leaf traits between sunlit and shaded environments, particularly in leaf thickness, specific leaf area, and water content. Tussock moth larvae preferred and performed better on sunlit leaves, whereas spongy moth caterpillars showed no preference. These results do not support leaf physical traits as defenses against leaf-chewers in our study system or explain the previously observed gradient in herbivore damage, where shaded understory leaves showed greater damage than sunlit canopy leaves (Hakimara & Despland, 2024).

In line with our initial hypothesis, sunlit leaves exhibited greater thickness and lower SLA compared to shaded leaves, consistent with the leaf economic spectrum (Messier et al., 2017; Reich, 2014). These morphological traits, driven by increased sunlight exposure, were predicted to serve as enhanced physical defenses, making the leaves less palatable to herbivores (Agrawal & Fishbein, 2006; Wright et al., 2004). In addition, theory suggests that trees should defend sun leaves more because they are more valuable (Dudt & Shure, 1994; Eisenring et al., 2021). However, despite these defenses, tussock moth caterpillars significantly preferred sunlit leaves, underscoring the complexity of herbivore-plant interactions.

Interestingly, while both caterpillar species consumed more sunlit leaf area, only tussock moths showed a statistically significant preference for these leaves. PCA findings suggest that leaf consumption increases with water content in the tussock moth, followed by leaf thickness, illustrating a clear pattern of trait preference. Conversely, no single leaf trait appeared strongly associated with feeding preferences in the spongy moth. This outcome is somewhat paradoxical, given sunlit leaves' lower water content and higher physical defenses, which would typically deter herbivory. The nutritional benefits, possibly from higher nitrogen content, as suggested by previous studies (Fortin & Mauffette, 2002; Levesque et al., 2002), might outweigh the deterrent effects of physical defenses for tussock moth caterpillars. For the invasive spongy moth, sugar maple is a relatively novel and intermediate host (Mauffette et al., 1983), and the absence of specific defensive traits in sugar maple leaves against spongy moths may contribute to this lack of strong preference. As suggested by studies on herbivore-host interactions, the broader host adaptability of invasive species allows them to utilize hosts with different structural and chemical traits effectively, regardless of the typical deterrents in native environments (Keena & Richards, 2020).

The performance bioassay results further support the tussock moth narrative. In the first year, tussock moth caterpillars reared on sunlit leaves demonstrated significantly higher survival rates and pupal mass compared to those on shaded leaves, supporting the idea that sunlit leaves, despite their defenses, offer a more suitable food source for caterpillar development. The higher survival and growth observed could be attributed to the higher nutritional content, which may compensate for the physical traits of the leaves. A complex effect on feeding behaviour and growth of other leaf-feeding insects has also been documented. For instance, in the emerald ash borer (*Agrilus planipennis*), neither leaf age nor light intensity influenced feeding preference or development on green ash (*Fraxinus pennsylvanica*) (Chen & Poland, 2009). In contrast, in Aristotelia chilensis saplings, sawfly larvae consumed more shaded leaves than sunlit ones (Guerra et al., 2010). These findings emphasize that sun-induced changes in leaf traits, such as thickness, toughness, or water content, may not uniformly affect herbivore behavior across different host plant species. This variability underscores the significant role of light exposure in shaping plant-insect interactions. In other words, thicker leaves with lower SLA but no difference in toughness are not necessarily less palatable or less suitable for development. Thickness could occur by differences in anatomical arrangements in cell walls and the epidermis or could just mean more mesophyll tissue and, therefore, more nutrition per bite for a caterpillar (Kitajima et al., 2012; Nobel et al., 1975). For example, lower thickness in shaded leaves could be due to decreased leaf palisade development, corresponding to less photosynthetic activity (Witkowski & Lamont, 1991). The second-year bioassay, involving first instar larvae, did not reveal significant differences in survival rates between sunlit and shaded leaves, suggesting that these small early-instar caterpillars experience no difficulty in biting into the thicker sun leaves and establishing a feeding site. The lack of significant differences in leaf toughness between sunlit and shaded environments supports the interpretation that the increased thickness of sun leaves does not necessarily imply a physical barrier to leaf-chewer feeding, even for small early-instar larvae. This similarity in leaf toughness between sunlit and shaded environments contrasts with previous findings (Angulo-Sandoval & Aide, 2000; Fortin & Mauffette, 2002). This discrepancy might be due to the similarity in the age of sampled saplings, which reduces the ontogenetic effect as a substantial factor in leaf trait and herbivory pattern variation (Hanley et al., 2007; Thomas et al., 2010).

Our study suggests that the physical properties of leaves do not always predict herbivore performance or preference. The higher survival and better performance of tussock moth caterpillars on sunlit leaves, despite their greater thickness, highlight the nuanced balance between plant defenses and insect herbivory. This is particularly relevant given the significant decline in sugar maple populations due to insect defoliation (Duchesne et al., 2003; Payette et al., 1996). Higher herbivory on sugar maple saplings under full sunlight can have important implications for forest regeneration in human-disturbed landscapes, notably in fragmented forest patches (Roland, 1993) or in degraded stands with high mortality of mature trees (e.g., from emerald ash borer, Baron & Rubin, 2021). Under these situations of artificially increased sunlight, other native or invasive trees species may outcompete sugar maple saplings, jeopardizing the future position of this species in the forest ecosystems. Similarly, in plantations, sugar maples might perform better when planted under cover of fast-growing, shade-intolerant trees than in full sun (Comeau et al., 2009). However, the role of natural enemies must also be considered, as they are likely to increase top-dawn pressure on herbivores in sun-exposed saplings (Stoepler & Lill, 2013).

In conclusion, our findings contribute to the broader understanding of how intra-species variation in leaf traits, driven by light availability, influences herbivore-plant dynamics. The apparent preference of tussock moth caterpillars for sunlit leaves, coupled with their enhanced performance in these environments, suggests that managing light exposure in sugar maple forests could be a key strategy in mitigating the impact of leaf-cutter moths for shade-tolerant sugar maples. These tree species thrive in late-succession stages of forest development, often remaining under the canopy for decades before reaching the light-abundant upper layers (Bray, 1956; Godman et al., 1990). Further research should explore the long-term implications of these interactions, considering the potential for changes in forest structure and light availability under shifting climate conditions.

These results highlight the importance of considering within-species variation in leaf traits when examining the interactions between maple trees and their insect herbivores. The differential performance of defoliators on sun versus shaded leaves may have implications for these insects' spatial distribution and outbreak dynamics within maple-dominated forests, as suggested in the previous study (Roland, 1993). Further research is needed to elucidate the specific mechanisms underlying the divergent responses of these herbivores in nature to sun and shade leaves by leaf chemical traits, direct abiotic effects of high sunlight or natural enemies (Guerra et al., 2010; Hochuli, 2001), as well as the broader ecological consequences of these interactions.

3.7 Supplementary information

Table S 3.1 Linear mixed model coefficient results for the effect of sugar maple tree sapling position (sunlit vs. shaded) on leaf traits in 2021 (n = 216 leaves, d.f. = 22) and in 2022 (n = 40 leaves, d.f. = 38) both collected from 12 sites at Kenauk nature.

Year		**Leaf thickness**			**Leaf water content**			**Specific leaf area**			**Leaf toughness**		
	Predictors	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P
2021	Shaded leaves (Intercept)	0.10	0.003	<0.001*	62.70	0.95	<0.001*	335.1	6.047	<0.001*			
	Sunlit leaves	0.021	0.004	<0.001*	-2.81	1.34	0.048*	-78.76	8.55	<0.001*			
2022	Shaded leaves (Intercept)	0.13	0.003	<0.001*	39.1	4.20	<0.001*	262.92	11.84	<0.001*	3.45	0.26	<0.001*
	Sunlit leaves	0.011	0.004	0.024 *	-4.40	5.64	0.44	-68.06	16.75	<0.001*	0.45	0.37	0.24

Note: Response variables, including leaf thickness, leaf water content, SLA, and leaf toughness, fit in the model: **lmer (leaf trait ~ treatment + (1|site), data)**. Significant results shown by * = P < 0.05.

Table S 3.2 This table summarizes the Principal Component Analysis (PCA) performed on the data from various experiments evaluating the performance and preference of tussock moth caterpillars (Orgyia leucostigma) and spongy moths (Lymantria dispar) when exposed to sunlit versus shaded sugar maple sapling leaves. The PCA includes three physical leaf traits: specific leaf area (SLA), water content, and thickness for three experiments, including tussock moth performance indicating caterpillar survival rate and pupal mass, tussock moth preference test measured by leaf area consumed and spongy moth preference test with again measuring leaf area consumed.

Experiment	**Variables**	**Components**				
		PC1	*PC2*	*PC3*	*PC4*	*PC4*
Preference 2021- Spongy moth	Proportion of Variance	0.56	0.25	0.10	0.06	
	Leaf consumption	-0.04	0.97	0.03	-0.20	
	SLA	-0.59	0.13	-0.11	0.78	
	Water content	-0.57	-0.11	-0.63	-0.51	
	Thickness	0.57	0.11	-0.76	0.29	
Preference 2021- Tussock moth	Proportion of Variance	0.34	0.25	0.23	0.16	
	Leaf consumption	0.37	-0.26	-0.88	0.10	
	SLA	-0.64	-0.17	-0.30	-0.67	
	Water content	-0.16	-0.91	0.23	0.28	
	Thickness	0.64	-0.25	0.26	-0.67	
Performance 2021- Tussock moth	Proportion of Variance	0.66	0.14	0.10	0.02	0.05
	Survival rate	-0.48	0.14	-0.12	-0.85	0.05
	Pupal mass	-0.45	0.35	-0.52	0.36	-0.50
	SLA	0.51	-0.001	0.06	-0.34	-0.78
	Water content	0.33	0.90	0.07	-0.02	0.23
	Thickness	-0.42	0.16	0.83	0.13	-0.27

Note: Data from Lymantria dispar performance tests are excluded due to virus infection.

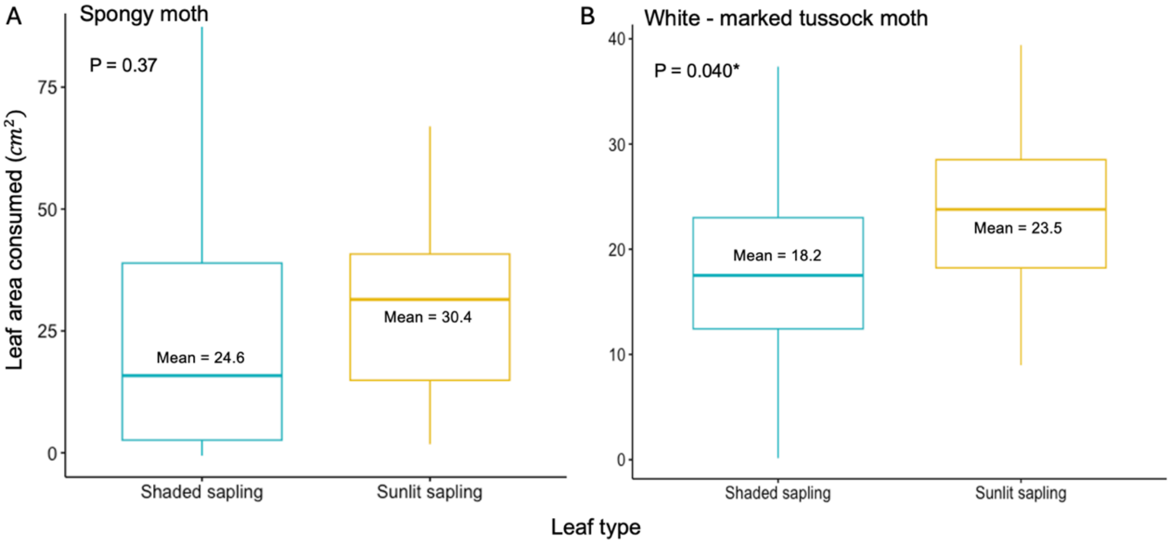


Figure S 3.1 Box plots showing two moth caterpillar species preference over sunlit leaves versus shaded leaves by measuring the area of leaves consumed per testing container (n = 25) (A) Leaf area of sunlit leaves versus shaded leaves consumed by a spongy moth (Lymantria dispar) and (B) Leaf area of sunlit leaves versus shaded leaves consumed by white-marked tussock moth (Orgyia leucostigma). The central line within each box represents the median (50th percentile). The lower and upper edges of the box show the first quartile (Q1, 25th percentile) and the third quartile (Q3, 75th percentile), respectively. The whiskers extend to the most extreme data points within 1.5 times the interquartile range (IQR) from Q1 and Q3. Data points outside this range are considered outliers and are represented by individual points. Mean values are presented inside the box plots, and P-values are shown above each plot.

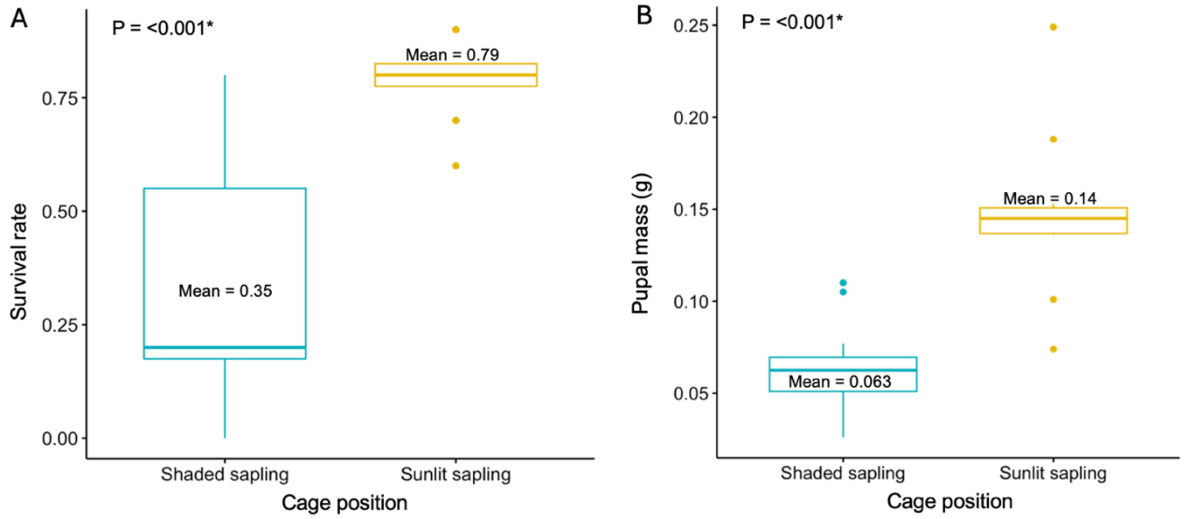


Figure S 3.2 Box plots showing the performance of white marked tussock moth caterpillar (Orgyia leucostigma) in cages on sunlit saplings versus in cages on shaded saplings (n =120 caterpillars per sapling) in 2021 measured by (A) survival rate of caterpillars from late 3rd instar to pupation and (B) pupal mass of caterpillars survived from late 3rd instar to pupation (g) (Lymantria dispar performance test failed due to virus infection; therefore, its figures are not included here). The central line within each box represents the median (50th percentile). The lower and upper edges of the box show the first quartile (Q1, 25th percentile) and the third quartile (Q3, 75th percentile), respectively. The whiskers extend to the most extreme data points within 1.5 times the interquartile range (IQR) from Q1 and Q3. Data points outside this range are considered outliers and are represented by individual points. Mean values are presented inside the box plots, and P-values are shown above each plot.

4 Chapter 4: Influence of Vertical Stratification and Light Gradient on Insect Herbivores’ Natural Enemies in Sugar Maple Temperate Forests

The following chapter is under preparation to be submitted as: Hakimara, M., & Despland, E. (2025). Influence of Vertical Stratification and Light Gradient on Insect Herbivores’ Parasitoid and Predation Rates in Sugar Maple Temperate Forests, in *The Canadian Entomologist.*

4.1 Abstract

Forest ecosystems are a mosaic of habitats shaped by structural and light variability, which in turn influence insect herbivores and their interactions with natural enemies. This study examines how light gradients affect predation and parasitism rates on insect herbivores feeding on sugar maple (*Acer saccharum*). From 2023 to 2024, we conducted field experiments in the Kenauk Nature Reserve, Quebec, across vertical strata (sunlit canopy, shaded canopy, understory) and between sunlit and shaded saplings.

Light intensity and canopy openness showed the expected clear differences among vertical strata, with sunlit canopies receiving the most light. Total predation rates measured using artificial caterpillars did not show significant differences. However, arthropod predation was higher in the understory, while bird predation was mainly confined to the canopy. A similar experiment with saplings showed no significant differences in predation rates between sunlit and shaded saplings.

Parasitism proved far more difficult to measure. Despite extensive efforts to assess parasitism rates of larvae and to sample parasitoid communities, the numbers were too low for meaningful analysis, only 13 parasitoids from sleeve cages containing sentinel larvae and 32 from suction traps.

Overall, these findings suggest that, as bird predation is concentrated in the canopy, the understory becomes a refuge for both herbivorous and predatory arthropods and thus constitutes an important reservoir for biodiversity. The minor differences between sunlit and shaded saplings suggest that vertical gradients in predator activity were due to distance from the ground and tree architecture rather than to vertical light gradients per se.

4.2 Introduction

The structural diversity within and between trees in forest ecosystems creates heterogeneous food and habitat resources for herbivores and their natural enemies. These dynamics strongly influence the bottom-up and top-down forces that shape insect herbivore communities (Schowalter et al., 1986; Singer & Stireman, 2005; Ulyshen, 2011; Wilkinson & Sherratt, 2016). Intra-specific variability in foliar physical defenses is often shaped by the light gradient in forest environments, leading to significant differences in leaf quality between sunlit and shaded foliage (Choong, 1996; Coley et al., 1985; Murakami & Wada, 1997). While numerous studies have examined how these leaf trait gradients affect the distribution and behavior of insect defoliators (Gripenberg et al., 2010; Mayhew, 1997; Thompson, 1988), there is comparatively limited research on how top-down forces, often stronger determinants of herbivore population dynamics than bottom-up factors (Vidal & Murphy, 2018), are influenced by stratification and light conditions.

Herbivorous insects face selective pressures from bottom-up forces via host plant quality and top-down forces via predators and parasitoids (Singer & Stireman, 2005). Bottom-up forces directly affect herbivore preferences and performance, as demonstrated by controlled experiments in enemy-free environments, where leaf traits optimized under different light conditions influence herbivore responses (Morrow et al., 2022; Stoepler & Lill, 2013). However, when natural enemies are present, herbivores may experience trade-offs between the nutritional quality of host plants and the risk of predation, influencing their feeding site choices and foraging mechanism (Heinrich, 1993; Mooney et al., 2012; Murphy & Loewy, 2015; Thompson, 1988). The enemy-free space hypothesis posits that herbivores may favor habitats with lower predation risk, even if such habitats offer lower nutritional quality (Jeffries & Lawton, 1984).

Predators and parasitoids play critical roles in regulating herbivore populations and influencing host plant use (Low et al., 2014; Sigut et al., 2018; Zvereva et al., 2020). Research highlights that interactions between parasitoids, predators, and herbivores vary with habitat heterogeneity, such as the vertical gradient in temperate forests (Ulyshen, 2011). However, studies exploring this heterogeneity remain fragmented and present mixed findings. For instance, in Lanzhot, Czech Republic deciduous forest, hymenopteran parasitism rates decreased from the low canopy level to the higher canopy with no understory data (Sigut et al., 2018). In a temperate forest in Italy, Dipteran parasitoids were more abundant in the understory compared to the canopy (Stireman et al., 2012). Contrarily, a study from Great Lakes St. Lawrence region of southcentral Ontario, Canada reports higher abundance of hymenopteran parasitoids in the canopy than the understory in maple stands (Vance et al., 2007). Additionally, within the understory, sun-exposed habitats may experience greater parasitism rates than shaded ones (Stoepler & Lill, 2013). Predation studies also yield varying results. For example, arthropod predation rates in a sugar maple stand were higher in the understory (Aikens et al., 2013), while in boreal forests, predation rates by birds and arthropods were higher on larger mature trees compared to smaller saplings (Zvereva et al., 2020). Interestingly, light conditions within the understory did not influence bird predation rates on saplings (Barber & Marquis, 2011). These scattered and occasionally contradictory findings illustrate the complexity of top-down forces in temperate forests, emphasizing a lack of a consistent pattern, particularly regarding the natural enemies of sugar maple herbivores.

This study investigates how sunlight exposure affects predation and parasitism rates on insect defoliators feeding on sugar maple (*Acer saccharum*). Specifically, it examines the impact of a vertical gradient from understory saplings to the canopy of mature trees and the differences between shaded and sunlit saplings. Sugar maple, a culturally, economically, and ecologically significant species, has experienced declines in Eastern Canada's deciduous forests since the late 1970s, partly due to insect defoliation (Côté & Ouimet, 1996; Duchesne et al., 2003; Horsley et al., 2002; Payette et al., 1996). Recent studies have shown that vertical stratification of leaf physical traits exerts bottom-up pressures on insect herbivory (Hakimara & Despland, 2024). In addition, works by the same authors shows that light intensity influences leaf traits, with caterpillar preferring and performing better on sunlit foliage (Hakimara & Despland, in press). Building on this foundation, the current research adds a new dimension by focusing on top-down forces, testing the enemy-avoidance hypothesis as an explanation for the higher herbivore damage observed in the understory. This hypothesis integrates existing findings on leaf trait variation with the spatial dynamics of natural enemy pressures.

Aligned with this hypothesis we predict to see higher predation and parasitism rates in the canopy level and higher enemy pressure on sun-exposed saplings than shaded ones. By testing these predictions, this study examines how forest structural heterogeneity drives variation in natural enemy pressures on insect herbivores feeding on saplings and mature trees. Given the shade tolerance of sugar maple and its importance in late-succession forests (Bray, 1956; Godman et al., 1990), understanding these dynamics is crucial for developing effective conservation and management strategies.

4.3 Materials and Methods

The field experiments were conducted in 2023 and 2024 at the Kenauk Nature property, an old-growth forest stretching from the Outaouais River Valley to the Laurentian Mountains in southwestern Quebec, Canada (45°42'N; 74°53'W). This maple/bitternut hickory temperate deciduous forest (Quebec Ministry of Forests, 2023) provide a consistent setting for investigating sugar maple (*Acer saccharum*, family Sapindaceae) associated herbivores and their natural enemies. We tested top-down effects on herbivory first along a vertical gradient and second between sun-exposed and shaded saplings. No significant herbivorous insect outbreaks were observed during the study. For a complete overview of sampling timelines and experimental setups, please refer to the infographic provided in **Error! Reference source not found.**.

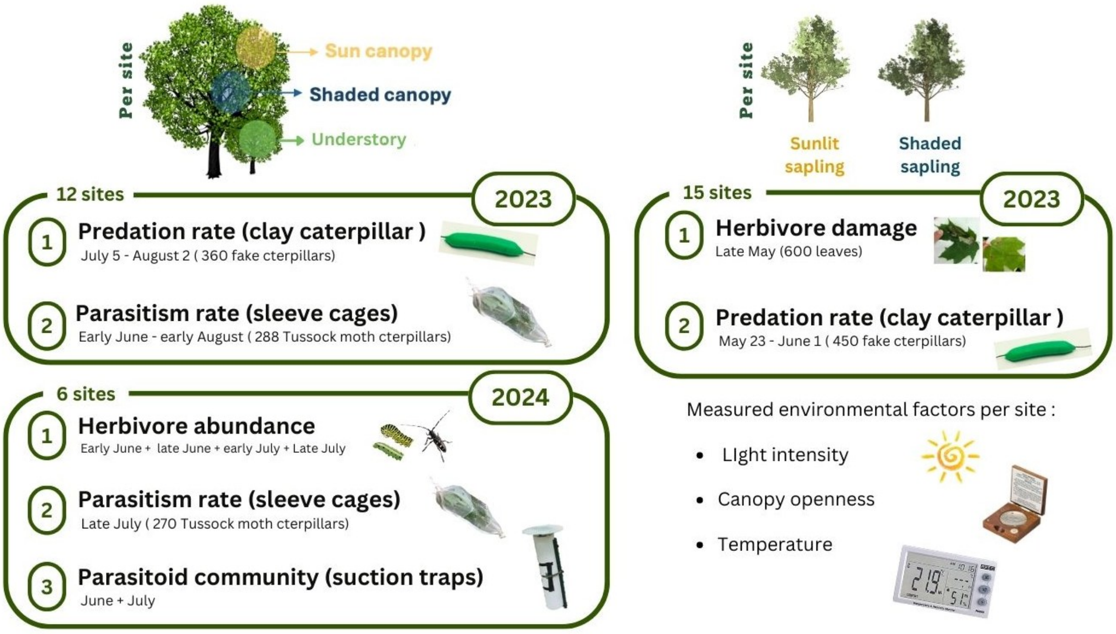


Figure 4.1 Infographic detailing the data collections used in 2023 and 2024 study in Kenauk nature. This graphic overview the experimental setup, timeline, data collection techniques, and key variables measured.

4.3.1 *Vertical Gradient (2023 and 2024)*

Study sites: To explore the impact of vertical stratification on the natural enemies of insect herbivores, we initially selected and sampled 12 sites spaced at least 40 meters apart, with the furthest distance between sites reaching 9 kilometers. Sites were chosen to maintain uniform tree species composition, similar tree sizes, and accessibility for climbing. Each site included three strata: the canopy (sunlit and shaded) and the understory. Canopy sampling heights ranged from 10–13 meters on mature trees with an average height of 16 meters, while the understory consisted of sugar maple saplings measuring 1–3 meters tall. For the second year of sampling, just 6 sites out of 12 were used.

Light intensity and temperature were measured with Reed light meter and Reed temperature meter on June 10, 2024, to capture variation in light conditions across the vertical strata and control for changes in the temperature. Canopy openness was also assessed along with light and temperature, using a spherical densiometer at targeted branches.

Insect herbivory: Insect herbivores were sampled across three vertical strata (understory, shaded canopy, and sunlit canopy) at 6 sugar maple sites in 2024. Sampling took place on June 11, June 25, July 8, and July 22, with two branches per stratum per site sampled using the beat sheeting method (10 beats per branch). All samples were preserved in 75% ethanol and transported to the lab for identification. Herbivores were identified to the lowest possible taxonomic level and grouped by feeding guilds for analysis.

Predation rate: Predation rates were evaluated using artificial polymer caterpillars mounted on tree branches, following established protocols (Howe et al., 2009; Low et al., 2014). Caterpillars were crafted from green, non-toxic modeling clay (Sculpey III, String Bean color), measuring ~30 mm in length and 5 mm in diameter, and attached using 12-cm, non-reflective metallic wires (*Quebec 4-H Clubs*, 2019; Stemmelen et al., 2022). Predation marks by mammals, birds, and arthropods were documented following retrieval and used as a measure of predation pressure (Ayotte-Beaudet et al., 2021; Low et al., 2014; *Quebec 4-H Clubs*, 2019; Stemmelen et al., 2022).

In 2023, 10 fake caterpillars were installed at 12 sites (10 clay caterpillars × 3 strata × 12 sites = 360 clay caterpillars per sampling). They were deployed on June 15 for the first round and collected on July 5. A second round followed, with installation on July 5 and collected on August 2, yielding another 360 caterpillars. The repetition and length of experiment allowed us to account for possible seasonal variations in predation (Zvereva et al., 2020). Unfortunately, one site was destroyed due to a storm, and the analysis was conducted using the data collected from 11 sites. During collection, caterpillars were carefully placed in foam holders labeled with the date, site, stratum, or sapling type to ensure treatment integrity. In the lab, predation marks were examined, photographed, and recorded as a measure of predation rates per treatment. For each clay prey, any predation marks (mammals, birds, or arthropods) were recorded as either present or absent in the data sheet. If more than one predation mark type was observed on a single clay prey and the marks were distinguishable, all types were recorded separately (Ayotte-Beaudet et al., 2021; Low et al., 2014; *Quebec 4-H Clubs*, 2019; Stemmelen et al., 2022).

Parasitism rate: To assess parasitism rates across sugar maple strata, we focused on leaf-cutting Lepidopteran caterpillars that are common in temperate forests (Thomas et al., 2010). The white-marked tussock moth (*Orgyia leucostigma*) as a generalist herbivore, non-outbreaking and late-season caterpillar from the family Erebidae (Hebert et al., 2017), was selected as the cutter herbivores representative. It is a suitable species for lab rearing and is prevalent in our study area. Diapausing egg masses were sourced from the Insect Production and Quarantine Laboratories (IPQL) in Ontario, Canada. After hatching, caterpillars were raised to the 3rd instar in the lab, initially on a Bell artificial diet and subsequently on non-experimental red oak (*Quercus rubra*) and Norway maple (*Acer platanoides*) leaves. Then they were moved to the field and transfered to the sleeve cages installed on the branches of sugar maples. In 2023, two branches per strata were selected and we put four caterpillars, all in 3rd instar, per cage from June 7 to June 12. A second round with the same number of caterpillars but at fifth instars was conducted in August (4 larvae × 2 cages × 3 strata × 12 sites = 288). Based on preliminary tests done in 2022, small holes in the mesh cage were used to avoid caterpillars' escape while letting parasitoids in.

Given that no parasitoids were collected in 2023, the experiment was repeated in 2024 with larger holes on mesh sleeve cages on three sites: ten caterpillars per cage and three cages per stratum (10 larvae × 3 cages × 3 strata × 3 sites = 270). Caterpillars were set on July 12 and collected on July 23. To complement these efforts, suction traps (Favret et al., 2019) were installed at each stratum of sugar maples at three sites (different from the sites used for parasitism rate experiment), from June 10 to 25 and from July 12 to 24. Insects captured were sorted into Hymenoptera (to superfamily) and Diptera (to family), with further sorting into morphospecies.

4.3.2 *Saplings in different light conditions (2023)*

Study sites: For the sapling experiments in 2023, we chose 15 sites, distinct from those used for the vertical gradient experiments, and ensured a minimum distance of 10 meters between them. All sites were located along a narrow path (approximately 2.5 m wide) in a mature forest, and this allowed us to have sun exposure as the only real difference between two saplings per site. All sites maintained consistent tree species composition and were selected for uniformity in tree size (1–2 meters in height) and branch accessibility (Guerra et al., 2010). At each site, we identified one shaded sapling growing under dense canopy cover and one sunlit sapling along the path and exposed to reduced canopy cover.

Light intensity and temperature were measured twice (on May 23 and 30) and canopy openness was evaluated once, on May 23, using a spherical densiometer to validate treatment consistency during this experiment.

Insect herbivory: For the sapling experiment, herbivory damage was visually assessed as a measure of insect herbivore activity (Schowalter, 2006; Schowalter et al., 1986). Observation was conducted on May 23 across 15 sites, with one shaded and one sunlit sugar maple sapling per site and repeated once more on June 1. Damage was categorized into nine feeding guilds, including leaf rollers, leaf skeletonizers, leaf cutters, leaf stipplers, spindle galls, erineum galls, ocellate galls, maple leaf-cutter miners, and maple leaf-blotch miners (Houston et al., 1990; M. T. J. Johnson et al., 2016; Landsberg, 1989; Thomas et al., 2010; Wint, 1983). The percentage of leaf area damaged was visually estimated for ten fully expanded leaves per sapling, and the scores were averaged for each sapling (10 leaves× 2 sapling type × 15 sites × 2 repeat = 600 leaves) (Hakimara & Despland, 2024; Thomas et al., 2010).

Predation rate: 15 fake caterpillars were installed per sapling across 15 sites. Each site had three branches on sun-exposed sapling and three branches on shaded sapling selected for installation (5 caterpillars × 3 branches × 15 saplings × 2 treatments). A total of 450 clay caterpillars were exposed to predators from May 23 to June 1. The same steps as in vertical gradient experiment were followed for collecting fake caterpillars and assessing predation marks on them in the lab.

We did not attempt to check parasitism rate for saplings under sun and shade treatments due to the challenges encountered in the vertical gradient experiments. The low parasitism rates observed in vertical gradient experiment, combined with difficulties in rearing sufficient white-marked tussock moth caterpillars, made extending the experiments to saplings impractical.

4.4 Statistical analysis

Statistical analyses were performed using R and RStudio (version 2024.04.1+748), and visualizations were created with the ggplot 2 package in R (Wickham, 2016).

4.4.1 *Vertical Gradient (2023 and 2024)*

Herbivore community: To analyze variation in insect herbivore abundance from different feeding guilds across the vertical gradient (collected in 2024), we used generalized linear mixed models (GLMMs) implemented in the glmmTMB package (Brooks et al., 2017). The site was included as a random factor to account for site variability. Due to the low abundance of leaf blotch miners and leaf miner maple leaf cutters, these guilds were excluded from the analysis. Models were fitted using the Poisson family for total herbivore abundance, leaf cutter abundance, and leaf stippler abundance. Date was added as a fixed factor in the initial model but was excluded during model simplification due to its lack of significant contribution to explaining the response variation. We used the DHARMa package to evaluate model assumptions by performing simulation-based residual diagnostics (Hartig, 2022), which is particularly suited for GLMMs as it detects deviations from expected distributions, including issues like overdispersion and non-normality.

Predation rate: Predation rates from birds, mammals, arthropods, and all predators combined were analyzed using logistic regression with a binomial error distribution and logit link function (Zvereva et al., 2020). Predation occurrence per clay prey (1 = predation, 0 = no predation) was the binary response variable. Vertical stratification (three strata: sunlit canopy, shaded canopy, and understory) was included as a fixed effect, with the site as a random factor to account for variability among locations. Date was initially included as a fixed factor but removed during model simplification as it did not significantly influence response variation.The models were implemented using the glmmTMB package, and assumptions were validated with the DHARMa package (Hartig, 2022).

Parasitism rate: Parasitism rates did not fit in any model due to low number of samples. Information regarding the individual parasitoids collected at our sites, is included in the supplementary information (Table S 4.3).

4.4.2 *Saplings in different light conditions (2023)*

Insect herbivory: The sapling experiment calculated total herbivory damage by summing the damage percentages from nine feeding guilds for each leaf. The average total damage across ten leaves provided an estimate for each sapling. Individual damage types were also analyzed, and no correlations were observed between damage types during exploratory data analysis.

Given the percentage nature of the response variables and zero-inflated data for some damage types, generalized mixed models (glmmTMB) were used, with the site included as a random factor (Brooks et al., 2017). For total damage, leaf cutter, and leaf stippler damage, models were fitted using the beta distribution, including light gradient effects (sunlit sapling vs. shaded sapling) on insect herbivore damage. Zero-inflated beta models were applied for rare damage types with over 50% zeros (e.g., leaf skeletonisers, and gall makers). No significant trend was shown for individual gall maker types (spindle, erineum, and ocellate galls); therefore, their values were pooled and analyzed as single response variables using a zero-inflated beta family in the glmmTMB model. The analysis did not include leaf miners with no damage and leaf rollers were also eventually removed due to having very few numbers of recorded values in our study sites. Model assumptions were assessed using the DHARMa package (Hartig, 2022).

Predation rate: The same logistic regression approach as in predation rate over vertical gradient was used for sapling experiments, with sapling type (sunlit vs. shaded) as the predictor variable.

4.5 Results

4.5.1 *Vertical Gradient (2023-2024)*

Significant differences in light intensity and canopy openness were observed among the three strata of sugar maple trees in 2024 with an average light intensity of 2845 ± 682 lux in sun canopy, 1267 ± 181 lux in shaded canopy and 835 ± 93.8 lux in understory of sugar maple trees. Canopy openness was recorded as 28.9 ± 5.34% in the sun canopy, 9.71 ± 1.13% in the shaded canopy and 12.2 ± 1.41% in understory (Figure S 4.1)

Insect herbivory: We collected 136 insect herbivores across all 12 sites in 2024, including 79 stippling herbivores and 57 leaf cutters. However, no significant differences were observed among the three strata of sugar maple trees (Table S 4.2). The information regarding the collected samples is provided in Table S 4.1.

Predation rate: The analysis of 660 artificial caterpillars placed across the vertical gradient of sugar maple trees in 2023 revealed no statistically significant difference in total predation rates among tree strata. However, individual predation rates varied by vertical gradient. Arthropod predation, the most commonly recorded mark, was significantly higher in the understory compared to the sun canopy (z = 4.15, P < 0.001), with no significant difference between the two canopy strata. From arthropod collection in 2024, we identified some predators including: 114 spiders, 6 predatory mites, 4 hemipteran Reduviidae and 4 coleopteran Cantharidae (70 out of 128 individual arthropods were collected from understory). Mammal predation marks were more frequent in the understory, though the difference with canopy level was not statistically significant (z = 1.47, P = 0.13). In contrast, mammal predation in the shaded canopy was significantly higher than in the sun canopy (z = 2.67, P = 0.007). Bird predation was significantly lower in the understory compared to the sun canopy (z = -5.67, P < 0.001), with a non-significant reduction in the shaded canopy relative to the sun (z = -0.69, P = 0.48) (Table 4.1; Figure 4.2).

Table 4.1 Generalized linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) on predation rate of insect herbivore of 12 sugar maple sites and the impact of light (sunlit sapling vs. shaded sapling) on predation rate of insect herbivore of 15 sugar maple sapling sites at Kenauk nature in 2023.

Experiment		*GlmmTMB binomial distribution model*											
	Predictors	*Estimate*	*Std. Error*	*p*	*Estim ates*	*Std. Error*	*P*	*stimates*	*Std. Error*	*p*	*Estimates*	*Std. Error*	*p*
		Total predation			**Arthropod predation**			**Mammal predation**			**Bird predation**		
Vertical gradient	(Intercept)	1.53	0.17	< 0.001*	0.21	0.13	0.10	-3.27	0.36	< 0.001*	-0.48	0.13	< 0.001*
	Shaded canopy	0.35	0.26	0.18	-0.33	0.19	1.00	1.12	0.42	0.007 *	-0.13	0.19	0.48
	Understory	0.19	0.25	0.44	0.85	0.20	< 0.001*	0.66	0.44	0.13	-1.36	0.24	< 0.001*
Light intensity on sapling	(Intercept)	0.36	0.13	0.006	-0.29	0.13	0.028*	-3.06	0.32	< 0.001*	-1.53	0.17	< 0.001*
	Shaded sapling	0.13	0.19	0.49	0.28	0.18	0.13	0.19	0.43	0.66	-0.50	0.27	0.063

Note: The results include estimates, standard errors, and p-values for different predictors. In the vertical gradient effect, the intercept is sun canopy strata and response variables with binomial distribution fit in the model: **GlmmTMB (Predation ~ tree strata + (1|site), family = binomial(link = "logit"), data)**. In the light intensity effect between two saplings, intercept is sunlit sapling and Response variables with binomial distribution fit in the model: **GlmmTMB (Predation ~ sapling type + (1|site), family = binomial(link = "logit"), data).** Significant results are shown by * = P < 0.05.

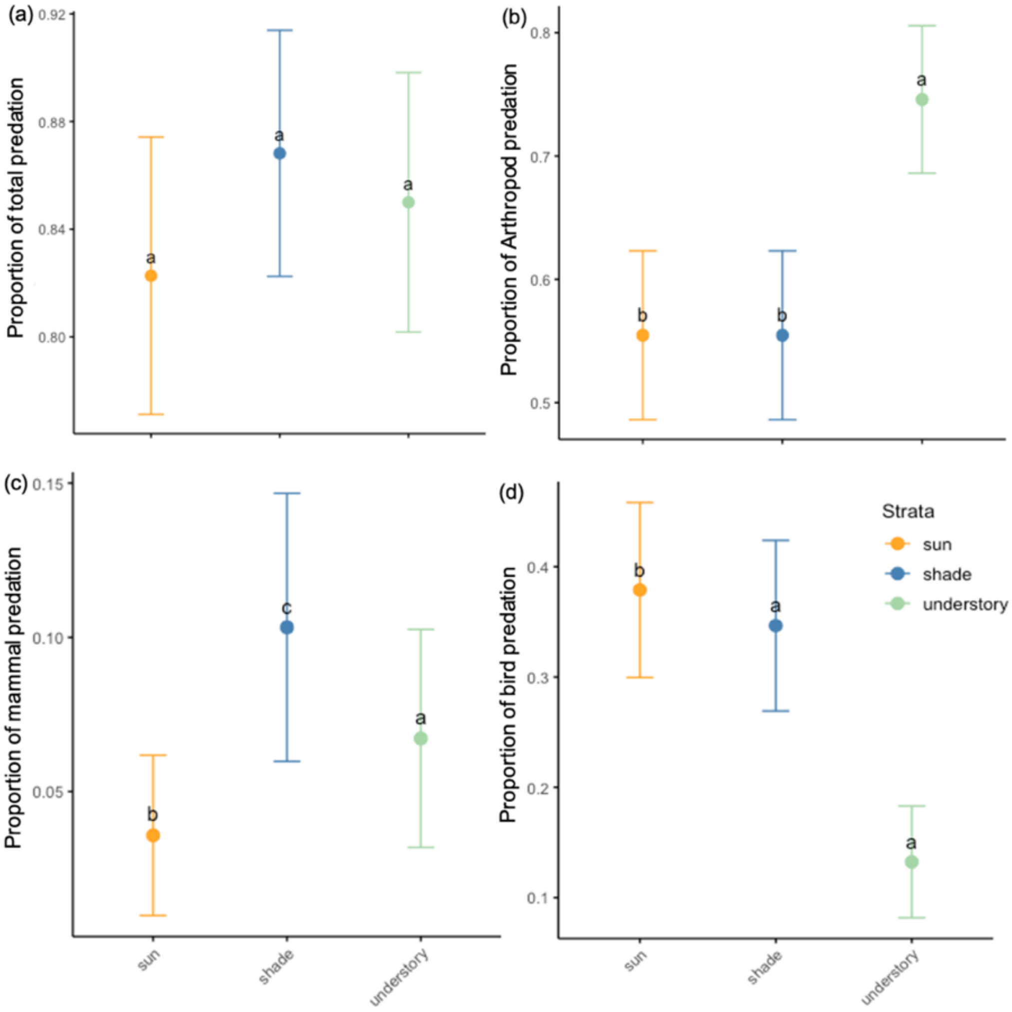


Figure 4.2 Estimated marginal means (with 95% confidence intervals) for predation rates by all predators combined (a), arthropod predators (b), mammal predators (c), and bird predators (d) on modeling clay caterpillars attached to three strata of sugar maple trees (sun canopy, shaded canopy, and understory) across 12 sites in 2023. Values marked with different letters indicate significant differences within strata (Tukey–Kramer test, adjusted P < 0.05).

Parasitism rate: In 2024, sleeve cages yielded a total of 13 parasitoid individuals, comprising 4 Hymenoptera from the Braconidae family (1 from the understory, two from the shaded canopy, and two from the sunlit canopy) and 9 Ichneumonidae (2 from the understory, 4 from the shaded canopy, and 3 from the sunlit canopy). The sample sizes were insufficient for statistical analysis, limiting the ability to interpret parasitism rate patterns meaningfully. Similarly, suction traps captured 32 parasitoids, including Diptera and Hymenoptera, with 20 individuals recorded at the canopy level (8 in the shaded canopy and 12 in the sunlit canopy) and 12 in the understory. Given the low sample size, no statistical analyses were performed on these data. Although suction traps are typically considered effective for capturing adequate samples for robust comparisons across forest habitat types (Favret et al., 2019), they did not yield the expected number of samples in this study.

4.5.2 *Saplings in different light conditions (2023)*

Temperature did not vary between sunlit and shaded habitats. However, light intensity and canopy openness differed significantly. Sun-exposed saplings had a light intensity of 5612 ± 735 lux and a canopy openness of 20.0 ± 4.22%, compared to shaded saplings, which exhibited a light intensity of 4063 ± 491 lux and a canopy openness of 7.90 ± 1.87% (Figure S 4.2)

Insect herbivory: Leaf damage assessments conducted on 600 leaves indicated slightly higher total damage levels in shaded saplings, driven by a few feeding guilds (cutters and stipplers) as shown in **Error! Reference source not found.**. However, statistical analysis revealed no significant differences in herbivory between the two treatments (Table S 4.4).

Predation rate: For the 450 artificial caterpillars placed on saplings in sunlit and shaded conditions, statistical analysis showed no significant differences in total predation rates or individual predation types (arthropod, mammal, and bird predation marks). However, the plots suggested slightly higher total predation in shaded saplings, primarily driven by arthropod predators and mammals, and marginally lower bird predation rates (Table 4.1; Figure 4.3).

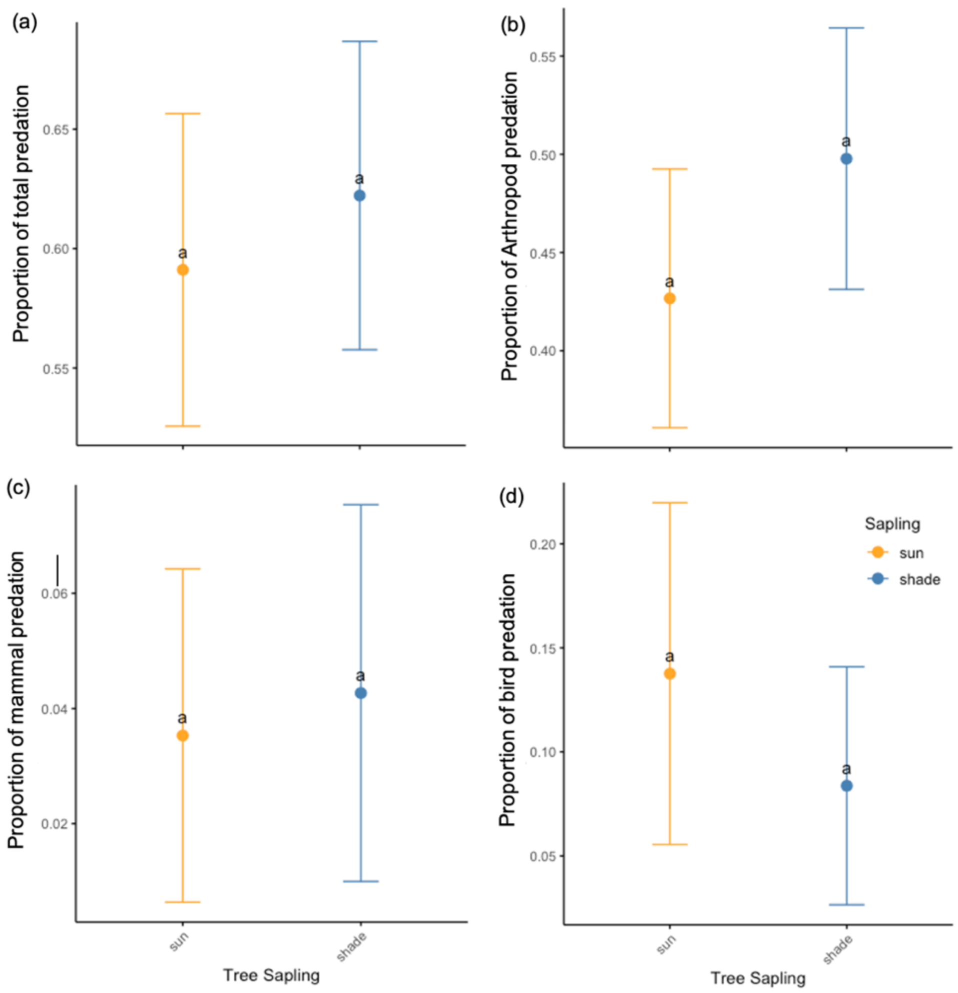


Figure 4.3 Estimated marginal means (with 95% confidence intervals) for predation rates by all predators combined (a), arthropod predators (b), mammal predators (c), and bird predators (d) on modeling clay caterpillars attached to two sugar maple saplings under different light intensity (sunlit sapling vs. shaded sapling) across 15 sites in 2023. Values marked with different letters indicate significant differences between two saplings (Tukey–Kramer test, adjusted P < 0.05).

4.6 Discussion

Our findings reveal distinct vertical stratification, primarily among arthropods and birds, with mammals playing a relatively minor role. However, we observed no influence of sun/shade conditions, suggesting that vertical positioning itself is the main factor. Arthropods are predominantly concentrated near the ground, while birds remain largely confined to the canopy. While bird predation supports the enemy-escape hypothesis by potentially driving insect herbivores downward into the understory, the higher activity of arthropod predators in the understory introduces an added layer of complexity to this expected pattern.

The sapling experiment showed no statistically significant differences in predation rates or herbivory between sunlit and shaded saplings, suggesting that the variations observed along the vertical gradient are more likely driven by proximity to the ground and overall spatial structure rather than direct light exposure. However, it is worth noting that the "sun" sites in this study were within a forested habitat, and the results might differ in an open forest edge environment.

Birds showed a clear preference for sun canopy-level prey, supporting the previous findings (Zvereva et al., 2020), a pattern that is likely due to increased prey visibility and higher productivity in upper foliage layers, offering more abundant foraging opportunities for birds due to dense foliage (Robinson & Holmes, 1982; Van Bael et al., 2003). Mammal predation showed a more complex pattern, being more frequent in the shaded canopy and understory compared to the sunlit canopy, possibly reflecting differences in accessibility and height from the ground or preferences for microhabitats that offer more cover from sun. On the other hand, arthropod predation peaked in the understory compared to the sunlit canopy. This aligns with one study in a Québec sugar maple temperate forests (Aikens et al., 2013) but differs from results found in other forest types (Zvereva et al., 2020). This may be attributed to the higher activity levels or greater abundance of ground based arthropod predators in lower strata that prefer to be closer to the forest floor due to their life cycle (Aikens et al., 2013; Zvereva et al., 2020).

It is also crucial to add that insectivore mammals and birds can target both predatory and herbivore arthropods. This could explain the low number of arthropod predators in the highest level of canopy, which means they use understory as a refuge. This has been confirmed in a bird exclusion experiment showed positive effect on arthropod predator abundance, which relates to the results we observed in the understory level (Aikens et al., 2013; Maguire et al., 2015; Mooney et al., 2012). In addition, our earlier work highlighted that sunlit canopy leaves possess greater physical defenses, including increased thickness and toughness, than shaded or understory leaves (Hakimara & Despland, 2024). These traits likely deter herbivores and may indirectly shape predator-prey dynamics by influencing herbivore distribution. Finally, the fake caterpillars made of clay are missing movement and chemical signals and this could lead to underestimation of predation rate in different tree strata (Zvereva & Kozlov, 2023).

Parasitism rates in our study were extremely low, with insufficient data for robust statistical analysis. This outcome echoes previous research highlighting the difficulties in capturing parasitoid-host interactions in natural settings (Sigut et al., 2018; Stireman et al., 2012). Additionally, the design of the sleeve cages, the timing of the experiment, and caterpillar performance within the cages could have influenced parasitoid behavior and potentially skewed results. Although suction traps provided some insights about the parasitoids in the study sites, perhaps timing or trap numbers need to be manipulated to get a better result. Future research could resolve these issues by combining several improved methodologies. Increasing the variety and number of traps, like flight interception traps or CD sticky traps, can enhance parasitoid sampling (Bar-Ness et al., 2012). Timing experiments to coincide with peak parasitoid activity periods, informed by pilot studies or existing literature, would also be beneficial. Open-environment exposure of caterpillars instead of sleeve cages may allow more natural parasitoid interactions, like using live sentinel prey (Libra et al., 2019), though it requires balancing practical constraints and risks of predation.

The differences in predation across strata emphasize the importance of top-down forces in shaping herbivore populations. While bottom-up pressures, such as variations in leaf traits influenced by light availability, play a critical role in herbivore fitness (Hakimara & Despland, 2024; Murakami & Wada, 1997a), given that over 70% of installed clay preys were attacked at least once, our findings demonstrate that predation by natural enemies can exert equally significant if not stronger influences align with the meta-analysis results (Vidal & Murphy, 2018). In particular, bird predation in the canopy appears to be critical regulators of herbivore populations in our study site, considering the high number of recorded birds in Kenauk Nature (*Bird Diversity in Kenauk Nature*, 2024), while arthropod predation dominates in the understory.

From a practical perspective, these findings offer valuable insights for forest management. While our results indicate that sunlight exposure does not exacerbate herbivore damage or reduce the effectiveness of natural enemies, broader ecosystem considerations remain critical. Adjusting canopy structure through selective thinning or other light-management strategies could support tree growth and health without increasing herbivory. However, it is important to balance these interventions to avoid excessive sunlight penetration, which could favor invasive species such as glossy buckthorn (*Rhamnus cathartica*), potentially competing with sugar maples and altering understory dynamics (Hamelin et al., 2016). Maintaining a dense and healthy canopy is equally vital, as it provides critical support for vertebrate predators while allowing arthropod predators to thrive in the understory by limiting competition from higher level of predators. This balance of top-down forces helps regulate herbivory and mitigate damage. Furthermore, shaded understory habitats are particularly important to conserve, given the shade tolerance and late-succession nature of sugar maples. A balanced approach to canopy management, one that preserves the ecological benefits of both sunlight exposure and shaded habitats, can enhance the resilience of sugar maple forests, which face significant challenges from defoliation (Duchesne et al., 2003; Payette et al., 1996).

This study provides new insights into how forest structural diversity influence natural enemy pressures on herbivores in sugar maple forests. The stratification of predation rates highlights the complex interactions between light availability, predator activity, and herbivore vulnerability. Although parasitism rates were too low for detailed analysis, the findings underscore the need for further research to uncover the factors shaping these interactions. By linking top-down pressures to the forest structure, this work contributes to a more comprehensive understanding of forest ecosystem dynamics and offers practical strategies for conserving sugar maple-dominated forests in Eastern Canada.

4.7 Supplementary information

Table S 4.1 Abundance of insect herbivores collected in 2024 over the sampling season from six sites each includes three strata (sun canopy, shaded canopy and understory) of sugar maple trees at Kenauk nature. The abundance number per row represent the same morphotypes. Most samples specially in early season were either nymph or at early larval stage so we were not able to identify them to the lower taxonomic level than family.

DATE	SITE	STRATA	FAMILY	ORDER	ABUNDANCE	STAGE OF LIFE	FEEDING GUILD
EARLY.JUNE	2	Shade	Aphididae	Hemiptera	1	Adult	Stippler
EARLY.JUNE	2	Shade	Nuctoidae	Lepidoptera	1	Caterpillar	Cutter
EARLY.JUNE	5	Understory	Miridae	Hemiptera	1	Nymph	Stippler
EARLY.JUNE	2	Shade	Aphididae	Hemiptera	1	Nymph	Stippler
EARLY.JUNE	2	Understory	Aphididae	Hemiptera	3	Nymph	Stippler
EARLY.JUNE	2	Understory	Thripidae	Thysanoptera	1	Nymph	Stippler
EARLY.JUNE	2	Understory	Geometridae	Lepidoptera	1	Caterpillar	Cutter
EARLY.JUNE	2	Understory	Meloidae	Coleoptera	1	Nymph	Cutter
EARLY.JUNE	2	Understory	Geometridae	Lepidoptera	1	Caterpillar	Cutter
EARLY.JUNE	2	Understory	Aphididae	Hemiptera	4	Nymph	Stippler
EARLY.JUNE	2	Understory	Nuctoidae	Lepidoptera	1	Caterpillar	Cutter
EARLY.JUNE	2	Understory	Thripidae	Thysanoptera	2	Nymph	Stippler
EARLY.JUNE	2	Shade	Aphididae	Hemiptera	4	Adult	Stippler
EARLY.JUNE	4	Shade	Miridae	Hemiptera	1	Adult	Stippler
EARLY.JUNE	6	Sun	Aphididae	Hemiptera	2	Adult	Stippler
EARLY.JUNE	6	Understory	Aphididae	Hemiptera	1	Nymph	Stippler
EARLY.JUNE	6	Understory	Curcolionidae	Coleoptera	1	Adult	Cutter
EARLY.JUNE	6	Shade	Aphididae	Hemiptera	2	Adult	Stippler
EARLY.JUNE	6	Shade	Psyllidae	Hemiptera	1	Adult	Stippler
EARLY.JUNE	7	Sun	Psyllidae	Hemiptera	1	Adult	Stippler
EARLY.JUNE	7	Sun	Aphididae	Hemiptera	1	Nymph	Stippler
EARLY.JUNE	7	Shade	Geometridae	Lepidoptera	1	Caterpillar	Cutter
EARLY.JUNE	7	Shade	Cicadellidae	Hemiptera	2	Adult	Stippler
EARLY.JUNE	7	Shade	Nuctoidae	Lepidoptera	1	Caterpillar	Cutter
EARLY.JUNE	7	Understory	Miridae	Hemiptera	1	Adult	Stippler
EARLY.JUNE	8	Sun	Aphididae	Hemiptera	6	Adult	Stippler
EARLY.JUNE	8	Sun	Nuctoidae	Lepidoptera	1	Caterpillar	Cutter
EARLY.JUNE	8	Sun	Geometridae	Lepidoptera	1	Caterpillar	Cutter
EARLY.JUNE	8	Shade	Aphididae	Hemiptera	9	Nymph	Stippler
EARLY.JUNE	8	Understory	Aphididae	Hemiptera	1	Nymph	Stippler
EARLY.JUNE	8	Understory	Curcolionidae	Coleoptera	1	Adult	Cutter
EARLY.JUNE	8	Understory	Psyllidae	Hemiptera	1	Adult	Stippler

EARLY.JUNE	8	Understory	Miridae	Hemiptera	1	Adult	Stippler
LATE.JUNE	6	Shade	Aphididae	Hemiptera	2	Nymph	Stippler
LATE.JUNE	6	Sun	Aphididae	Hemiptera	1	Nymph	Stippler
LATE.JUNE	6	Sun	Geometridae	Lepidoptera	1	Caterpillar	Cutter
LATE.JUNE	6	Understory	Cicadellidae	Hemiptera	2	Nymph	Stippler
LATE.JUNE	6	Understory	Tingidae	Hemiptera	1	Adult	Stippler
LATE.JUNE	4	Understory	Miridae	Hemiptera	3	Nymph	Stippler
LATE.JUNE	2	Sun	notodontidae	Lepidoptera	1	Caterpillar	cutter
LATE.JUNE	2	Shade	noctuidae	Lepidoptera	2	Caterpillar	cutter
LATE.JUNE	5	Shade	Aphididae	Hemiptera	1	Adult	Stippler
LATE.JUNE	8	Sun	Cicadellidae	Hemiptera	2	Nymph	Stippler
LATE.JUNE	8	Shade	Miridae	Hemiptera	1	Nymph	Stippler
LATE.JUNE	8	Shade	Cicadellidae	Hemiptera	1	Nymph	Stippler
LATE.JUNE	8	Shade	Geometridae	Lepidoptera	1	Caterpillar	Cutter
LATE.JUNE	7	Shade	Geometridae	Lepidoptera	1	Caterpillar	Cutter
LATE.JUNE	7	Shade	Aphididae	Hemiptera	1	Adult	Stippler
LATE.JUNE	8	Understory	Curcolionidae	Coleoptera	1	Adult	Cutter
LATE.JUNE	8	Understory	Geometridae	Lepidoptera	1	Adult	Cutter
LATE.JUNE	8	Understory	Nuctoidae	Lepidoptera	1	Adult	Cutter
LATE.JUNE	7	Understory	Notodontidae	Lepidoptera	1	Caterpillar	cutter
EARLY.JULY	8	understory	Curculionidae	Coleptera	2	Adult	Cutter
EARLY.JULY	8	Shade	Notodontidae	Lepidoptera	1	Caterpillar	Cutter
EARLY.JULY	4	Sun	Notodontidae	Lepidoptera	3	Caterpillar	Cutter
EARLY.JULY	4	understory	Aphididae	Hemiptera	1	Adult	Stippler
EARLY.JULY	4	Shade	Tortricidae	Lepidoptera	1	Caterpillar	roller
EARLY.JULY	4	Sun	Miridae	Hemiptera	2	Nymph	Stippler
EARLY.JULY	4	Shade	Thripidae	Thysanoptera	1	Adult	Stippler
EARLY.JULY	4	Shade	Notodontidae	Lepidoptera	1	Caterpillar	Cutter
LATE.JULY	4	Sun	Notodontidae	Lepidoptera	1	Caterpillar	Cutter
LATE.JULY	4	Sun	Noctuidae	Lepidoptera	2	Caterpillar	Cutter
LATE.JULY	2	Sun	Geometridae	Lepidoptera	1	Caterpillar	Cutter
LATE.JULY	2	Understory	Thripidae	Thysanoptera	1	Adult	Stippler
LATE.JULY	2	Understory	Aphididae	Hemiptera	2	Adult	Stippler
LATE.JULY	2	Understory	Geometridae	Lepidoptera	2	Caterpillar	Cutter
LATE.JULY	4	Understory	Geometridae	Lepidoptera	3	Caterpillar	Cutter
LATE.JULY	4	Understory	Cicadellidae	Hemiptera	1	Adult	Stippler
LATE.JULY	6	Sun	Cicadellidae	Hemiptera	1	Adult	Stippler
LATE.JULY	6	Sun	Aphididae	Hemiptera	1	Adult	Stippler
LATE.JULY	6	Sun	Aphididae	Hemiptera	3	Adult	Stippler
LATE.JULY	6	Shade	Notodontidae	Lepidoptera	1	Caterpillar	Cutter
LATE.JULY	5	Sun	Notodontidae	Lepidoptera	1	Caterpillar	Cutter
LATE.JULY	5	Understory	Cicadellidae	Hemiptera	1	Adult	Stippler
LATE.JULY	5	Understory	Notodontidae	Lepidoptera	1	Caterpillar	Cutter

LATE.JULY	7	Understory	Curcolionidae	Coleoptera	1	Adult	Cutter
LATE.JULY	8	Sun	Notodontidae	Lepidoptera	1	Caterpillar	Cutter
LATE.JULY	8	Sun	Geometridae	Lepidoptera	1	Caterpillar	Cutter
LATE.JULY	8	Understory	Notodontidae	Lepidoptera	1	Caterpillar	Cutter
LATE.JULY	8	Shade	Notodontidae	Lepidoptera	2	Caterpillar	Cutter
LATE.JULY	8	Shade	Aphididae	Hemiptera	1	Adult	Stippler
LATE.JULY	8	Shade	Notodontidae	Lepidoptera	1	Caterpillar	Cutter

Table S 4.2 Generalized linear mixed model coefficient results for the effect of stratification (sun canopy, shaded canopy, and understory) on insect herbivore abundance in six sugar maple sites at Kenauk nature in 2024.

	GlmmTMB Poisson distribution model								
Predictors	*Estimates*	*Std. Error*	*p*	*Estimates*	*Std. Error*	*P*	*Estimate*	*Std. Error*	*P*
	Total abundance			Leaf cutters			Leaf stipplers		
(Intercept)	0.97	0.21	< 0.001*	0.084	0.31	0.78	0.51	0.22	0.022 *
Shaded canopy	0.065	0.23	0.77	-0.23	0.38	0.54	0.21	0.29	0.45
Understory	0.085	0.22	0.70	0.060	0.35	0.86	0.048	0.29	0.86

Note: The results include estimates, standard errors, and p-values for different predictors. Intercept is sun canopy strata. Leaf cutter, leaf stipplers, and total insect herbivores with count distribution fit in the model: **GlmmTMB (Abundance ~ tree strata + (1|site), family = Poisson, data).**

Table S 4.3 Abundance of parasitoids collected in 2024 over the sampling season from 12 sites each includes three strata (sun canopy, shaded canopy and understory) of sugar maple trees at Kenauk nature.

YEAR	METHOD	DATE	SITE	STRATA	FAMILY	ORDER	ABUNDANCE
2024	Suction traps	mid.July	5	Shade	Phoridae	Diptera	1
	Suction traps	mid.July	2	Shade	Phoridae	Diptera	2
	Suction traps	mid.July	5	Understory	braconidae	Hymenoptera	1
	Suction traps	mid.July	7	Shade	Tachinidae	Diptera	1
	Suction traps	mid.July	2	Sun	Eulophidae	Hymenoptera	2
	Suction traps	mid.July	2	Sun	braconidae	Hymenoptera	1
	Suction traps	mid.July	2	Sun	Phoridae	Diptera	1
	Suction traps	mid.July	2	Understory	Platygastridae	Hymenoptera	1
	Suction traps	early. June	7	Sun	braconidae	Hymenoptera	1
	Suction traps	early. June	5	Sun	Phoridae	Diptera	1
	Suction traps	early. June	5	Understory	Mymaridae	Hymenoptera	2
	Suction traps	early. June	5	Shade	Phoridae	Diptera	1
	Suction traps	early. June	5	Shade	Mymaridae	Hymenoptera	2
	Suction traps	early. June	5	Shade	Eulophidae	Hymenoptera	1
	Suction traps	early. June	2	Understory	Eulophidae	Hymenoptera	1
	Suction traps	early. June	7	Understory	Phoridae	Diptera	2
	Suction traps	early. June	5	Sun	Tachinidae	Diptera	1
	Suction traps	early. June	5	Sun	Eulophidae	Hymenoptera	1
	Suction traps	early. June	7	Sun	Eulophidae	Hymenoptera	2
	Suction traps	early. June	7	Sun	Phoridae	Diptera	1

	Suction traps	early. June	2	Understory	Phoridae	Diptera	1
	Suction traps	early. June	2	Understory	Chalicididae	Hymenoptera	2
	Suction traps	early. June	2	Sun	Chalicididae	Hymenoptera	1
	Suction traps	early. June	2	Understory	Mymaridae	Hymenoptera	2
	Sleeve cages	mid.July	4	Understory	Braconidae	Hymenoptera	1
	Sleeve cages	mid.July	8	Sun	Ichneumonidae	Hymenoptera	3
	Sleeve cages	mid.July	8	Sun	Braconidae	Hymenoptera	2
	Sleeve cages	mid.July	8	Shade	Braconidae	Hymenoptera	1
	Sleeve cages	mid.July	8	Shade	Ichneumonidae	Hymenoptera	4
	Sleeve cages	mid.July	8	Understory	Ichneumonidae	Hymenoptera	2

Table S 4.4 Generalized linear mixed model coefficient results for the effect of light condition (sunlit sapling vs. shaded sapling) on insect herbivore damage in 15 sugar maple sites at Kenauk nature in 2023.

	GlmmTMB beta distribution model						*GlmmTMB zero-inflation beta distribution model*					
Predictors	*Estimates*	*Std. Error*	*p*	*Estimates*	*Std. Error*	*p*	*Conditional model*			*Zero-inflation model*		
							Estimates	*Std. Error*	*p*	*Estimates*	*Std. Error*	*p*
	Total damage			**Leaf cutters**			**Sum skeletoniser**					
(Intercept)	-2.73	0.14	< 0.001*	-3.75	0.22	< 0.001*	-5.06	0.23	< 0.001*	1.01	0.41	0.014
Shaded sapling	0.20	0.17	0.24	-0.064	0.23	0.78	-0.27	0.26	0.30	-0.26	0.56	0.63
	Leaf stipplers						**Sum gall makers**					
(Intercept)	-3.85	0.18	< 0.001*				-5.49	0.23	< 0.001*	0.0013	0.42	0.99
Shaded sapling	0.27	0.19	0.1				0.17	026	0.52	-0.35	0.54	0.52

Note: The results include estimates, standard errors, and p-values for different predictors. Intercept is sunlit sapling. Leaf cutter, leaf stipplers, and total insect herbores with beta distribution fit in the model: **GlmmTMB (damage percentage~ tree sapling+ (1|site), family = beta_family(link = "logit"), data).** The zero-inflated response variables including leaf skeletonisers and gall makers fit in model: **GlmmTMB(damage percentage, ziformula=~ tree sapling + (1|site), family=beta_family(), data).**

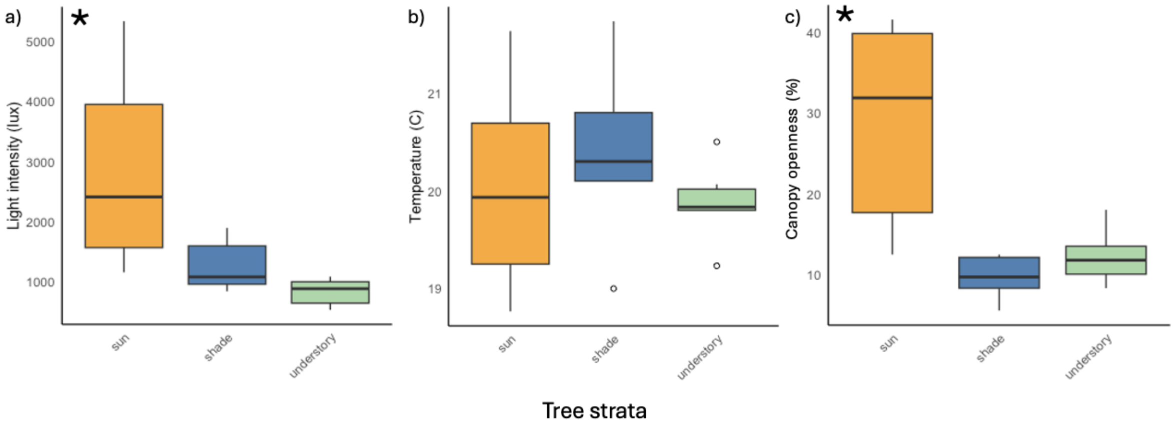


Figure S 4.1 Boxplots showing variation of measured environmental factors between three strata of sugar maple trees (sunlit canopy, shaded canopy and understory) in 6 sites in 2024: a) light intensity, b) temperature and c) canopy openness. Graphs with strike marks show significant difference between treatments.

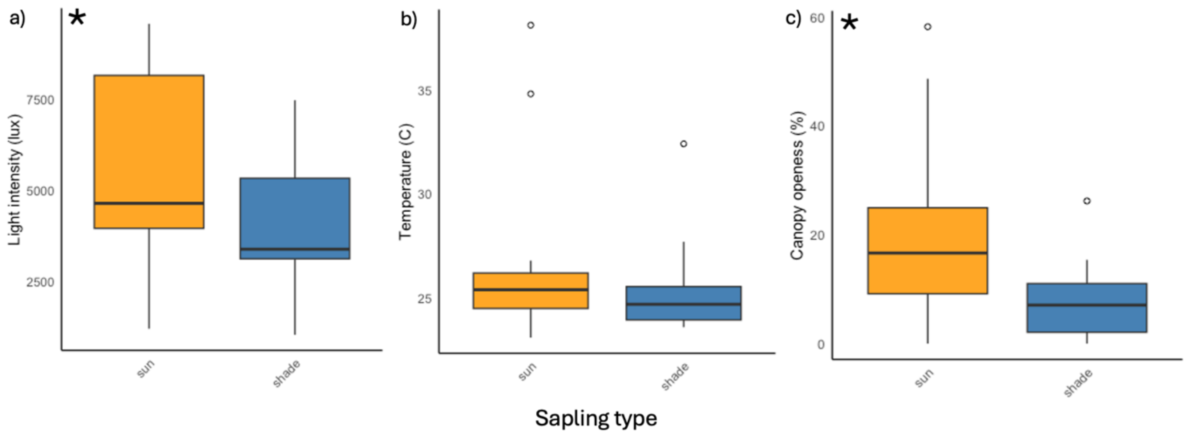


Figure S 4.2 Boxplots showing variation of measured environmental factors between two sugar maple saplings (sunlit sapling and shaded sapling) in 2023 done in 15 sites: a) light intensity, b) temperature and c) canopy openness. Graphs with strike marks show significant difference between treatments.

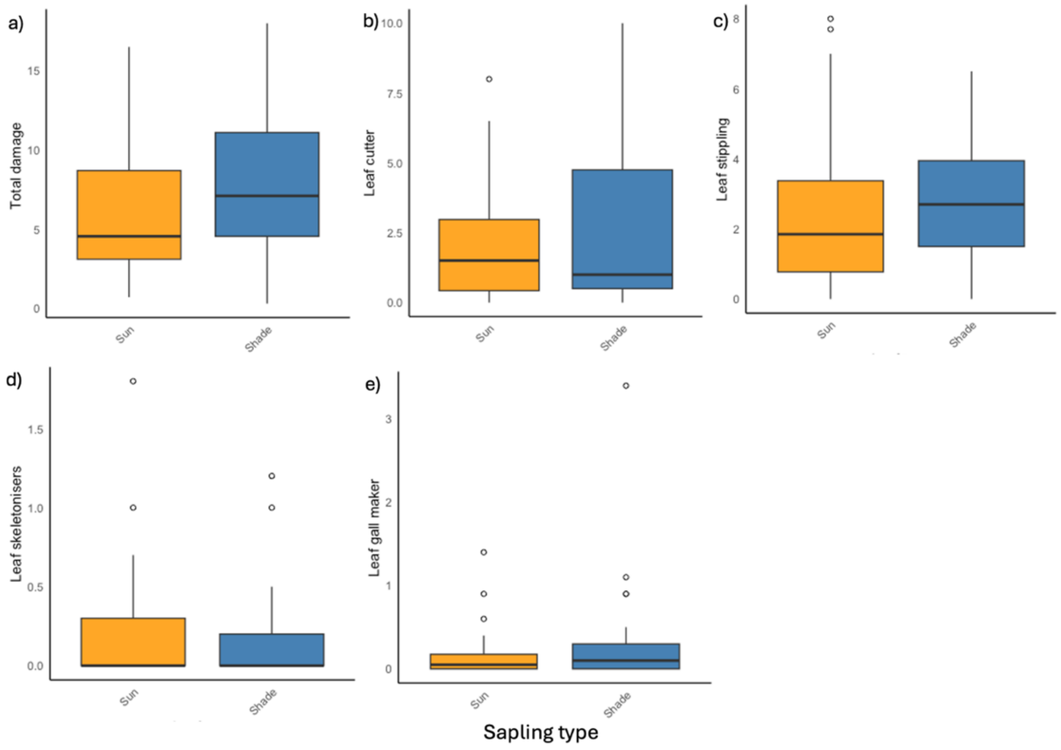


Figure S 4.3 Boxplots showing variation of five leaf damages caused by herbivores from different feeding guilds on two sugar maple saplings (sunlit sapling and shaded sapling) in 15 sites in 2023 (n= 10 leaves per type of sapling) a) total damage, b) leaf cutter damage, c) leaf stippling damage, d) leaf skeletoniser damage and e) leaf gall makers. No leaf miner damage and very few leaf rollers that were not included in the analysis.

5 Chapter 5: Bottom-up pressures on insect herbivory patterns on two closely related maple trees in a temperate forest in Quebec

The following chapter is based on the submitted manuscript: Hakimara, M., & Despland, E. (2024). Bottom-up pressures on insect herbivory patterns on two closely related maple trees in a temperate forest in Quebec, *Canadian Journal of Forest Research.*

5.1 Abstract

Closely related plant species often share similar leaf traits, experience same level of herbivore damage and support similar communities of herbivorous insects. The species pair of sugar maple (*Acer saccharum*) and black maple (*Acer nigrum*) provide an ideal system to test hypotheses about drivers of insect herbivory in long-lived forest trees, since they are closely related yet differ in leaf physical traits. We tested whether variations in foliar traits such as leaf thickness, toughness, specific leaf area (SLA), and trichome density influence insect herbivore damage, community composition and feeding behavior on these two closely related trees.

Field surveys over three years in two nature reserves involved visual assessments of ten insect herbivore damage types by different insect-feeding guilds and measurements of leaf traits. Results showed no significant differences in total herbivory damage and abundance of insect herbivores between the maple species. However, specific damage types varied, with less yellow blotches and stippler damage, but higher skeletoniser damage on sugar maples. Leaf traits differed consistently, with black maples having thicker and tougher leaves with lower SLA and higher trichome density. However, these traits did not correlate significantly with total herbivory damage. Laboratory bioassays with two common moth caterpillars revealed no significant preference or survival rate differences on foliage from the two maple species. These findings show that while foliar traits differ, they do not offer better defense in black

maples. Results do not support a role for insect herbivory in driving black maple's distinctive leaf traits or separation between the two maple species.

5.2 Introduction

Host plant defenses, as a bottom-up force, play a critical role in deterring herbivory by creating physical barriers or altering the feeding behavior of herbivores (Carmona et al., 2011; Hanley et al., 2007). These defenses can vary significantly between plant species and even among individuals within a species, contributing to differences in herbivory patterns (Agrawal, 2007; López-Carretero et al., 2016). Such variability is particularly relevant in closely related plant species, where divergence in physical traits may shape herbivore community composition and allow plants to escape shared herbivore pressures (Nakadai et al., 2014; Pearse & Hipp, 2009). This study examines the role of physical defenses, including trichomes, in influencing herbivore community structures in sugar maple (*Acer saccharum*) and black maple (*Acer nigrum*), offering insights into how subtle defensive trait differences affect patterns of herbivory.

Sugar maple and black maple, two species in the hard maple group, share an overlapping native range across North America (north of 35° latitude and east of 100° longitude) (Jackson, 2020). As a dominant species in northern forests, sugar maple is vital both ecologically and economically (Godman et al., 1990; Jackson, 2020), while black maple shares many similar traits, such as height, leaf structure, and sap used in syrup production (Gabriel, 1990; Hilaire & Graves, 1999). Despite these similarities, black maple possesses notable morphological differences, including darker green leaves with more trichomes, less lobing, and bark with deeper furrows (Gabriel, 1990). Some researchers have considered them a single species due to genetic compatibility (Skepner, A. P., 1997), but these morphological differences, particularly those related to leaf physical traits, warrant their classification as separate species

(Pugeault et al 2024). The genetic similarity in two targeted maple trees helps to minimize the confounding effects of phylogenetic distance, while physical trait differences make the two species ideal for examining how slight but significant trait variations may influence herbivory (Pearse & Hipp, 2009).

The phylogenetic composition of host plant communities shapes herbivore communities, with closely related plants often hosting similar herbivores due to shared traits (Schowalter & Ganio, 1998; Volf et al., 2017). Several studies have reported significant differences in herbivory severity among more distantly related tree species within the same forest (Carmona et al., 2011; Harrow, 2014). Yet, for closely related species, the structure of herbivore communities is often reported similar, linked to shared chemical and physical defenses (Pearse & Hipp, 2009; Rasmann & Agrawal, 2011; Southwood et al., 2005). Comparison of 14 *Acer* species in a temperate forest of Japan showed that more phylogenetically close *Acer* species hosted similar herbivore communities. Leaf physical traits showed phylogenetic signals and influenced herbivorous insect community assembly (Nakadai et al., 2014). However, other studies have shown divergence in leaf defensive traits among closely related plants, allowing these hosts to effectively 'escape' shared insect herbivory pressure. As a result, such divergence shapes distinct herbivory patterns within plant communities (Basset & Novotny, 1999; Endara et al., 2017).

In the context of plant defenses, while chemical defenses have been extensively studied, physical traits also play an essential role in influencing herbivory patterns and are sometimes even better predictors of herbivory (Carmona et al., 2011; Hanley et al., 2007; Salgado-Luarte et al., 2023). Leaf physical defenses such as toughness, thickness, and trichome density can act as significant bottom-up forces, affecting the palatability of leaves and deterring herbivores (Hairston et al., 1960; Schowalter et al., 1986; Singer & Stireman, 2005). In the case of sugar maple and black maple, previous research has reported differences in physical traits like leaf toughness, thickness, and trichome density, with black maple generally having higher values for these traits (Gabriel, 1973, 1990). These traits, along with specific leaf area (SLA) and water content, may influence herbivore performance and preference by altering leaf quality and palatability (Björkman et al., 2008; Caldwell et al., 2016; N. M. Johnson & Baucom, 2023; Salgado-Luarte et al., 2023). Sugar and black maple thus represent two very closely related species that differ significantly in leaf physical traits and constitute an ideal system to test the role of these traits in driving insect herbivory, as distinct from general phylogenetic signals.

Furthermore, the abundance of tree species can also influence herbivory patterns. More abundant species, like sugar maple, may experience higher herbivory rates, as larger populations can attract more herbivores and create more structurally complex habitats that support a higher insect diversity (Jactel & Brockerhoff, 2007; Kennedy & Southwood, 1984; Volf et al., 2019). In contrast, black maple, which is much rarer in Québec, may experience lower herbivore pressure.

The focus on sugar and black maples is also motivated by concerns over climate resilience. Sugar maples are vulnerable to drought, heat, and urbanization (Godman et al., 1990; Graves, 1994; Kriebel, 1957), which makes them particularly susceptible to displacement as climate change reduces suitable habitat (Iverson et al., 2008; Parry, 2007). Additionally, sugar maple populations have shown signs of decline partly due to insect herbivory in southern Quebec, where this study was conducted (Côté & Ouimet, 1996; Duchesne et al., 2003; Horsley et al., 2002; Payette et al., 1996). Though drought, competition, and other herbivory like deer browsing are also contributing factors (Boerner & Brinkman, 1996; Hett & Loucks, 1971; Taylor & Aarssen, 1989), the impact of insect herbivory on sapling mortality remains understudied, particularly for tiny seedlings (Gardescu, 2003). Meanwhile, black maple, though currently stable internationally and listed as "Least concern" at IUCN (Crowley &

Barstow, 2017) but is vulnerable in Quebec mostly due to development (*Black Maple-Endangered species in Quebec*, 2024; Potter & Hargrove, 2013). Furthermore, black maple's tolerance for flooding and drought makes it a viable option for conservation and breeding programs to increase resilience in maple populations (Gabriel, 1990; Graves, 1994).

This study aims to deepen our understanding of herbivore host selection by analyzing the defensive strategies of sugar and black maples in temperate forests. Specifically, we investigate whether variations in leaf physical traits between these two species influence the insect herbivore community patterns. Our objectives are to (1) quantify and compare leaf physical defenses between sugar and black maple in urban and natural forest sites, (2) assess and compare the diversity of insect herbivores on each maple species, and (3) test whether observed herbivory patterns align with caterpillar behavior in controlled lab conditions. We hypothesize that black maple's higher physical defenses make it less susceptible to insect herbivores than sugar maple, a dominant species in both parks studied. Furthermore, we predict that the performance and preference of two representative caterpillar species in lab tests will reflect the field damage patterns observed between the two maples.

5.3 Methods

5.3.1 *Study site and plant species*

The initial two years of field surveys took place within the temperate sugar maple forest of Kenauk Nature Property, situated in southwestern Quebec, Canada (45°42'N; 74°53'W). This property spans two regions, extending from the Outaouais River valley to the Laurentian Mountains. As indicated on the domains map provided by the Quebec Ministry of Forests, the area falls within the temperate deciduous forest's maple/bitternut hickory domain (Quebec Ministry of Forests, 2023). Despite the rarity of black maple in Quebec, Kenauk is uniquely endowed with an entire forest of these trees, designated as an ecological reserve. While access to the denser areas of mature black maple forest in Kenauk was restricted due to conservation considerations, our sampling was conducted near the forest margin, where black maple and sugar maple saplings were present together. We carried out the third year of fieldwork in the black maple section of Bois des Liesse Nature Park (45° 29' N; 73°46' W), recognized as an urban forest predominantly dominated by sugar maples (Ville de Montreal, 2023). This park was selected due to its mixed stand of black maple and sugar maple trees. We identified 12 sites in Kenauk (added two more for the second year) and 20 in Bois des Liesse, each containing one sugar maple and one black maple sapling (mean height 1.8 m) to sample. Knowing that saplings in understory receive more herbivory damage in sugar maple forest (Hakimara & Despland, 2024), we focused on sapling trees for all the measurements and experiments. For visual clarity and to present all sampling, measurement and experiments and their respective timings in one place, an infographic has been included as Figure 5.1.

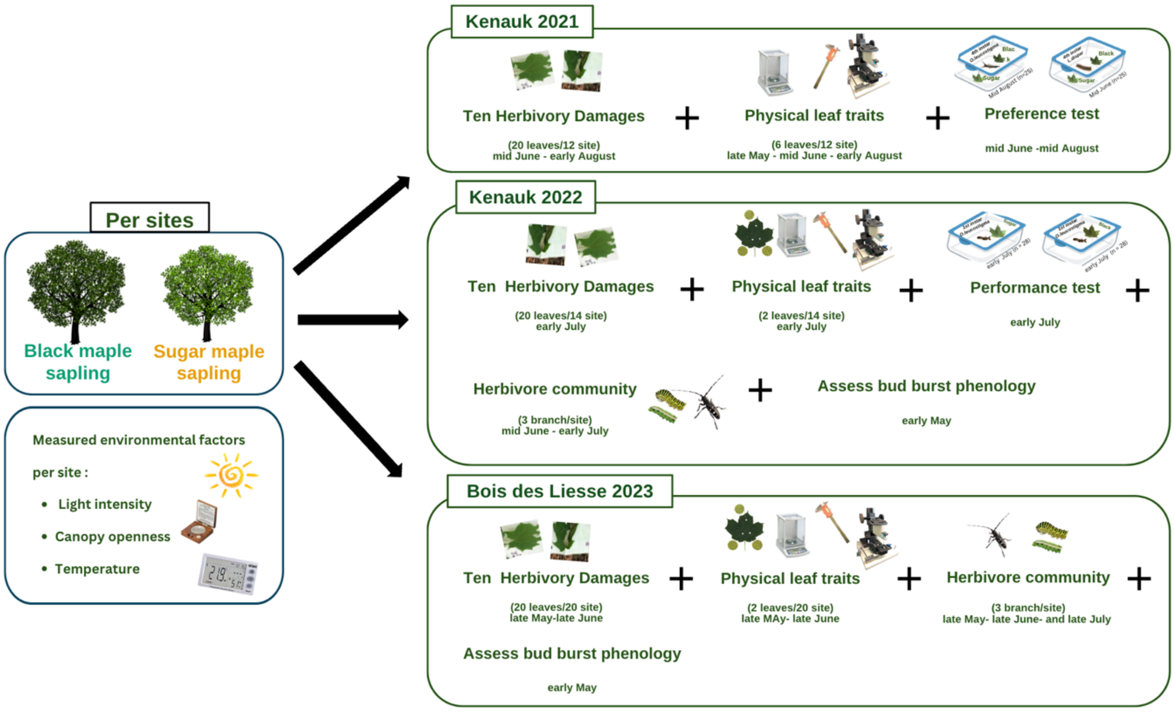


Figure 5.1 Infographic detailing the data collections used in the 2021 and 2022 study in Kenauk nature, as well as 2023 study in Bois des Liesse. This graphic overview the experimental setup, timeline, data collection techniques, and key variables measured.

5.3.2 *Assessment of insect herbivore damages*

The visual assessment of insect herbivory damage on leaves provides a reliable method for experiments to compare damage values across different treatments (M. T. J. Johnson et al., 2016; Landsberg, 1989; Schowalter, 2006). Consequently, we visually quantified ten types of herbivory damage as the percentage of leaf area affected by distinct insect feeding guilds, including leaf rollers, skeletonisers, cutters, stipplers, spindle galls, erineum gall, ocellate gall, leaf miners, maple leaf-cutters, maple leaf-blotch miners and yellow blotch mark (caused by pear thrips *Taeniothrips inconsequens* (Uzel)) (Houston et al., 1990; M. T. J. Johnson et al., 2016; Thomas et al., 2010; Wint, 1983). All assessments were conducted by a trained intern for each study location per year based on maple leaf damage guidelines provided (Houston et al., 1990), where the previous year's intern monitored the new intern in the subsequent year to measure around 20 first leaf damages together and assure consistency in their estimations. In 2021, we surveyed 12 sites at Kenauk Nature repeated twice over the sampling season (17 June and 10 August), and in 2022, we expanded to 14 sites, visually inspecting ten leaves per species at each site on a single date during the season (July 7). Sampling throughout the season was not repeated at the second year due to the challenges encountered in reaching the sites. We calculated the sum of all damages per leaf per sapling as the total damage value, and these values were averaged for ten observed leaves per sapling to be used for the analysis of total and separate damage types. The same insect herbivory damage assessment was repeated the following year at Bois des Liesse Nature Park on 20 sites, each with one black maple and one sugar maple sapling. Sampling was conducted twice during 2023, in May and June to get more robust insect herbivory damage data (Schowalter et al., 1986; Turcotte et al., 2014; Zehnder et al., 2009).

5.3.3 *Leaf physical traits and phenology of leaf bud burst*

To evaluate differences in defensive traits between two congeneric Acer species, in both Kenauk Nature and Bois des Liesse Nature Park, we conducted measurements of various leaf physical traits, including thickness, water content, specific leaf area (SLA), toughness, and leaf trichome density. At Kenauk, all traits were assessed on May 21, June 17 and August 10, 2021, where three fully expanded leaves were collected per species per site, resulting in a total of 72 leaves (3 leaves * 2 species * 12 sites). Leaf toughness was measured in the last sampling date on mature leaves because it increases as leaves mature. In 2022, the same leaf traits were measured for one leaf per sapling per site on July 6 (1 leaf * 2 sapling * 14 sites = 28 leaves). Access to the sites was restricted access and further sampling was not possible. The Kenauk leaf trait analysis results identified leaf toughness as a potentially more effective defense in black maples than in sugar maples. Therefore, in the following sampling in Bois des Liesse, this specific leaf trait was measured twice, in late May and late June (with the average used for analysis), on two leaves collected per sapling per site each time (a total of 80 leaves = 2 leaves * 2 species * 20 sites). Other traits were assessed once in late June 2023, with one leaf collected per sapling per site, resulting in 40 leaves (1 leaf * 2 species * 20 sites). Given the conservation status of black maple trees, extensive leaf trait sampling was not feasible at either location.

In both sampling years, randomly collected leaves were brought to the lab for fresh weighing and thickness measurement using a digital caliper. In the first year, leaf toughness was measured using a digital penetrometer burrowed from Janice Cooke's laboratory at the University of Alberta, Canada. In the second year, we used a penetrometer built at Concordia University following thorough instructions provided by Janice Cooke's lab (Yataco et al., 2024). Subsequently, leaves were scanned, labeled, and dried in an oven for 72 hours. We recorded the weights of the dried leaves to determine water content and SLA values. Trichome density on the abaxial surface area was measured in 2022 and 2023 on a separate leaf collected from each sapling per site to avoid any possible trichome loss from other measurements. From each leaf, three disks, each with 6 mm diameter ($28.27mm^2$ area), were manually punched out - one on the central vein and two in areas without veins (Figure 5.2).

These disks were then observed under a Nikon SMZ1500 stereomicroscope and photographed using a Nikon digital sight DS-Fi1 camera to count the number of trichomes on each. An average number of trichomes from three disks per leaf was used for the analysis (Hilaire & Graves, 1999).

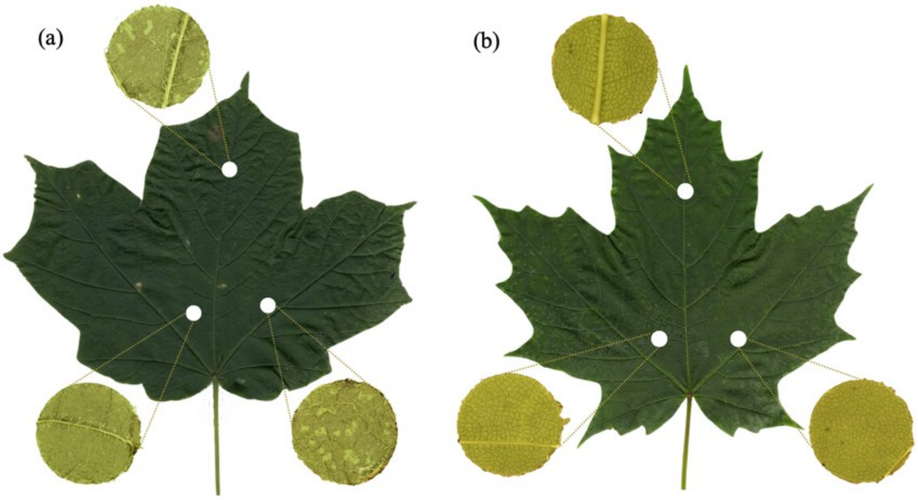


Figure 5.2 Illustration of sampling areas on black maple and sugar maple leaves for trichome density measurement in 2022 at Kenauk and in 2023 at Bois des Liesse. Three punched circles on each leaf were used to count trichomes, as shown in (a) Black maple leaf and (b) Sugar maple leaf.

Additionally, we scored the phenology of leaf-flush on sugar maple and black maple saplings on 10-May-2022 in Kenauk and on 10-May-2023 in Bois des Liesse by observing ten buds on each maple saplings. The bud burst ranking was assessed on a scale of 0 to 30, with 0 indicating small closed buds covered in brown scales, ten indicating swollen buds that rounded at the tips, 20 indicating small leaves left the bud casing, and 30 indicating full-size expanded leaves (Hannerz, 1999; Meier et al., 2009). Along with all leaf budburst phenology measurements, environmental factors, including light intensity (using a Reed light meter model R 1930), temperature (Using a Reed temperature monitor model R6000), and canopy openness (using a spherical densitometer) also were recorded to ensure consistency of those factors between two tree species in all sampling sites. Canopy openness was only measured at Bois des Liesses in 2023. These environmental conditions can affect leaf traits and leaf buds opening phenology (Choong, 1996; Coley et al., 1985; Murakami & Wada, 1997).

5.3.4 *Insect herbivore species identification*

To assess the impact of leaf traits on the herbivore community, we conducted insect collections in mid-June and early July of 2022 at the same 14 sites as leaf trait measurement sites in Kenauk. Due to the small size of the saplings and conservation concerns, samples were opted to be collected by hand. We thoroughly searched three branches per sapling for insect herbivores, and each branch was searched for 3-4 minutes, removing any specimens found and transferring them to micro vials filled with 75% ethanol for preservation and future identification in the lab. Caterpillars were reared in separate containers in the lab till pupation and adult emergence and were identified to the lowest possible taxon. In the following year, insect collection was conducted by hand and through beat sheeting on three occasions: late May, late June, and late July, at the same 20 sites used for damage and leaf trait measurements in Bois des Liesse. All collected insects were subsequently identified as morphotypes and categorized based on their feeding guilds.

5.3.5 *Preference and Performance lab tests*

To further explore potential differences in leaf palatability and susceptibility to insect herbivores between sugar maple and black maple in Kenauk, we focused on insect leaf cutters which are dominated by lepidopteran caterpillars in sugar maple forests (Thomas et al., 2010). As a representative of these lepidopterans, we conducted bioassays to examine one typical early-season and one late-season Erebid caterpillar: invasive spongy moth (*Lymantria dispar*) and native, white-marked tussock moth (*Orgyia leucostigma*) caterpillars' performance on, and preference for two conspecific maple species. For the spongy moth, egg masses were collected from Angrignon Park in Montreal, QC, Canada, in late January 2021, and tussock moth egg masses were obtained in diapause from Insect Production and Quarantine Laboratories (IPQL) in Ontario, Canada, and hatched and reared to the ideal larval stage in our lab. We fed them all on an artificial Bell diet until they finished the first instar and were reared on non-experimental red oak (*Quercus rubra*) and Norway maple (*Acer platanoides*) leaves before the experiment.

The preference experiment was performed on June 17, 2021, using 4th instar spongy moth caterpillars (early season caterpillar) and on August 12, using 4th instar tussock moth caterpillars (late season caterpillar) to test host selection. From all the 16 sites previously sampled in Kenauk, we collected some sugar maple and black maple undamaged leaves from various sizes, storing each leaf with its petiole fixed in a hole punched into the lid of 2.0 ml microcentrifuge tubes containing water to maintain freshness until transferred to the lab. Subsequently, leaves were sorted by size in the lab. 25 sugar maple and 25 black maple leaves were chosen and scanned, and their area was measured using Image J. We placed one black maple and one sugar maple leaf on opposite ends of each of 25 plastic containers (19cm L * 13cm W), with similar leaf area in each container (with an average area of ~$102cm^2$ for black maple leaves and ~$95cm^2$ for sugar maple leaves). A 4th instar spongy moth caterpillar was then introduced to each container, positioned equidistantly between the two maple leaves to allow free choice. After 48 hours or after more than 50% of the leaf area was consumed, we removed caterpillars, and the consumed leaf area, as a measure for host preference, was measured by rescanning the leaves (Fortin et al., 1997; Foss & Rieske, 2003; Morrow et al., 2022). This process was repeated later in the season using white-marked tussock moth caterpillars.

Following the preference test results in 2021, we conducted a performance test using white-marked tussock moth caterpillars the following year, 2022, at the beginning of July, which coordinated with the natural life cycle of these samples in our study site (Hebert et al., 2017). Since mortality in lepidopteran first instar larvae is reported to be highest compared to the other stages of their life (Zalucki et al., 2002), here we aimed to determine whether small leaf cutters would be more affected by the differences detected in leaf physical defenses between the two closely related maple species compared to the larger leaf chewers. The same protocol as the previous year was followed for obtaining and rearing tussock moth caterpillars until they reached their first instar and collecting and storing sugar maple and black maple leaves in Kenauk. For this bioassay, a total of 56 caterpillars at their first instar larval stage were moved to the plastic containers (19cm L * 13cm W) and were supplied with fresh black maple leaves (2 caterpillars in each of 28 plastic containers), and the same number of caterpillars were fed on sugar maple leaves (28 containers * 2 first instar caterpillars). Due to our focus on the evaluation of the survival rate of young 1st instar insect herbivores, we observed caterpillars after 48 hours and recorded their survival rate at the time point (DiTommaso & Losey, 2003; Murakami & Wada, 1997; Zalucki et al., 2001).

5.4 Data analysis

All statistical analyses were performed using R and RStudio (version 2024.04.1+748). Graphical representations of total and individual damages, leaf traits, and insect herbivore abundance were created with the ggplot2 package in R (Wickham, 2016).

5.4.1 *Insect herbivory damages*

Given that the number of sites, the number of leaves and sampling dates were different in 2021 and 2022 in Kenauk and also in 2023 in Bois des Liess, separate analysis were conducted for each year's data. Initially, for each year, we combined the percentages of nine types of insect herbivory damage to compute the total herbivory damage for each leaf observed. These totals were then averaged across all ten leaves to gauge the overall herbivory damage on each sapling for every sampling site. In Kenauk data in 2021 and Bois des Liesse data in 2023, with observations repeated over the sampling season, date was initially included as a fixed factor in the model. However, as it did not contribute to explaining the response variation by sampling date and was not central to the study's main question, it was excluded from model. As initially planned, sampling date was treated as a measure of sampling effort, and the average measurements across sampling dates were used for the analysis.

Due to the presence of response variables expressed as percentages (damage types), some zero-inflated damage types, and the site's inclusion as a random factor, we used a generalized mixed model with the glmmTMB package (B. Bolker, 2019). The initial model accounted for the effects of tree species (black maple vs sugar maple) and total insect herbivore damages, and we built separate models for damage types in each sampling year. Our model utilized the beta family for the total damage types, leaf cutters, leaf skeletonisers, and leaf stipplers, which followed a beta distribution. However, for the less common, zero-inflated damage types such as leaf rollers, gall makers, leaf miners, and yellow blotch mark values were transferred to a binomial distribution and fitted in a model with the binomial family. Then, with no notable trends between tree species displayed for gall makers and leaf miners, we grouped them based on similar feeding guilds. The sum of the percentage of gall damages (including spindle gall, erineum gall, and ocellate gall) and leaf miners (comprising maple leaf cutter and maple leaf-blotch miner) were utilized in the final statistical analysis fitting in a glmmTMB model with the binomial family. The DHARMa package was used to assess model assumptions, including residual diagnostics, dispersion, and goodness-of-fit, ensuring the validity of the GLMM results. DHARMa implements a simulation-based residual analysis (Hartig, 2022), which is particularly useful for GLMMs as it accounts for non-normality and overdispersion. Dependent variables that did not exhibit normality of residuals were transformed by the square root before analysis.

5.4.2 *Leaf physical traits and phenology of leaf bud burst*

To understand the trend in leaf traits, including specific leaf area, thickness, toughness, water content, and leaf hair density between two sapling species of sugar maple and black maple, we used separate linear mixed regression model for each year dataset (2021 and 2022 at Kenauk and data in 2023 at Bois des Liesse) by lme4 package (Bates et al., 2015). In each years' model, leaf traits fit as the response variables (using average amount of three measurements in 2021), tree species set as a fixed factor and site as a random factor. Sampling date did not significantly contribute to the response variables in the initial model and was not central to the study's main question. Therefore, for the years with more than one sampling date, average values across different dates were used as a measure of sampling effort in the analysis.

To model our response variables, including all herbivory damage types, as a function of predictor variables such as measured leaf traits and the two tree species, we conducted separate redundancy analyses (RDA) using the vegan package for each year individually (Zuur et al. 2007). We applied Scaling 2 in RDA, which preserves the correlation structure among variables, allowing for better visualization of the relationships between response and explanatory variables in ordination space.

Differences between black and sugar maple leaf bud burst phenology were assessed by conducting a Welch's t-test between two maple species with unequal variance (Welch, 1938).

5.4.3 *Insect herbivore community*

Due to the small number of insect herbivores collected in 2022 from Kenauk, those samples were not included in the analysis. As for the next sampling years, 2023 in Bois des Liesse, the variation in the overall abundance of insect herbivores and abundance of various feeding guilds was assessed between two closely related maple trees using generalized linear mixed model regression with glmmTMB package, with site as a random factor (B. Bolker, 2019). Due to the low abundance of leaf skeletonisers and lack of gall makers and leaf miners, they were not included in the statistical analysis. The total abundance of insect herbivores, leaf stippling, and yellow blotch makers fit in the model with the Poisson family. In contrast, leaf roller and cutter abundance, including many zeros, was fit in a zero-inflated generalized mixed model with a

Poisson family. All model assumptions were evaluated by the DHARMa package (Hartig, 2022).

5.4.4 *Preference and performance lab tests*

The lab preference test in 2021 test was structured so that each caterpillar could choose between a sugar maple leaf and a black maple leaf within the same container. This design allowed us to assess the effect of host tree species on the preference of fourth instar larvae over a 48-hour period, with the consumed leaf area serving as the response variable. We applied a paired t-test to control for individual caterpillar differences and accurately measure how tree species affected leaf area consumption. This approach allows for a direct comparison between the two leaf types within each caterpillar's feeding choice. To ensure the validity of the results, we tested for normality and equal variance across caterpillar species using Shapiro-Wilk's test and Levene’s test, respectively.

In the 2022 lab performance bioassay, placing two caterpillars in each container allowed the survival data to fit well within a binomial distribution, making it suitable for analysis using a generalized linear model (GLM) with a binomial family.

5.5 Results

5.5.1 *Insect herbivore damages*

Although the total damage on sugar maple was slightly higher in 2021, there was no significant difference in the total damage caused by herbivores between sugar maple and black maple saplings in any of the sampling years (glmm 2021: z = 0.86; P = 0.38; N = 12, glmm 2022: z = -0.58, P = 0.55, N = 14, glmm 2023: z = 0.86, P = 0.12, N = 20) (Table S 5.1; Figure 5.3). However, some individual leaf damage types, such as yellow blotch mark, showed higher probability of showing zeros values on sugar maples than black maples (z = -2.31, P = 0.020) in 2022 (Table S 5.1; Figure S 5.2). Likewise, stipplers showed less damage (z = -7., P <0.001) on sugar maples, while skeletonisers caused more damage (z = 2.07, P = 0.038) on these species than their conspecific black maple trees in 2023 (Table S 5.1; Figure S 5.3). Box plots for individual damage types in each sampling year are included in the supporting information (Figure S 5.1; Figure S 5.2; Figure S 5.3). Total damage in three years, as well as different types of herbivory damage are presented in Figure 5.3.

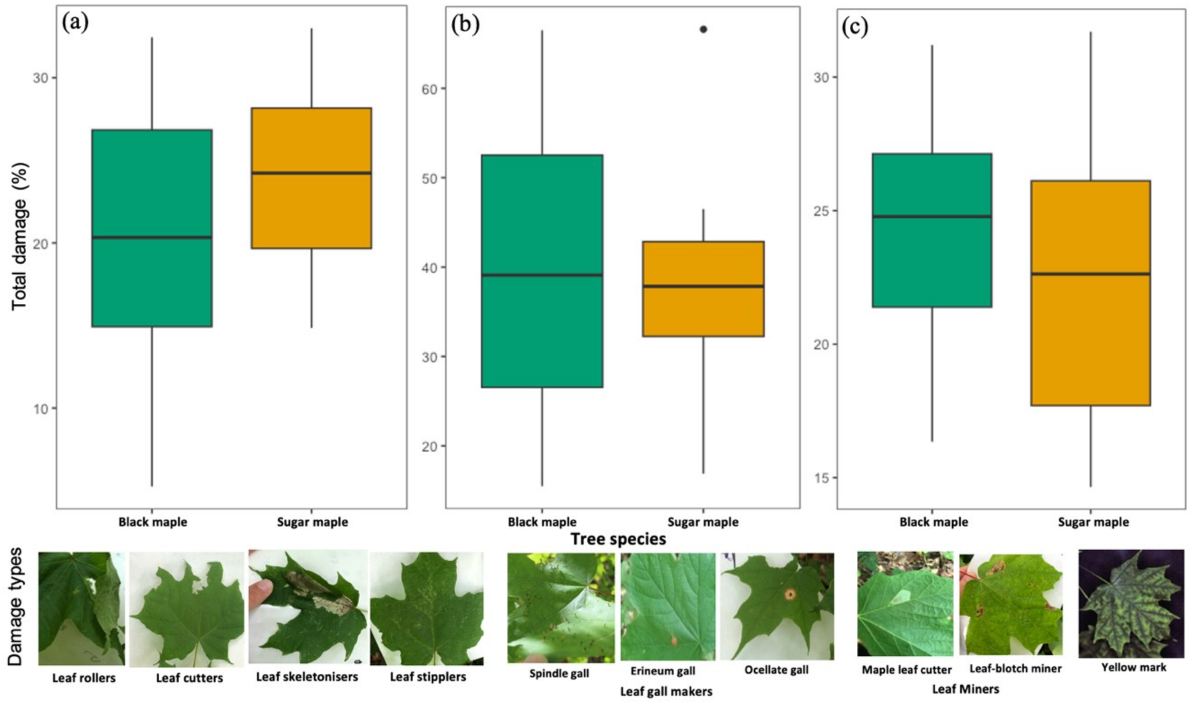


Figure 5.3 Boxplots showing the variation in the percentage of total insect herbivory damage on leaf surface over black (green box) and sugar maple (orange box) saplings. Images below graphs demonstrate ten different insect herbivore damage types estimated on leaves: a) Total insect herbivory damage in 2021 at Kenauk, b) Total insect herbivory damage in 2022 at Kenauk and c) Total insect herbivory damage in 2023 at Bois des Liesse.

5.5.2 *Leaf physical traits and phenology of leaf bud burst*

Starting with the leaf thickness, although it was consistently higher in the black maple leaves than in sugar maple leaves, the value was only significantly different in the first year of sampling, in 2021 (d.f.= 12, t = -4.49, P <0.001). We found leaf toughness to be also consistently higher in black maples compared to the sugar maple leaves, with significant variation in both the first (d.f. = 12, t = -4.49, P = 0.017) and second year of sampling (d.f.= 14, t = -2.22, P <0.001). The values showed the same trend for SLA with a slightly lower amount in sugar maple for all years, which was significantly different (d.f.= 20, t = -2.19, P =

0.040) in 2023. Trichome density, which was measured in the two last years, varied significantly between the two maple species (In 2022: d.f. = 12, t = -4.49, P <0.001; In 2023: d.f.= 40, t = 11.77, P <0.001) with a lower amount on sugar maple leaves compared to black maple leaves (Table 5.1; Figure 5.4).

Table 5.1 Linear mixed model coefficient results for the effect of tree species (Black maple vs. sugar maple saplings) on leaf traits for 12 sites in 2021 and 14 sites in 2022 in Kenauk nature. As well as for 20 sites at Bois des Liesse in 2023.

Year		**Leaf thickness**			**Leaf water content**			**Specific leaf area**			**Leaf toughness**			**Sqrt (Leaf trichome density)**		
	Predictors	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P
2021	Black maple (Intercept)	0.12	0.003	<0.001*	56.30	1.78	<0.001*	344.8	11.8	<0.001*	1.81	0.24	<0.001*			
	Sugar maple	-0.01	0.002	<0.001*	3.10	2.51	0.24	-4.92	9.40	0.61	-0.61	0.22	0.017			
2022	Black maple (Intercept)	0.17	0.008	<0.001*	49.95	2.24	<0.001*	259.42	15.31	<0.001*	4.31	0.39	<0.001*	4.76	0.30	<0.001*
	Sugar maple	-0.015	0.010	0.18	-4.47	2.30	0.072	-15.47	18.71	0.42	-1.13	0.51	0.046*	-4.59	0.41	<0.001*
2023	Black maple (Intercept)	0.16	0.031	<0.001*	63.85	1.78	<0.001*	383.86	8.01	<0.001*	2.22	0.090	<0.001*	7.20	0.39	<0.001*
	Sugar maple	0.061	0.044	0.17	-4.38	2.52	0.090	-22.52	10.25	0.040*	-0.18	0.088	0.051	-6.53	0.55	<0.001*

Note: Significant results shown by * = P < 0.05. Response variables, including leaf thickness, leaf water content, specific leaf area, leaf toughness, and leaf trichome density, fit in the model: **lmer (leaf trait ~ tree species + (1|site), data).** Trichome density was not measured in 2021.

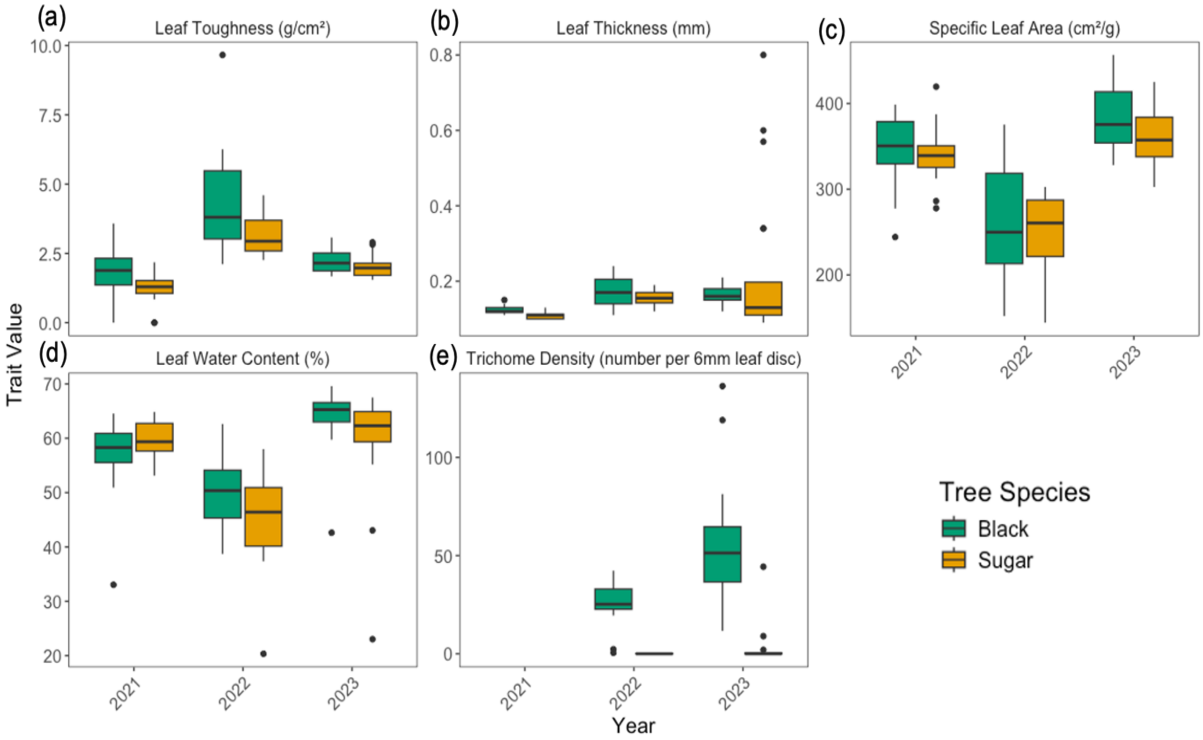


Figure 5.4 Box plots illustrate the variation in five leaf traits across three years (2021, 2022, and 2023) for black and sugar maple at two natural reserves (the first two years at Kenauk and last year at Bois des Liesse). The leaf traits measured: (a) leaf toughness (g/cm²), (b) leaf thickness (mm), (c) specific leaf area (cm²/g), (d) water content (%), and (e) trichome density (number per 6mm leaf disc). Each box plot represents the distribution of trait values for each year, with the fill color indicating the tree species: black maple (green) and sugar maple (orange).

The redundancy analysis (RDA) conducted in 2021 to assess the relationship between herbivore damage and leaf traits revealed that the first two axes explained 85% of the data variation. Axis 1 accounted for 63.8% of the variation and was mainly influenced by positive correlations with SLA and thickness, and negative correlations with leaf toughness and water content. Axis 2 explained 20.7% of the variation, with negative correlations to SLA, toughness, and water content, and a positive correlation to leaf thickness. The analysis of leaf damage scores showed that damage by stipplers, rollers, and cutters contributed most to the variation explained by Axis 1, positively associated with SLA and thickness but negatively linked to leaf toughness and water content. In contrast, damage from skeletonizers, yellow blotch marks, gall makers, and miners had a negative contribution to Axis 1, aligning negatively with SLA and thickness, and positively with toughness and water content. A permutation test indicates that the explanatory variables did not significantly account for the variation observed in the herbivore damage (P = 0.693) (**Error! Reference source not found.**; **Error! Reference source not found.**).

In 2022, the RDA showed the first two axes of the ordination accounted for 86.8% of the variation. RDA1 accounted for 73.3% of the variation and was positively correlated with leaf specific area and water content while negatively correlated with leaf toughness, thickness, and trichome density. RDA2 explained 13.6% of the variation and exhibited a positive correlation with leaf thickness and water content, but a negative correlation with toughness, trichome and SLA. Leaf total damage, rollers and yellow blotch makers negatively contributed to RDA1, correlated with tougher leaves, lower SLA, and higher trichome density. The permutation test showed P=0.089, indicating that leaf traits and tree species do not account for all the variability in the herbivore damage types (**Error! Reference source not found.**;**Error! Reference source not found.**).

For 2023, the RDA explained 81.4% of the variation with RDA1 accounting for 51.4%. It was positively correlated with thickness but negatively associated with SLA, water content, toughness and trichome density. RDA2 explained 30% of the variation and was positively associated with SLA, water content and thickness but showed a negative association with toughness and trichome. The leaf cutter, skeletonizer, and gallyellow blotch maker mark contributed most positively to the variation on RDA1, aligning with thickness. On the other hand, total damage, rollers and stippling exhibited negative contributions, showing associations with higher leaf toughness, water content, SLA and trichome density. The permutation test for the RDA model was not statistically significant (P=0.302), indicating that the explanatory variables only partially captured the patterns in the herbivory damage (**Error! Reference source not found.**; **Error! Reference source not found.**).

Regarding the bud burst phenology of two congeneric maple trees, the Welch t-test result shows no significant difference between the bud burst timing of sugar maple and black maple neither at Kenauk in 2022 (t = -0.746, d.f.= 29.968, P = 0.461), nor at Bois des Liesse in 2023 (t = -0.065, d.f. = 30.735, P = 0.948).

Light intensity and temperature were recorded as environmental factors on May 10, 2022, at Kenauk and on the same date in 2023 at Bois des Liesse. In both years, the results showed consistent light intensity and temperature between sugar maple and black maple saplings across all sites. The average light intensity was 4845 ± 403 lux for sugar maples and 3988 ± 457 lux for black maples, while the average temperature was 30.2 ± 0.61°C for sugar maples and 29.9 ± 0.54°C for black maples. In 2023, the average light intensity was 3890 ± 462 lux for sugar maples and 4531 ± 428 lux for black maples. The average temperature was 17.5 ± 0.50°C for sugar maples and 17.4 ± 0.49°C for black maples across all sites. In this year, canopy openness was also measured, averaging 41.6 ± 2.36% over sugar maples and 38.8 ± 2.54% over black maples.

5.5.3 *Insect herbivore community response*

In 2022, the number of insect herbivores collected on maple saplings was insufficient for analysis. Therefore, we simply included the analysis results of samples from the 2023 collection at Bois des Liesse National Park. From 7132 individuals we collected from sapling maple trees in 20 sites, the majority, around 6500, were collembola and fungivore Acari, and just 199 appeared to be insect herbivores. The rest were predators, including spiders and ants and hemipteran reduviids, hymenopteran parasitoids, and a very few detrivore dipterans. A complete list of herbivores we collected appeared in the supplementary information (Table S 5.3). Model results indicate no significant difference in the abundance of the total insect herbivores collected on two maple saplings (z = -1.38, P = 0.165) and not in any of the individual herbivore guilds identified in the sites (Table S 5.2; Figure 5.5).

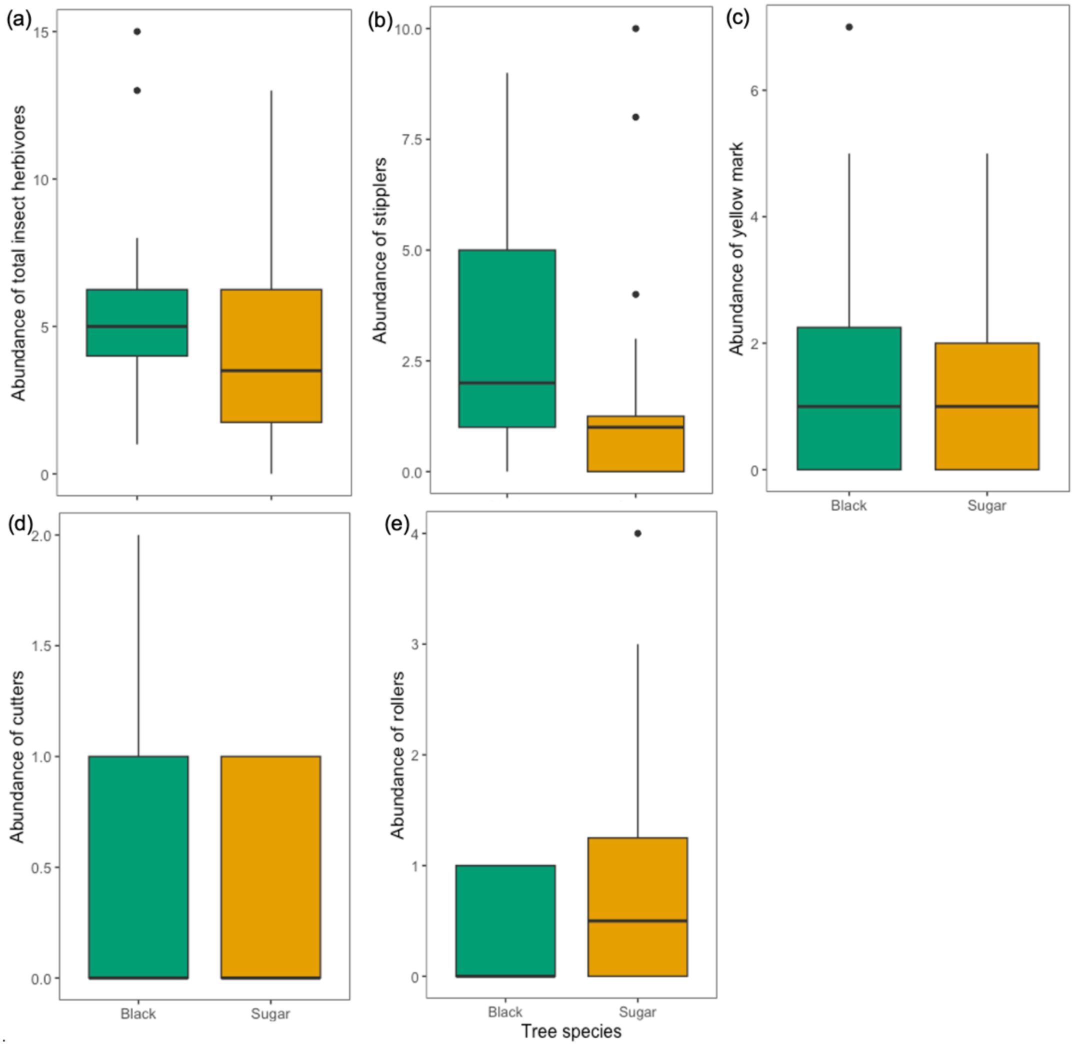
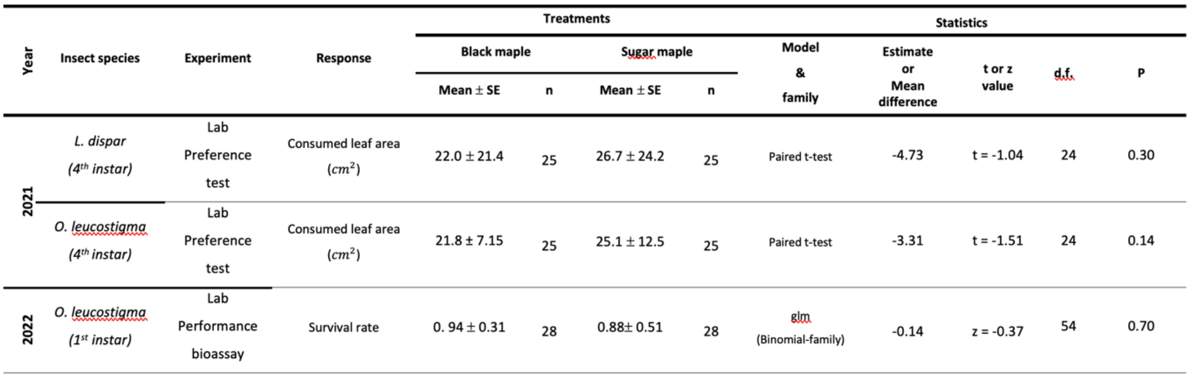


Figure 5.5 Box plots showing the abundance of insect herbivores collected from sugar maple and black maple trees, grouped by the type of damage they cause, at Bois des Liesse in 2023. Each plot represents data collected from 20 sites. (a) Average abundance of insect herbivores, (b) Abundance of leaf stippling herbivores, (c) Abundance of yellow blotch makers, (d) Abundance of leaf cutters, and (e) Abundance of leaf rollers.

5.5.4 *preference and performance lab test*

Regarding the preference test, although the proportion of leaf area consumed by 4th instar spongy moth caterpillars (mean consumed leaf area for sugar maple = 26.7 ± 24.2cm^2, mean consumed leaf area for black maple = 22.0 ± 21.4cm^2) and by 4th instar white-marked tussock moth caterpillars (mean consumed leaf area for sugar maple = 25.1 ± 12.5cm^2, mean consumed leaf area for black maple = 21.8 ± 7.15cm^2) was slightly higher for sugar maple leaves than for black maples, the difference was not statistically significant for neither early nor late season caterpillars (spongy moth P = 0.30, white-marked tussock moth P= 0.14) (Table 5.2; Figure 5.6).

Table 5.2 Mean ± SE and the results of statistical analysis for preference and performance responses of spongy moth (Lymantria dispar) and white-marked tussock moth (Orgyia leucostigma) on the leaves of black maple versus sugar maple saplings collected from Kenauk nature sites in 2021 and 2022.



Year	Insect species	Experiment	Response	Treatments				Statistics				
				Black maple		Sugar maple		Model & family	Estimate or Mean difference	t or z value	d.f.	P
				Mean ± SE	n	Mean ± SE	n					
2021	*L. dispar* *(4th instar)*	Lab Preference test	Consumed leaf area (cm^2)	22.0 ± 21.4	25	26.7 ± 24.2	25	Paired t-test	-4.73	t = -1.04	24	0.30
	O. leucostigma *(4th instar)*	Lab Preference test	Consumed leaf area (cm^2)	21.8 ± 7.15	25	25.1 ± 12.5	25	Paired t-test	-3.31	t = -1.51	24	0.14
2022	*O. leucostigma* *(1st instar)*	Lab Performance bioassay	Survival rate	0. 94 ± 0.31	28	0.88± 0.51	28	glm (Binomial-family)	-0.14	z = -0.37	54	0.70

Note: Analyses compare mean value of consumed leaf area (cm²) for the lab preference test analyzed by **paired t-test** and the mean value of survival rate (first instar caterpillars surviving after 48 hours) in 2022 lab performance test which fit in the model: **glm (survival ~ treatment, family = binomial (link = "logit"), data).** Significant results shown by * = P < 0.05.

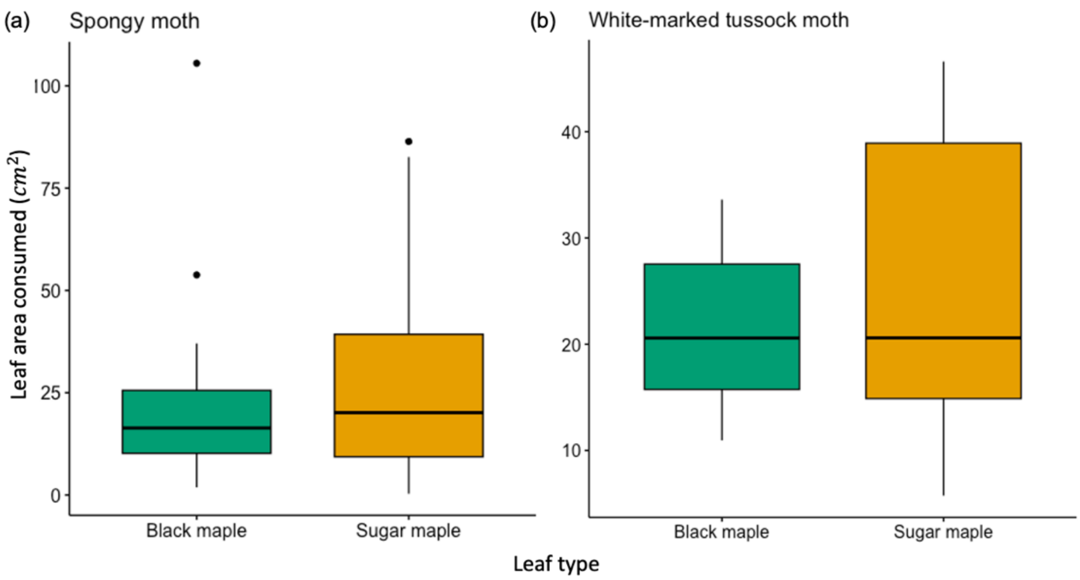


Figure 5.6 Box plots showing two moth caterpillar species preference test between black maple leaves versus sugar maple leaves collected from Kenauk nature sites by measuring the area of leaves consumed (*n* = 25) *(a) Leaf area of black maple leaves versus sugar maple leaves consumed by a spongy moth (Lymantria dispar) and (b) Leaf area of black maple leaves versus sugar maple leaves consumed by white-marked tussock moth (Orgyia leucostigma).*

In addition, the examination of the 1st instar white-marked tussock moth's performance in 2022 showed no notable contrast in their survival rate when consuming black maple versus sugar maple leaves after 48 hours (P = 0.70).

5.6 Discussion

The physical traits of black maple, particularly toughness and trichome density, were expected to offer substantial defenses against herbivory. Some of the measured foliar morphological traits of sugar maples and black maples in this study, including thickness, toughness, SLA, and trichome density, showed differences between two closely related maple hosts as expected, albeit not all varied significantly. However, these traits were ineffective in reducing herbivory damage as revealed in redundancy analysis (RDA). Therefore, insect herbivores do not seem to differentiate between two maple trees and leaf physical traits were not the primary drivers of damage patterns caused by insect herbivores on the maple trees.

This result could be partly explained by the ability of two congeneric maple trees to make hybrids where their native range overlaps. These hybrids have been used to improve sugar production (Kriebel, 1989) but lead to misidentification of specimens in their region (Dansereau & Desmarais, 1947; Fleak, 1967). Although we chose individuals with distinctive phenotypes, leaf shape and trichomes, attempting to avoid sampling hybrids in our study sites, distinguishing some of them from the parental taxa was challenging, even for local experts. This uncertainty supports the hybrid bridge zone hypothesis, which proposes that plant hybrids fill the genetic gap between existing and potential host species, allowing herbivorous insects to broaden their host range in the presence of these hybrids (Floate & Whitham, 1993).

Environmental factors such as humidity, temperature, and light intensity were consistently similar for each pair of maples in our sampling sites, suggesting that these cannot explain observed differences in leaf traits. Studies on canopy versus understory variation in forest ecosystems demonstrate that changes in light intensity can alter leaf quality (Messier et al., 2017), thus structuring vertical patterns in insect herbivore communities (Hakimara & Despland, 2024; Murakami et al., 2005; Ulyshen, 2011). In addition, a study done on oak showed that temperature and humidity can influence species' investment in leaf defenses (Pearse & Hipp, 2012). These findings highlight the importance of abiotic factors in influencing insect herbivory patterns. In our study, since these factors did not vary between the two species, they likely did not contribute to variations in leaf traits or herbivory patterns. On top of that, tree ontogeny is known to influence insect leaf damage in temperate forests, with saplings receiving more damage than mature trees again due to the direct effect on leaf trait variation (Thomas et al., 2010). In our case, selected trees were all saplings with the same height, controlling for the ontogeny effect on insect herbivory.

Furthermore, bioassays showed caterpillars don’t distinguish black maple leaves from sugar maple leaves. Despite the fact that recent genetic work (Pugeaut et al., 2024) supports a separate species, the results of herbivores behavioral experiment suggest little or no chemical differences between these two trees. In general, chemical defenses are better preserved than physical defenses at the family level (Descombes et al., 2020). Although black maple leaves appear less palatability due to their higher thickness, toughness, and trichome density, performance and preference tests did not support the hypothesis that black maple leaves are better defended against large cutter insect herbivores. Previous studies suggest that high trichome density offers better defense against herbivory, particularly in species where leaves are fully covered with hairs like milkweeds (Agrawal & Fishbein, 2006). However, in the black maple leaves collected in our study sites, trichomes were predominantly around veins rather than uniformly distributed on the lower surface, which may reduce their effectiveness against herbivores. This finding align well with previous work in which trichomes were considered to be mainly playing role in reducing water loss than reducing insect herbivory (Hilaire & Graves, 1999).

Concerning leaf thickness and toughness, they were found not to affect insect herbivores from different feeding guilds except the ones with piercing-sucking mouth parts, which left stippling or yellow blotch mark damage. This result contradicts previous studies' observed insect herbivory negative correlation with leaf toughness (López-Carretero et al., 2016) and thickness (Caldwell et al., 2016). However, the mean toughness and thickness for the two maple trees were perhaps not different enough to yield an influential difference between the herbivory of these two species. Therefore, the absence of contrast in performance tests does not substantiate the idea that black maple leaves offer a superior defense.

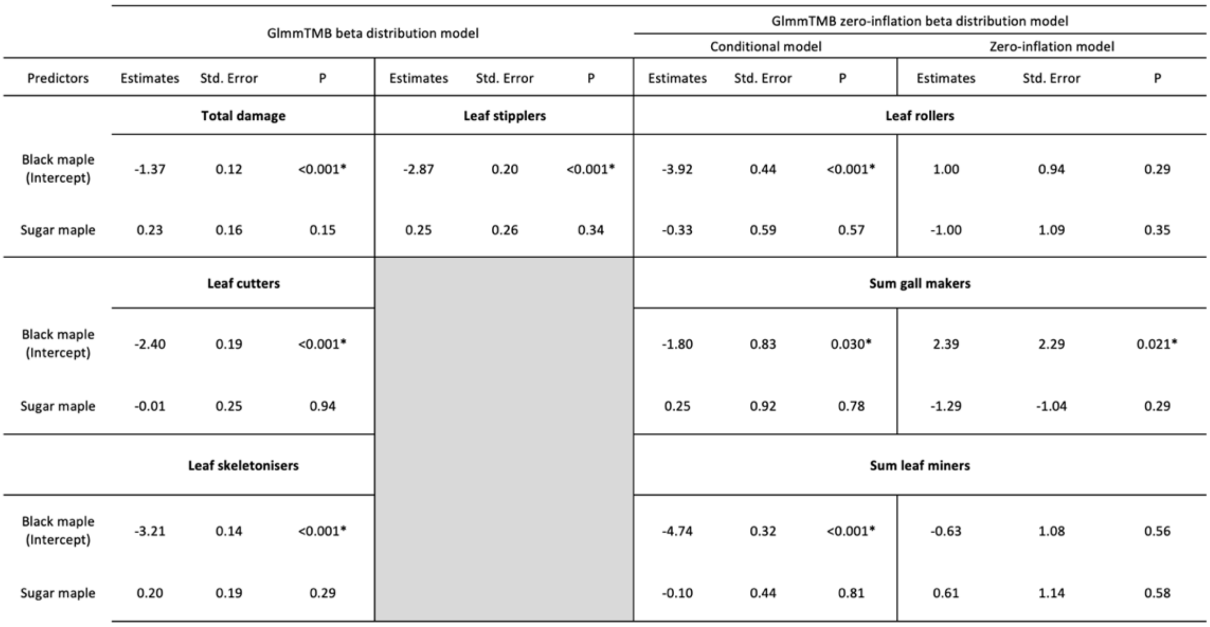
The influence of host species on the strength of interactions with herbivores can be affected by density-dependent processes. Research has shown that the most abundant plant species with the highest biomass or cover tend to be associated with more herbivore species (Marques et al., 2000; Whitfeld et al., 2012), likely because these species are more frequently encountered or have higher apparency. In the natural and urban forests, we studied sugar maples, the dominant species, while black maples were less abundant. As a result, it was expected that sugar maples would generally experience more insect herbivory damage and higher insect herbivore abundance. However, our findings did not align with this expectation.

In conclusion, our study suggests that observed defensive leaf physical traits on black maples, such as trichome density, are not providing better protection against insect herbivores. Despite differences in foliar physical traits of black maples with their close relative, sugar maple, these alone do not appear to drive insect herbivory patterns. Instead, these two closely related species seem not to be distinguished either by the two caterpillars tested in bioassays, or by the broader insect community. The close phylogenetic positioning of these two trees better explains their relationships with insect herbivores than do their differences in leaf traits. Future research should explore the roles of chemical defenses and other ecological factors in shaping these interactions. This study provides essential insights into the bottom-up pressures shaping herbivore dynamics in maple-dominated forests, showing that black maple is not likely protected from sugar maple insects, with implications for forest conservation and management strategies.

5.7 Supplementary information

Table S 5.1 Generalised linear mixed model coefficient results for the effect of tree species (Black maple and sugar maple) on insect herbivory damages on leaves. a) 2021 results at Kenauk nature in 2021 (Site No.= 12), b) 2022 results at Kenauk nature (Site No. = 14), c) 2023 results at Bois des Liesse (Site No. = 20).

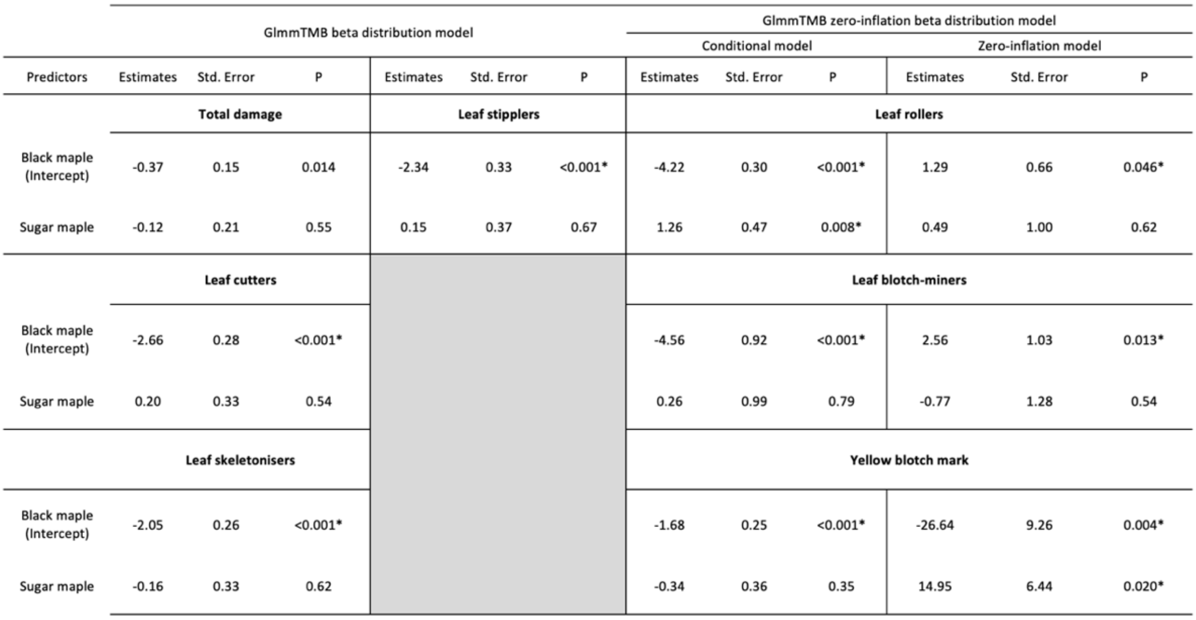
a) 2021 Kenauk



	GlmmTMB beta distribution model						GlmmTMB zero-inflation beta distribution model					
							Conditional model			Zero-inflation model		
Predictors	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P
	Total damage			**Leaf stipplers**			**Leaf rollers**					
Black maple (Intercept)	-1.37	0.12	<0.001*	-2.87	0.20	<0.001*	-3.92	0.44	<0.001*	1.00	0.94	0.29
Sugar maple	0.23	0.16	0.15	0.25	0.26	0.34	-0.33	0.59	0.57	-1.00	1.09	0.35
	Leaf cutters						**Sum gall makers**					
Black maple (Intercept)	-2.40	0.19	<0.001*				-1.80	0.83	0.030*	2.39	2.29	0.021*
Sugar maple	-0.01	0.25	0.94				0.25	0.92	0.78	-1.29	-1.04	0.29
	Leaf skeletonisers						**Sum leaf miners**					
Black maple (Intercept)	-3.21	0.14	<0.001*				-4.74	0.32	<0.001*	-0.63	1.08	0.56
Sugar maple	0.20	0.19	0.29				-0.10	0.44	0.81	0.61	1.14	0.58

Note: Significant results shown by * = P < 0.05. The table distinguishes between models with a beta distribution and zero-inflated beta distribution. The results include estimates, standard errors, and P-values for different predictors. Total damage of insect herbivores, leaf cutters, leaf skeletonisers and leaf stipplers fit in the model: **GlmmTMB (leaf damage ~ tree species + (1|site), family = gaussian, data)**. The zero inflated leaf rollers, leaf gall makers, leaf miners and yellow marks fit in model: **GlmmTMB (leaf damage ~ tree species + (1|site), ziformula = tree species + (1|site), family = beta_family(), data).** Yellow blotch mark had a very few non-zero values, so we excluded that from the analysis

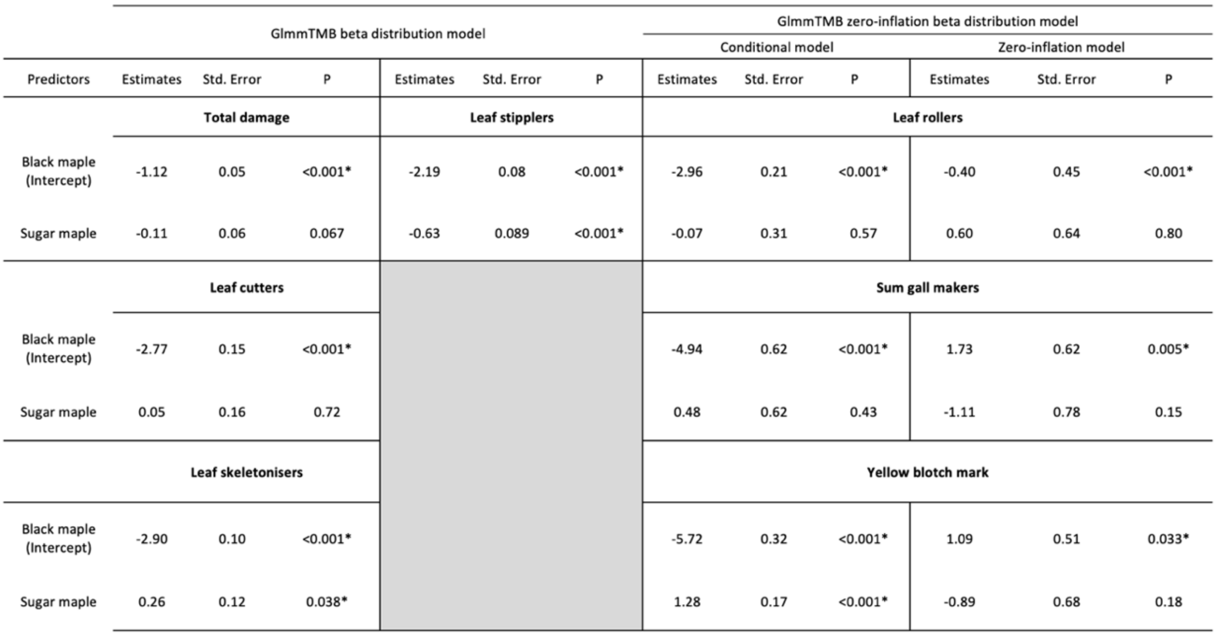
b) 2022 Kenauk



	GlmmTMB beta distribution model						GlmmTMB zero-inflation beta distribution model					
							Conditional model			Zero-inflation model		
Predictors	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P
	Total damage			**Leaf stipplers**			**Leaf rollers**					
Black maple (Intercept)	-0.37	0.15	0.014	-2.34	0.33	<0.001*	-4.22	0.30	<0.001*	1.29	0.66	0.046*
Sugar maple	-0.12	0.21	0.55	0.15	0.37	0.67	1.26	0.47	0.008*	0.49	1.00	0.62
	Leaf cutters						**Leaf blotch-miners**					
Black maple (Intercept)	-2.66	0.28	<0.001*				-4.56	0.92	<0.001*	2.56	1.03	0.013*
Sugar maple	0.20	0.33	0.54				0.26	0.99	0.79	-0.77	1.28	0.54
	Leaf skeletonisers						**Yellow blotch mark**					
Black maple (Intercept)	-2.05	0.26	<0.001*				-1.68	0.25	<0.001*	-26.64	9.26	0.004*
Sugar maple	-0.16	0.33	0.62				-0.34	0.36	0.35	14.95	6.44	0.020*

Note: Note: Significant results shown by * = P < 0.05. The table distinguishes between models with a beta distribution and zero-inflated beta distribution. The results include estimates, standard errors, and P-values for different predictors. Total damage of insect herbivores, leaf cutters, leaf skeletonisers and leaf stipplers fit in the model: **GlmmTMB (leaf damage ~ tree species + (1|site), family = gaussian, data)**. The zero inflated leaf rollers, leaf gall makers, leaf miners and yellow marks fit in model: **GlmmTMB (leaf damage ~ tree species + (1|site), ziformula = tree species + (1|site), family = beta_family(), data).** Leaf miner-maple leaf cutter damage had zero values and sum of gall makers had a very few non-zero values, so we excluded them from the analysis

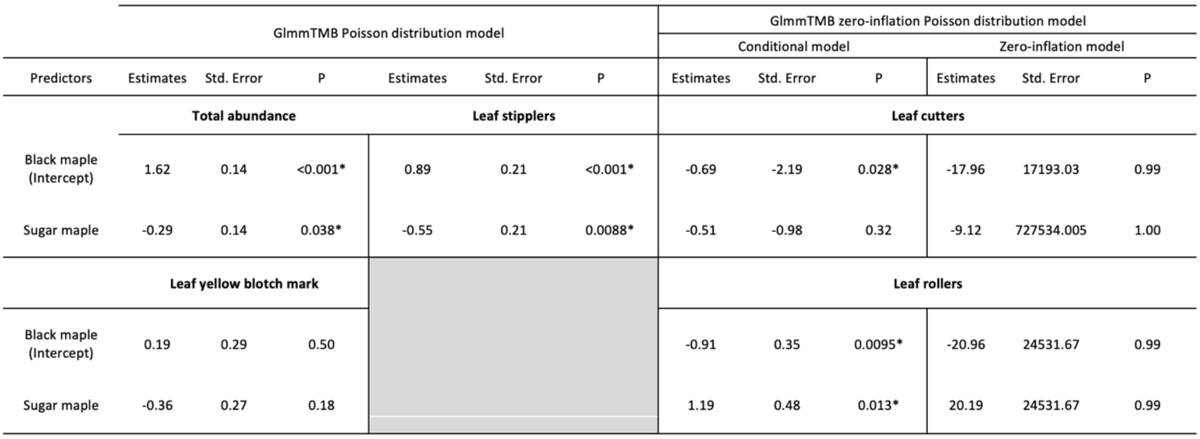
c) 2023 Bios des Liesse



	GlmmTMB beta distribution model						GlmmTMB zero-inflation beta distribution model					
							Conditional model			Zero-inflation model		
Predictors	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P
	Total damage			Leaf stipplers			Leaf rollers					
Black maple (Intercept)	-1.12	0.05	<0.001*	-2.19	0.08	<0.001*	-2.96	0.21	<0.001*	-0.40	0.45	<0.001*
Sugar maple	-0.11	0.06	0.067	-0.63	0.089	<0.001*	-0.07	0.31	0.57	0.60	0.64	0.80
	Leaf cutters						Sum gall makers					
Black maple (Intercept)	-2.77	0.15	<0.001*				-4.94	0.62	<0.001*	1.73	0.62	0.005*
Sugar maple	0.05	0.16	0.72				0.48	0.62	0.43	-1.11	0.78	0.15
	Leaf skeletonisers						Yellow blotch mark					
Black maple (Intercept)	-2.90	0.10	<0.001*				-5.72	0.32	<0.001*	1.09	0.51	0.033*
Sugar maple	0.26	0.12	0.038*				1.28	0.17	<0.001*	-0.89	0.68	0.18

Note: Significant results shown by * = P < 0.05. The table distinguishes between models with a beta distribution and zero-inflated beta distribution. The results include estimates, standard errors, and p-values for different predictors. Total damage of insect herbivores, leaf cutters, leaf skeletonisers and leaf stipplers fit in the model: **GlmmTMB (leaf damage ~ tree species + (1|site), family = gaussian, data)**. The zero inflated leaf rollers, leaf gall makers, leaf miners and yellow marks fit in model: **GlmmTMB (leaf damage ~ tree species + (1|site), ziformula = tree species + (1|site), family = beta_family(), data).** Leaf miners had a very few non-zero values, so we excluded that from the analysis.

Table S 5.2 Generalized linear mixed model coefficient results for the effect of tree species (black maple and sugar maple) on the abundance of insect herbivores collected in 2023 at Bois des Liesse.



	GlmmTMB Poisson distribution model						GlmmTMB zero-inflation Poisson distribution model					
							Conditional model			Zero-inflation model		
Predictors	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P	Estimates	Std. Error	P
	Total abundance			Leaf stipplers			Leaf cutters					
Black maple (Intercept)	1.62	0.14	<0.001*	0.89	0.21	<0.001*	-0.69	-2.19	0.028*	-17.96	17193.03	0.99
Sugar maple	-0.29	0.14	0.038*	-0.55	0.21	0.0088*	-0.51	-0.98	0.32	-9.12	727534.005	1.00
	Leaf yellow blotch mark						Leaf rollers					
Black maple (Intercept)	0.19	0.29	0.50				-0.91	0.35	0.0095*	-20.96	24531.67	0.99
Sugar maple	-0.36	0.27	0.18				1.19	0.48	0.013*	20.19	24531.67	0.99

Note: Total abundance of insect herbivores, leaf stipplers and yellow blotch mark makers fit in the model: **GlmmTMB (insect abundance ~ tree species + (1|site), family = Poisson, data)**. The zero inflated leaf rollers and leaf cutters fit in model: **GlmmTMB (insect abundance ~ tree species + (1|site), ziformula = tree species + (1|site), family = Poisson, data)**. No skeletonisers, gall maker or leaf miners were collected and those were not included in the analysis. Significant results shown by * = P < 0.05.

Table S 5.3 Abundance of Insect herbivore species collected from two types of maple trees (sugar maple and black maple) in 20 sites through the sampling season at Bos des Liesse in 2023.

Date	Tree type	Order/ Family	Species	Feeding guild	Life stage	Method	Abundance
May	Black	Thysanoptera/ Thripidae	*Taeniothrips sp.*	Yellow blotch mark	Nymph	beat sheeting	5
May	Black	Lepidoptera/ Tortricidae	*Not.Identified*	Roller	Caterpillar	by hand	1
May	Sugar	Lepidoptera/ Tortricidae	*Catastega aceriella*	Skeletoniser	Caterpillar	by hand	1
May	Sugar	Lepidoptera/ Tortricidae	*Sparganothis sp.*	Roller	Caterpillar	by hand	1
May	Black	Hemiptera/ Pentatomidae	*Not.Identified*	Stippling	Adult	by hand	1

May	Sugar	Lepidoptera/ Tortricidae	*Not.Identified*	Roller	Caterpillar	by hand	1
May	Black	Lepidoptera/ Tortricidae	*Sparganothis sp.*	Roller	Caterpillar	by hand	3
May	Black	Thysanoptera/ Thripidae	*Taeniothrips sp.*	Yellow blotch mark	Adult	beat sheeting	10
May	Black	Coleoptera/ Curculionidae/	*Not.Identified*	Cutter	Adult	beat sheeting	1
May	Black	Thysanoptera/ Thripidae	*Taeniothrips sp.*	Yellow blotch mark	Adult	beat sheeting	4
May	Black	Thysanoptera/ Not.Identified	*Not.Identified*	Yellow blotch mark	Nymph	beat sheeting	10
May	Black	Lepidoptera/ Geometridae	*Alsophila pometaria*	Cutter	Caterpillar	beat sheeting	3
May	Black	Lepidoptera/ Geometridae	*Phigalia titea*	Cutter	Caterpillar	beat sheeting	1
May	Sugar	Thysanoptera/ Not.Identified	*Not.Identified*	Yellow blotch mark	Nymph	beat sheeting	7
May	Sugar	Hemiptera/ Aphididae	*Not.Identified*	Stippling	Adult	beat sheeting	1
May	Sugar	Lepidoptera/ Tortricidae	*Sparganothis sp.*	Roller	Adult	by hand	1
May	Sugar	Lepidoptera/ Noctuidae	*Not.Identified*	Cutter	Caterpillar	beat sheeting	1
May	Black	Hemiptera/ Miridae	*Not.Identified*	Stippling	Nymph	beat sheeting	1
May	Black	Hemiptera/ Cicadellidae	*Not.Identified*	Stippling	Nymph	beat sheeting	1
May	Black	Hemiptera/ Lygaeidae	*Not.Identified*	Stippling	Nymph	beat sheeting	1
May	Black	Thysanoptera/ Not.Identified	*Not.Identified*	Yellow blotch mark	Nymph	beat sheeting	3
May	Sugar	Lepidoptera/ Tortricidae	*Sparganothis sp.*	Roller	Caterpillar	beat sheeting	10
May	Sugar	Thysanoptera/ Thripidae	*Taeniothrips sp.*	Yellow blotch mark	Adult	beat sheeting	10
May	Sugar	Hemiptera/ Cicadellidae	*Not.Identified*	Stippling	Nymph	beat sheeting	1
May	Sugar	Hemiptera/ Pentatomidae	*Not.Identified*	Stippling	Adult	beat sheeting	1
May	Black	Lepidoptera/ Tortricidae	*Sparganothis sp.*	Roller	Caterpillar	beat sheeting	3
May	Sugar	Lepidoptera/ Geometridae	*Alsophila pometaria*	Cutter	Caterpillar	beat sheeting	1
May	Sugar	Thysanoptera/ Thripidae	*Taeniothrips sp.*	Yellow blotch mark	Nymph	beat sheeting	3
May	Sugar	Lepidoptera/ Geometridae	*Not.Identified*	Cutter	Caterpillar	beat sheeting	1
May	Sugar	Lepidoptera/ Tortricidae	*Pseudexentera oregonana*	Roller	Caterpillar	by hand	1
May	Black	Lepidoptera/ Geometridae	*Hypagyrtis unipunctata*	Cutter	Caterpillar	beat sheeting	1
May	Black	Lepidoptera/ Noctuidae	*Not.Identified*	Cutter	Caterpillar	beat sheeting	1
May	Black	Hemiptera/ Aphididae	*Not.Identified*	Stippling	Adult	beat sheeting	2
June	Sugar	Thysanoptera/ Thripidae	*Taeniothrips sp.*	Yellow blotch mark	Adult	beat sheeting	1
June	Black	Hemiptera/ Miridae	*Not.Identified*	Stippling	Nymph	beat sheeting	5
June	Black	Coleoptera/ Elateridae	*Athous rufifrons*	Cutter	Adult	beat sheeting	1
June	Black	Hemiptera/ Miridae	*Not.Identified*	Stippling	Adult	beat sheeting	1
June	Sugar	Hemiptera/ Aphididae	*Not.Identified*	Stippling	Adult	beat sheeting	1
June	Sugar	Lepidoptera/ Noctuidae	*Not.Identified*	Cutter	Caterpillar	beat sheeting	1
June	Black	Coleoptera Curculionidae	*Not.Identified*	Cutter	Adult	beat sheeting	1
June	Black	Lepidoptera/ Geometridae	*Ennomos subsignaria*	Cutter	Caterpillar	by hand	1
June	Black	Lepidoptera/ Geometridae	*Not.Identified*	Cutter	Caterpillar	beat sheeting	1

June	Sugar	Aphididae/ Not.Identified	*Not.Identified*	Stippling	Adult	beat sheeting	1
June	Black	Hemiptera/ Miridae	*Lygus lineolaris*	Stippling	Adult	beat sheeting	3
June	Black	Hemiptera/ Cicadellidae	*Not.Identified*	Stippling	Nymph	beat sheeting	3
June	Black	Hemiptera/ Cicadellidae	*Not.Identified*	Stippling	Nymph	beat sheeting	3
June	Sugar	Lepidoptera/ Noctuoidea	*Lymantria dispar*	Cutter	Caterpillar	beat sheeting	1
June	Black	Hemiptera/ Miridae	*Not.Identified*	Stippling	Nymph	beat sheeting	3
June	Sugar	Hemiptera/ Cicadellidae	*Erythroneura sp.*	Stippling	Adult	beat sheeting	1
June	Sugar	Coleoptera/ Curculionidae	*Not.Identified*	Cutter	Adult	beat sheeting	1
July	Black	Hemiptera/ Cicadellidae	*Not.Identified*	Stippling	Nymph	beat sheeting	27
July	Sugar	Hemiptera/ Cicadellidae	*Not.Identified*	Stippling	Nymph	beat sheeting	9
July	Sugar	Hemiptera/ Aphididae	*Not.Identified*	Stippling	Nymph	beat sheeting	2
July	Black	Hemiptera/ Aphididae	*Not.Identified*	Stippling	Nymph	beat sheeting	4
July	Black	Hemiptera/ Aphididae	*Not.Identified*	Stippling	Adult	beat sheeting	7
July	Sugar	Hemiptera/ Aphididae	*Not.Identified*	Stippling	Adult	beat sheeting	11
July	Black	Hemiptera/ Cicadellidae	*Not.Identified*	Stippling	Adult	beat sheeting	4
July	Black	Hemiptera/ Miridae	*Not.Identified*	Stippling	Nymph	beat sheeting	1

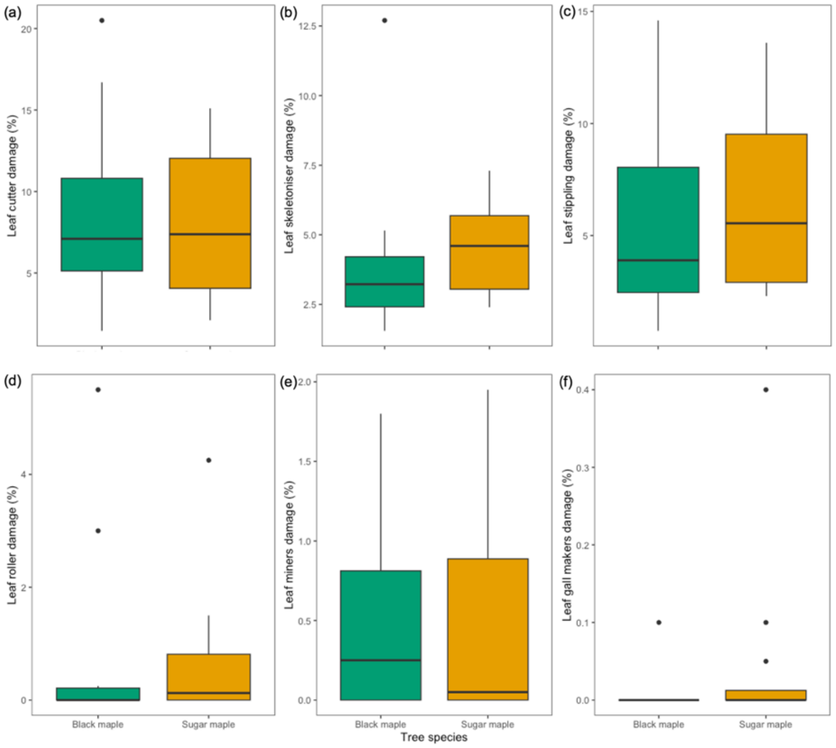


Figure S 5.1 Box plots showing the variation of average percentage of leaf surface damaged by different insect herbivores between two tree species (black maple and sugar maple) on 12 sites at Kenauk nature in 2021. a) leaf cutters damage, b) leaf skeletonisers damage, c) leaf stippling damage, d) leaf rollers damage e) leaf miners damage, f) leaf gall maker damage. (Yellow blotch mark damage is excluded due to very low number of non-zero values).

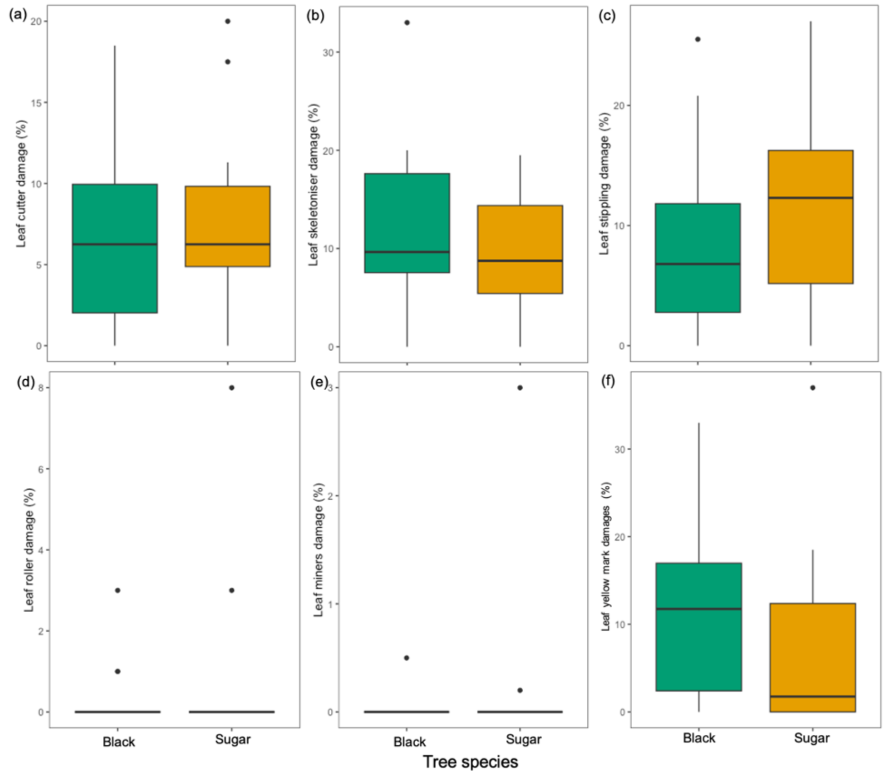


Figure S 5.2 Box plots showing the variation of average percentage of leaf surface damaged by different insect herbivores between two tree species (black maple and sugar maple) on 14 sites at Kenauk nature in 2022. a) leaf cutters damage, b) leaf skeletonisers damage, c) leaf stippling damage, d) leaf rollers damage e) leaf miners damage, f) leaf yellow blotch makers. (gall makers are excluded due to the low number of non-zero values).

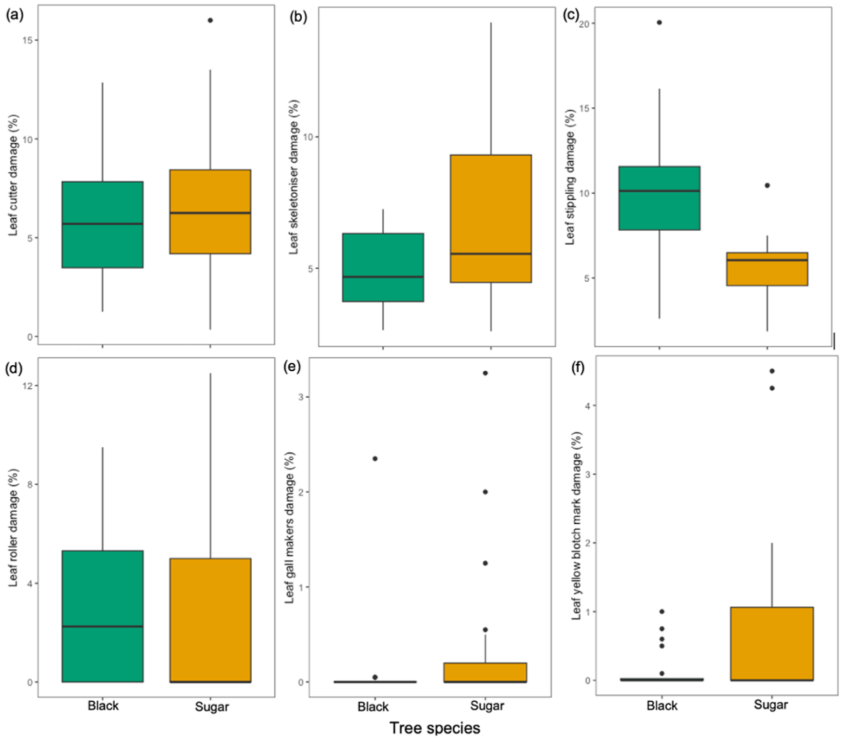


Figure S 5.3 Box plots showing the variation of average percentage of leaf surface damaged by different insect herbivores between two tree species (black maple and sugar maple) on 20 sites at Bois des Liesse nature park in 2023. a) leaf cutters damage, b) leaf skeletonisers damage, c) leaf stippling damage, d) leaf rollers damage e) leaf gall makers damage, f) Yellow blotch makers damage. (Leaf miners damage is excluded due to low number of non-zero values).

6 Chapter 6: General Discussion

6.1 Thesis objectives and results

This thesis explored the interplay of bottom-up and top-down forces shaping insect herbivory and predation in sugar maple-dominated forests of Eastern Canada, focusing on vertical stratification and light availability. The goal was to understand how these ecological pressures influence herbivore and predator dynamics across different strata and between sunlit and shaded conditions. Overall, herbivory was concentrated in the understory, despite bottom-up forces generally favoring herbivores in the canopy. This herbivory pattern is partly attributed to top-down pressure, primarily exerted by birds. Align with "green world" hypothesis, insect herbivore populations are regulated by combined bottom-up and top-down forces, sustaining the structure and function of sugar maple forests.

In Chapter 2, we investigated how vertical gradients in light availability influence leaf traits and herbivory in sugar maples. The results revealed significant differences in leaf morphology, with sunlit canopy leaves being thicker and having lower specific leaf area (SLA) and water content than understory leaves. These traits, consistent with the leaf economics spectrum, align with stronger physical defenses in sunlit leaves. Indeed, herbivore damage was lower in the sunlit canopy compared to the understory. However, no significant differences were detected between shaded and sunlit canopy leaves. This finding suggests that ontogeny, the development stage of the tree, may play a more critical role than light intensity in shaping herbivory patterns. Additionally, earlier budburst in understory saplings compared to canopy trees likely increases their susceptibility to herbivory early in the season, when leaves are most vulnerable. These results underscore the complex interplay between leaf traits, phenology, and herbivore pressures within vertical gradients, showing the contribution of bottom-up pressure in shaping stratified pattern in insect herbivory align with green world hypothesis.

In Chapter 3, we build on the preceding result to examine relationships between differences in leaf traits and herbivore responses to sunlit and shaded sugar maple saplings. As predicted, sunlit sapling leaves exhibited greater thickness and lower SLA, which are associated with enhanced physical defenses. However, contrary to expectations, tussock moth larvae preferred and performed better on sunlit leaves, while spongy moth caterpillars showed no clear preference. Performance bioassays demonstrated higher survival and pupal mass for tussock moths on sunlit leaves, suggesting that nutritional benefits, such as higher nitrogen content (Ellsworth & Reich, 1992), outweighed the deterrent effects of physical defenses. These findings emphasize the nuanced relationship between leaf traits and herbivory, where increased thickness or lower SLA does not necessarily translate to reduced herbivory. The lack of strong preference in spongy moths could reflect their broader host adaptability, typical of invasive species, while tussock moths' preferences highlight the importance of light-driven leaf trait variation in influencing herbivore behavior. These results highlight the complexity of bottom-up influences on herbivory and suggest that, in this case, leaf traits alone may not be sufficient to regulate herbivore populations as predicted by the green world hypothesis.

In Chapter 4, we focused on predation rates across vertical strata and between sunlit and shaded saplings to assess top-down pressures on herbivores as a possible explanation for the gradient observed in Chapter 2. Our findings revealed a clear pattern of vertical stratification: arthropod predation was highest in the understory, while bird predation dominated in the sunlit canopy. Mammal predation followed a more complex pattern, being higher in the shaded canopy and understory compared to the sunlit canopy. No significant differences in total predation rates were observed between saplings. These results provide support for the enemy escape hypothesis, suggesting that herbivores are pushed to the understory primarily due to bird predation. However, the increased presence of arthropod predators in the understory complicates this dynamic, making it challenging to draw strong conclusions about the role of top-down forces in structuring the observed insect herbivory patterns. The concentration of herbivory in the understory despite favorable bottom-up conditions in the canopy (shown in chapter 2) combined with this predation pattern suggests that top-down force, likely from predators, help regulating herbivore populations and preventing excessive plant damage, aligning with the green world hypothesis.

In Chapter 5, we compared foliar traits and herbivore damage between sugar maples and black maples. While leaf traits, such as thickness, SLA, and trichome density, differed between the two species, these variations did not significantly influence herbivore damage patterns. This result could stem from the high potential for hybridization between the two species, which may obscure differences in defense traits. Although black maple leaves appeared less palatable due to their higher toughness and trichome density, performance tests showed no significant differences in caterpillar survival between the two species. These findings suggest that the physical traits of black maple leaves do not play an important role in shaping herbivory patterns between these congeneric species, which does not support the expected role of bottom-up pressure in insect herbivory regulation as proposed by the green world hypothesis.

6.1 Limitations

While this thesis provides valuable insights into the interplay of top-down and bottom-up forces in sugar maple-dominated forests, several challenges and limitations emerged during the research that shaped the scope and interpretation of the findings.

Vertical Stratification (Chapter 2): The study of vertical stratification faced limitations due to the seasonal timing of data collection. While differences in leaf physical traits and herbivory damage across strata were captured, the lack of early-season sampling may have missed the critical period of budburst when young leaves are most susceptible to herbivory (Murakami et al., 2005). Furthermore, only physical leaf traits were measured, leaving gaps in understanding the potential influence of chemical defenses, such as phenolics or tannins, which are known to play roles in herbivory patterns (Fortin & Mauffette, 2002; Levesque et al., 2002).

Preference and Performance Tests (Chapter 3): In the preference and performance tests, a limitation arose from the controlled laboratory environment, which cannot fully replicate the complexity of field conditions. While laboratory settings allowed for controlled comparisons of caterpillar performance on sunlit versus shaded leaves, they excluded natural factors like predator presence, abiotic stressors, or other herbivore competitors, which could influence caterpillar behavior and development. Additionally, the absence of detailed chemical analysis of the leaves, such as nitrogen or secondary metabolite content, limited our understanding of the nutritional drivers behind herbivore preferences.

Predation and Parasitism (Chapter 4): Challenges in assessing predation rates included the reliance on clay caterpillars, which lack the chemical and behavioral cues of live prey, potentially underestimating predation by certain predators like arthropods (Vet & Dicke, 1992). The method was effective for comparing predation rates across strata but may not reflect absolute predation pressure in natural conditions. Additionally, low parasitism rates limited the statistical analysis and interpretation of parasitism patterns. The design of sleeve cages, while useful for exposing caterpillars to parasitoids, may have inadvertently influenced their behavior or access, introducing potential bias.

Herbivory Between Maple Species (Chapter 5): Distinguishing between sugar maple and black maple leaves proved challenging due to the presence of hybrids in the study sites, which may have confounded trait and herbivory comparisons. While physical traits like SLA and toughness were measured, the exclusion of chemical traits meant the study could not fully explore potential differences in plant defense strategies. Moreover, the lack of significant differences in herbivore damage between the two species could reflect similarities in their environmental conditions and herbivore communities, underscoring the need for more nuanced analysis of biotic and abiotic factors. Although chemical analysis could help to provide more information about bottom-up factors effect on herbivory, it was beyond the scope of this study.

Methodological and Temporal Constraints: Across all chapters, although we made all the efforts to provide the experimental designs regarding the amount of time and effort could be put in this work, for some experiments we were limited to a single-season data collection restricted the ability to capture interannual variability or long-term trends influenced by climatic fluctuations or forest dynamics. Logistical challenges, such as limited access to forest canopies and the labor-intensive nature of certain methodologies, further constrained the sample sizes and replication in some experiments.

These challenges highlight the complexities of ecological research in forest systems and the need for continued efforts to refine methodologies, expand temporal and spatial scales, and incorporate additional variables to build a more comprehensive understanding of these interactions.

6.2 Implications for management and conservation purposes

This thesis offers insights into the intricate interplay of top-down and bottom-up forces within sugar maple-dominated forests. These findings are particularly relevant for developing strategies aimed at reducing herbivory damage in sapling, which are shown to be more vulnerable strata in this work and supporting forest health and regeneration in temperate ecosystems.

Strengthening Natural Enemy Effectiveness Through Canopy conservation: The results of this study highlight the critical role of natural enemies in regulating herbivorous insect populations. Bird predation dominated in the sunlit canopy, while arthropod predation was most prevalent in the understory. This dynamic suggests that while birds may push herbivores downward to understory saplings, insect herbivory at this level is partially balanced by arthropod predators, which themselves are driven to the understory by their own predators. Moreover, increasing sunlight exposure would not deter these top-down forces. Therefore, preserving mature trees in the canopy can better protect saplings by maintaining intense bird predation higher in the canopy while supporting arthropod biodiversity, including predators, in the shaded understory. This approach also preserves insect herbivore diversity, which, as shown in Chapter 1, does not cause threatening levels of damage to sapling leaves. Sugar maples are indeed among the most important hosts for herbivorous insect diversity (Narango et al., 2020).

Management Using Leaf Trait Insights: The influence of leaf traits such as thickness, toughness, and specific leaf area (SLA) on herbivory patterns provides a pathway for refining forest management practices. Leaves in sunlit conditions often showed stronger physical defenses, which in some plant species deterred herbivores. By maintaining a mosaic of sunlit and shaded areas, forest managers can foster a diversity of leaf traits that collectively reduce herbivore impact. For plantations, planting sugar maple saplings under the cover of fast-growing species could mimic natural understory conditions, enhancing defenses and minimizing herbivory. These measures could be especially valuable in areas prone to herbivore outbreaks.

Promoting Biodiversity for Natural Pest Control: The importance of biodiversity in regulating forest ecosystems is evident in this research. Predation pressure in the understory, combined with the diversity of herbivores observed, highlights the need to conserve habitat heterogeneity (Narango et al., 2020). Management approaches that enhance structural and species diversity, such as maintaining multi-aged stands and protecting understory vegetation, could boost predator populations and reduce herbivory. These practices align with broader goals of conserving forest ecosystem services, which are critical for long-term resilience.

Insights for Sugar Maple Regeneration: The study's findings on higher herbivory damage in shaded saplings stress the importance of targeted interventions to support sugar maple regeneration. Saplings in shaded environments are particularly vulnerable, suggesting that selective planting in moderately open areas could reduce herbivore damage and improve growth. Monitoring predator-prey dynamics in these areas would also help forest managers anticipate potential challenges and develop timely responses.

These implications highlight the potential for forest management practices that leverage natural processes to sustain sugar maple forests. By integrating these findings into conservation strategies, forest managers can not only mitigate the impacts of herbivory but also ensure the ecological and economic sustainability of these important forest ecosystems. This approach underscores the value of understanding ecological dynamics in developing effective, adaptable management practices.

6.3 Future research

Building upon the findings and addressing the limitations encountered in this thesis, several promising avenues for future research have emerged:

Vertical Stratification of Herbivory and Natural Enemy Pressures: The results in Chapter 2 highlight the importance of vertical stratification in shaping herbivory patterns and natural enemy interactions. However, the exclusion of early-season sampling limits our understanding of how budburst timing influences herbivory. Future studies could initiate sampling earlier in the season, capturing the critical window when young, nutritionally rich leaves are most vulnerable. Incorporating data on herbivore phenology and leaf chemical traits, such as secondary metabolites, would further illuminate the interplay between bottom-up and top-down forces.

Additionally, the study of natural enemy pressures could be expanded by incorporating live prey models alongside clay caterpillars to better capture predation dynamics influenced by chemical and behavioral cues. Advances in remote sensing technologies, such as drones equipped with imaging sensors, could be used to quantify fine-scale canopy structure and its effects on predator-prey interactions.

Sapling Growth and Herbivory Under Variable Light Conditions: Chapter 3 demonstrated the complexity of herbivore preferences for sunlit versus shaded saplings. However, the controlled lab environment excluded the influence of field conditions such as abiotic stressors, competitors, and predators. Future experiments could involve field-based mesocosm setups to evaluate herbivore performance in a semi-natural context, where these factors are present. Including a broader range of herbivore species would also help generalize the findings.

Long-term monitoring of sapling growth under different light conditions would be particularly valuable in assessing how herbivory impacts regeneration and competition among sugar maple saplings and other tree species. Integrating tree physiological measures, such as carbohydrate reserves and growth allocation, would provide insights into how saplings compensate for herbivore damage.

Predator Communities in Forest Canopies and Understory: The findings in Chapter 4 reveal intriguing patterns of predation across strata but also underline the need for more targeted studies on predator communities. A detailed analysis of predator assemblages, using molecular gut content analysis or stable isotope techniques, could identify the specific predators contributing to observed predation rates. These methods would also help differentiate between generalist and specialist predators and their impacts on herbivore populations.

The development of automated camera traps and acoustic sensors could provide non-invasive methods to monitor bird and mammal activity in different strata, offering a more comprehensive picture of predator-prey dynamics. Further, linking predation data to microclimatic conditions such as temperature, humidity, and light intensity could reveal how abiotic factors shape predator behavior and efficacy.

Comparing Sugar Maple and Black Maple Defense Mechanisms: Chapter 5 raises questions about the role of hybridization and chemical defenses in shaping herbivory patterns. Future research could focus on hybrid mapping using genomic tools to distinguish hybrids from parental species more accurately. Measuring a broader suite of traits, including secondary metabolites and nutrient content, would provide a more holistic understanding of defense strategies.

In addition, experiments manipulating environmental conditions, such as light intensity or nutrient availability, could test their influence on trait expression and herbivore responses in sugar and black maples. A reciprocal transplant experiment involving saplings in different forest habitats would further elucidate the relative roles of genetics and environment in determining herbivory patterns.

Integrating Long-Term and Climate-Driven Studies: This thesis provides a snapshot of herbivory and natural enemy interactions in sugar maple forests. Expanding this work to include long-term monitoring would capture interannual variability and the potential impacts of climate change. For example, tracking herbivore damage over multiple years and correlating them with climatic variables such as temperature and precipitation would enhance our understanding of how environmental fluctuations influence these dynamics.

Additionally, modeling studies could predict how future climate scenarios might shift the balance between top-down and bottom-up forces in these ecosystems. Incorporating climate-driven changes in forest structure and herbivore phenology into these models would make them more robust and relevant for forest management planning.

These future research directions aim to address the current knowledge gaps and extend the implications of this thesis, contributing to a deeper understanding of the ecological dynamics in sugar maple-dominated forests.

6.4 Conclusion

The findings from this thesis shed light on the intricate interplay of bottom-up and top-down forces driving insect herbivory patterns in a sugar maple-dominated temperate forest. Through the exploration of vertical stratification, leaf trait variations, and the dynamics of natural enemies, this research highlights the complexity of trophic interactions that regulate herbivore pressure within and between maple trees.

Key results reveal that bottom-up forces, shaped by leaf physical traits like thickness, SLA, and water content, significantly influence herbivory patterns. Sunlit leaves, characterized by higher physical defenses, consistently exhibited reduced damage compared to shaded leaves. Yet, despite these defenses, certain defoliators displayed a preference for these high-value leaves, underscoring the nuanced balance between plant defenses and herbivore feeding behavior. Top-down pressures also played a critical role, with predation rates differing across strata, suggesting that predators like birds and arthropods adapt their foraging behavior based on canopy structure.

This work shows that the relative roles of top-down and bottom-up forces differ in vertical gradients in different forest types. In the tropics, the understory is a predator refuge with only low-quality old tough leaves. It was different in the boreal where leaf quality did not vary so much (Zvereva et al., 2020). This study in a temperate deciduous forest shows different patterns in which differences in leaf quality do not limit herbivores in the understory. Mechanisms at play in these different ecosystems are the same (leaf age, leaf economic spectrum, ground-based vs arboreal predators) but their relative strengths vary and lead to different patterns.

This work underscores the dual importance of bottom-up and top-down factors in shaping herbivory patterns and provides critical insights into the spatial and temporal variability of these interactions. The integration of these findings into forest management strategies has the potential to enhance ecosystem resilience, guiding conservation practices that consider both the physiological responses of trees and the ecological dynamics of their associated fauna. In doing so, this thesis contributes to the broader understanding of how trophic interactions regulate forest health, offering a foundation for sustainable forest management and biodiversity conservation in the face of ongoing environmental change.

Bibliography

Agrawal, A. A. (2007). Macroevolution of plant defense strategies. *Trends in Ecology & Evolution*, *22*(2), 103–109. https://doi.org/10.1016/j.tree.2006.10.012

Agrawal, A. A., & Fishbein, M. (2006). Plant Defense Syndromes. *Ecology*, *87*(sp7), S132–S149. https://doi.org/10.1890/0012-9658(2006)87[132:PDS]2.0.CO;2

Aikens, K. R. (2008). *Heterogeneity in a temperate forest canopy: Describing patterns of distribution and depredation of arthropod assemblages*. McGill University.

Aikens, K. R., Timms, L. L., & Buddle, C. M. (2013). Vertical heterogeneity in predation pressure in a temperate forest canopy. *PeerJ*, *1*, e138.

Angulo-Sandoval, P., & Aide, T. M. (2000). Effect of plant density and light availability on leaf damage in *Manilkara bidentata* (Sapotaceae). *Journal of Tropical Ecology*, *16*(3), 447–464. https://doi.org/10.1017/S0266467400001504

Ashton, L. A., Nakamura, A., Basset, Y., Burwell, C. J., Cao, M., Eastwood, R., Odell, E., de Oliveira, E. G., Hurley, K., Katabuchi, M., Maunsell, S., McBroom, J., Schmidl, J., Sun, Z., Tang, Y., Whitaker, T., Laidlaw, M. J., McDonald, W. J. F., & Kitching, R. L. (2016). Vertical stratification of moths across elevation and latitude. *Journal of Biogeography*, *43*(1), 59–69. https://doi.org/10.1111/jbi.12616

Awmack, C. S., & Leather, S. R. (2002). Host Plant Quality and Fecundity in Herbivorous Insects. *Annual Review of Entomology*, *47*(Volume 47, 2002), 817–844. https://doi.org/10.1146/annurev.ento.47.091201.145300

Ayotte-Beaudet, J.-P., Chastenay, P., Beaudry, M.-C., L'Heureux, K., Giamellaro, M., Smith, J., Desjarlais, E., & Paquette, A. (2021). Exploring the impacts of contextualised outdoor science education on learning: The case of primary school students learning about ecosystem relationships. *Journal of Biological Education*, 1–18. https://doi.org/10.1080/00219266.2021.1909634

Barber, N. A., & Marquis, R. J. (2011). Light environment and the impacts of foliage quality on herbivorous insect attack and bird predation. *Oecologia*, *166*(2), 401–409. https://doi.org/10.1007/s00442-010-1840-9

Bar-Ness, Y. D., McQuillan, P. B., Whitman, M., Junker, R. R., Cracknell, M., & Barrows, A. (2012). Sampling forest canopy arthropod biodiversity with three novel minimal-cost trap designs. *Australian Journal of Entomology*, *51*(1), 12–21. https://doi.org/10.1111/j.1440-6055.2011.00836.x

Baron, J. N., & Rubin, B. D. (2021). Secondary invasion? Emerald ash borer (Agrilus planipennis) induced ash (Fraxinus spp.) mortality interacts with ecological integrity to facilitate European buckthorn (Rhamnus cathartica). *Canadian Journal of Forest Research*, *51*(3), 455–464. https://doi.org/10.1139/cjfr-2020-0134

Barton, K. E., & Koricheva, J. (2010). The Ontogeny of Plant Defense and Herbivory: Characterizing General Patterns Using Meta-Analysis. *The American Naturalist*, *175*(4), 481–493. https://doi.org/10.1086/650722

Basset, Y. (1991). The Spatial Distribution of Herbivory, Mines and Galls Within an Australian Rain Forest Tree. *Biotropica*, *23*(3), 271. https://doi.org/10.2307/2388204

Basset, Y., & Novotny, V. (1999). Species richness of insect herbivore communities on Ficus in Papua New Guinea. *Biological Journal of the Linnean Society*, *67*(4), 477–499. https://doi.org/10.1111/j.1095-8312.1999.tb01943.x

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using **lme4**. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Björkman, C., Dalin, P., & Ahrné, K. (2008). Leaf trichome responses to herbivory in willows: Induction, relaxation and costs. *New Phytologist*, *179*(1), 176–184. https://doi.org/10.1111/j.1469-8137.2008.02442.x

Black Maple-Endangered species in Quebec. (2024, December 11). CDPNQ-Ministry of the Environment, the Fight against Climate Change, Wildlife and Parks in Quebec. https://www.environnement.gouv.qc.ca/biodiversite/especes-designees-susceptibles/acer-nigrum/index.htm

Boege, K., & Marquis, R. J. (2006). Plant quality and predation risk mediated by plant ontogeny: Consequences for herbivores and plants. *Oikos*, *115*(3), 559–572. https://doi.org/10.1111/j.2006.0030-1299.15076.x

Boerner, R. E., & Brinkman, J. A. (1996). Ten years of tree seedling establishment and mortality in an Ohio deciduous forest complex. *Bulletin of the Torrey Botanical Club*, 309–317.

Bolker, B. (2019). Getting started with the glmmTMB package. *Cran. R-Project Vignette*, *9*. https://cran.r-hub.io/web/packages/glmmTMB/vignettes/glmmTMB.pdf

Bolker, B. M. (2020). *Post-model-fitting procedures with glmmTMB models: Diagnostics, inference, and model output(pp. 1–18)*.

Boukouvala, M. C., Kavallieratos, N. G., Skourti, A., Pons, X., Alonso, C. L., Eizaguirre, M., Fernandez, E. B., Solera, E. D., Fita, S., Bohinc, T., Trdan, S., Agrafioti, P., & Athanassiou, C. G. (2022). Lymantria dispar (L.) (Lepidoptera: Erebidae): Current Status of Biology, Ecology, and Management in Europe with Notes from North America. *Insects*, *13*(9), Article 9. https://doi.org/10.3390/insects13090854

Bray, J. R. (1956). Gap Phase Replacement in a Maple-Basswood Forest. *Ecology*, *37*(3), 598–600. https://doi.org/10.2307/1930185

Brehm, G. (2007). Contrasting patterns of vertical stratification in two moth families in a Costa Rican lowland rain forest. *Basic and Applied Ecology*, *8*(1), 44–54. https://doi.org/10.1016/j.baae.2006.02.002

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378–400.

Caldwell, E., Read, J., & Sanson, G. D. (2016). Which leaf mechanical traits correlate with insect herbivory among feeding guilds? *Annals of Botany*, *117*(2), 349–361. https://doi.org/10.1093/aob/mcv178

Cánovas, F. M., Lüttge, U., & Matyssek, R. (Eds.). (2018). *Progress in Botany Vol. 79* (Vol. 79). Springer International Publishing. https://doi.org/10.1007/978-3-319-71413-4

Carmona, D., Lajeunesse, M. J., & Johnson, M. T. J. (2011). Plant traits that predict resistance to herbivores. *Functional Ecology*, *25*(2), 358–367. https://doi.org/10.1111/j.1365-2435.2010.01794.x

Chen, Y., & Poland, T. M. (2009). Interactive Influence of Leaf Age, Light Intensity, and Girdling on Green Ash Foliar Chemistry and Emerald Ash Borer Development. *Journal of Chemical Ecology*, *35*(7), 806–815. https://doi.org/10.1007/s10886-009-9661-1

Choong, M. F. (1996). What Makes a Leaf Tough and How This Affects the Pattern of Castanopsis fissa Leaf Consumption by Caterpillars. *Functional Ecology*, *10*(5), 668–674. https://doi.org/10.2307/2390178

Coley, P. D., & Barone, J. A. (1996). HERBIVORY AND PLANT DEFENSES IN TROPICAL FORESTS. *Annual Review of Ecology and Systematics*, *27*(1), 305–335. https://doi.org/10.1146/annurev.ecolsys.27.1.305

Coley, P. D., Bryant, J. P., & Chapin, F. S. (1985). Resource availability and plant antiherbivore defense. *Science (New York, N.Y.)*, *230*(4728), 895–899. https://doi.org/10.1126/science.230.4728.895

Comeau, P. G., Filipescu, C. N., Kabzems, R., & DeLong, C. (2009). Corrigendum to: Growth of white spruce underplanted beneath spaced and unspaced aspen stands in northeastern B.C.—10 year results. *Forest Ecology and Management*, *257*(7), 1629–1636. https://doi.org/10.1016/j.foreco.2009.01.025

Corff, J. LE., & Marquis, R. J. (1999). Differences between understorey and canopy in herbivore community composition and leaf quality for two oak species in Missouri. *Ecological Entomology*, *24*(1), 46–58. https://doi.org/10.1046/j.1365-2311.1999.00174.x

Côté, B., & Ouimet, R. (1996). Decline of the maple-dominated forest in southern Quebec: Impact of natural stresses and forest management. *Environmental Reviews*, *4*(2), 133–148. https://doi.org/10.1139/a96-009

Cribari-Neto, F., & Zeileis, A. (2010). Beta regression in R. *Journal of Statistical Software*, *34*, 1–24.

Crowley, D., & Barstow, M. (2017). IUCN Red List of Threatened Species: Acer nigrum. *IUCN Red List of Threatened Species*. https://www.iucnredlist.org/en

Dansereau, P., & Desmarais, Y. (1947). Introgression in Sugar Maples II. *The American Midland Naturalist*, *37*(1), 146–161. https://doi.org/10.2307/2421649

Descombes, P., Kergunteuil, A., Glauser, G., Rasmann, S., & Pellissier, L. (2020). Plant physical and chemical traits associated with herbivory in situ and under a warming treatment. *Journal of Ecology*, *108*(2), 733–749. https://doi.org/10.1111/1365-2745.13286

DiTommaso, A., & Losey, J. E. (2003). Oviposition preference and larval performance of monarch butterflies (Danaus plexippus) on two invasive swallow-wort species. *Entomologia Experimentalis et Applicata*, *108*(3), 205–209. https://doi.org/10.1046/j.1570-7458.2003.00089.x

Duchesne, L., Ouimet, R., & Morneau, C. (2003). Assessment of sugar maple health based on basal area growth pattern. *Canadian Journal of Forest Research*, *33*(11), 2074–2080. https://doi.org/10.1139/x03-141

Dudt, J. F., & Shure, D. J. (1994). The Influence of Light and Nutrients on Foliar Phenolics and Insect Herbivory. *Ecology*, *75*(1), 86–98. https://doi.org/10.2307/1939385

Eisenring, M., Unsicker, S. B., & Lindroth, R. L. (2021). Spatial, genetic and biotic factors shape within-crown leaf trait variation and herbivore performance in a foundation tree species. *Functional Ecology*, *35*(1), 54–66. https://doi.org/10.1111/1365-2435.13699

Ellsworth, D. S., & Reich, P. B. (1992). Leaf Mass Per Area, Nitrogen Content and Photosynthetic Carbon Gain in Acer saccharum Seedlings in Contrasting Forest Light Environments. *Functional Ecology*, *6*(4), 423–435. https://doi.org/10.2307/2389280

Endara, M.-J., Coley, P. D., Ghabash, G., Nicholls, J. A., Dexter, K. G., Donoso, D. A., Stone, G. N., Pennington, R. T., & Kursar, T. A. (2017). Coevolutionary arms race versus host defense chase in a tropical herbivore–plant system. *Proceedings of the National Academy of Sciences*, *114*(36), E7499–E7505. https://doi.org/10.1073/pnas.1707727114

Fauna. (2024, December 9). Kenauk Institute. https://institutkenauk.org/conservations/fauna/

Favret, C., Lessard, V., Trépanier, A., Eon-Le Guern, T., & Théry, T. (2019). Voegtlin-style suction traps measure insect diversity and community heterogeneity. *Insect Conservation and Diversity*, *12*(5), 373–381. https://doi.org/10.1111/icad.12368

Fleak, S. (1967). *Hybridization in Acer saccharum Marsh. And Acer nigrum Michx. F.* 12–16.

Floate, K. D., & Whitham, T. G. (1993). The “Hybrid Bridge” Hypothesis: Host Shifting via Plant Hybrid Swarms. *The American Naturalist*, *141*(4), 651–662. https://doi.org/10.1086/285497

Fortin, M., & Mauffette, Y. (2002). *The suitability of leaves from different canopy layers for a generalist herbivore (Lepidoptera: Lasiocampidae) foraging on sugar maple*. *32*, 13.

Fortin, M., Mauffette, Y., & Albert, P. J. (1997). The effects of ozone-exposed sugar maple seedlings on the biological performance and the feeding preference of the forest tent caterpillar (Malacosoma disstria Hbn.). *Environmental Pollution*, *97*(3), 303–309. https://doi.org/10.1016/S0269-7491(97)00079-1

Foss, L. K., & Rieske, L. K. (2003). Species-specific differences in oak foliage affect preference and performance of gypsy moth caterpillars: *Oak foliage differences and gypsy moth caterpillars*. *Entomologia Experimentalis et Applicata*, *108*(2), 87–93. https://doi.org/10.1046/j.1570-7458.2003.00067.x

Gabriel, W. J. (1973). *MORPHOLOGICAL DIFFERENCES BETWEEN BLACK MAPLE AND SUGAR MAPLE AND THEIR HYBRIDS.*

Gabriel, W. J. (1990). Acer nigrum Michx. F. Black maple. *Silvics of North America*, *2*, 46–52.

Gardescu, S. (2003). Herbivory, Disease, and Mortality of Sugar Maple Seedlings. *Northeastern Naturalist*, *10*(3), 253–268.

Gaucher, C., Gougeon, S., Mauffette, Y., & Messier, C. (2005). Seasonal variation in biomass and carbohydrate partitioning of understory sugar maple (Acer saccharum) and yellow birch (Betula alleghaniensis) seedlings. *Tree Physiology*, *25*(1), 93–100. https://doi.org/10.1093/treephys/25.1.93

Godman, R. M., Yawney, H. W., & Tubbs, C. H. (1990). Acer saccharum Marsh. Sugar Maple. *Silvics of North America*, *2*(654), 78.

Gonzáles, W. L., & Gianoli, E. (2003). Evaluation of induced responses, insect population growth, and host-plant fitness may change the outcome of tests of the preference-performance hypothesis: A case study. *Entomologia Experimentalis et Applicata*, *109*(3), 211–216. https://doi.org/10.1046/j.0013-8703.2003.00110.x

Graça, M. B., Pequeno, P. A. C. L., Franklin, E., & Morais, J. W. (2017). Coevolution between flight morphology, vertical stratification and sexual dimorphism: What can we learn from tropical butterflies? *Journal of Evolutionary Biology*, *30*(10), 1862–1871. https://doi.org/10.1111/jeb.13145

Graves, W. R. (1994). Seedling Development of Sugar Maple and Black Maple Irrigated at Various Frequencies. *HortScience*, *29*(11), 1292–1294. https://doi.org/10.21273/HORTSCI.29.11.1292

Grayson, K. L., Parry, D., Faske, T. M., Hamilton, A., Tobin, P. C., Agosta, S. J., & Johnson, D. M. (2015). Performance of Wild and Laboratory-Reared Gypsy Moth (Lepidoptera: Erebidae): A Comparison between Foliage and Artificial Diet. *Environmental Entomology*, *44*(3), 864–873. https://doi.org/10.1093/ee/nvv063

Grimbacher, P. S., & Stork, N. E. (2007). Vertical stratification of feeding guilds and body size in beetle assemblages from an Australian tropical rainforest. *Austral Ecology*, *32*(1), 77–85. https://doi.org/10.1111/j.1442-9993.2007.01735.x

Gripenberg, S., Mayhew, P. J., Parnell, M., & Roslin, T. (2010). A meta-analysis of preference–performance relationships in phytophagous insects. *Ecology Letters*, *13*(3), 383–393. https://doi.org/10.1111/j.1461-0248.2009.01433.x

Guerra, P. C., Becerra, J., & Gianoli, E. (2010). Explaining differential herbivory in sun and shade: The case of Aristotelia chilensis saplings. *Arthropod-Plant Interactions*, *4*(4), 229–235. https://doi.org/10.1007/s11829-010-9099-y

Hairston, N. G., Smith, F. E., & Slobodkin, L. B. (1960). Community Structure, Population Control, and Competition. *The American Naturalist*, *94*(879), 421–425. https://doi.org/10.1086/282146

Hakimara, M., & Despland, E. (2024). Vertical stratification of leaf physical traits exerts bottom–up pressures on insect herbivory in a sugar maple temperate forest. *Insect Conservation and Diversity*, *Online Version of Record before inclusion in an issue*. https://doi.org/10.1111/icad.12777

Hamelin, C., Truax, B., & Gagnon, D. (2016). Invasive glossy buckthorn impedes growth of red oak and sugar maple under-planted in a mature hybrid poplar plantation. *New Forests*, *47*(6), 897–911. https://doi.org/10.1007/s11056-016-9551-7

Hanley, M. E., Lamont, B. B., Fairbanks, M. M., & Rafferty, C. M. (2007). Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, *8*(4), 157–178. https://doi.org/10.1016/j.ppees.2007.01.001

Hannerz, M. (1999). Evaluation of temperature models for predicting bud burst in Norway spruce. *Canadian Journal of Forest Research*, *29*(1), 9–19.

Harrow, S. (2014). *Comparative Analysis of Four Tree Species in a Northern Hardwood Forest*. Kansas State University.

Hartig, F. (2022). Package 'DHARMa.' *R Package. Available Online: Https://CRAN. R-Project. Org/Package= DHARMa (Accessed on 5 September 2022)*. https://scholar.google.com/scholar?cluster=7277662741730037362&hl=en&oi=scholarr

Hébert, C., Comtois, B., & Morneau, L. (2017). *Insectes des arbres du Québec*. Les Publications du Québec.

Heinrich, B. (1993). How avian predators constrain caterpillar foraging. In *Caterpillars. Ecological and Evolutionary Constraints on Foraging In: Stamp, N.E. and Casey, T.M.* (pp. 224–247). Chapman and Hall.

Henry, C. R., Walters, M. B., Finley, A. O., Roloff, G. J., & Farinosi, E. J. (2021). Complex drivers of sugar maple (Acer saccharum) regeneration reveal challenges to long-term sustainability of managed northern hardwood forests. *Forest Ecology and Management*, *479*, 118541. https://doi.org/10.1016/j.foreco.2020.118541

Hett, J. M., & Loucks, O. L. (1971). Sugar maple (Acer saccharum Marsh.) seedling mortality. *The Journal of Ecology*, 507–520.

Hilaire, R. St., & Graves, W. R. (1999). Foliar Traits of Sugar Maples and Black Maples Near 43 °N Latitude in the Eastern and Central United States. *Journal of the American Society for Horticultural Science*, *124*(6), 605–611. https://doi.org/10.21273/JASHS.124.6.605

Hochuli, D. F. (2001). Insect herbivory and ontogeny: How do growth and development influence feeding behaviour, morphology and host use? *Austral Ecology*, *26*(5), 563–570. https://doi.org/10.1046/j.1442-9993.2001.01135.x

Hochwender, C. G., & Fritz, R. S. (2004). Plant genetic differences influence herbivore community structure: Evidence from a hybrid willow system. *Oecologia*, *138*(4), 547–557. https://doi.org/10.1007/s00442-003-1472-4

Horsley, S. B., Long, R. P., Bailey, S. W., Hallett, R. A., & Wargo, P. M. (2002). Health of Eastern North American Sugar Maple Forests and Factors Affecting Decline. *Northern Journal of Applied Forestry*, *19*(1), 34–44. https://doi.org/10.1093/njaf/19.1.34

Houston, D. R., Allen, D. C., & Lachance, D. (1990). *Sugarbush management: A guide to maintaining tree health* (NE-GTR-129; p. NE-GTR-129). U.S. Department of Agriculture, Forest Service, Northeastern Forest Experimental Station. https://doi.org/10.2737/NE-GTR-129

Howe, A., Lövei, G. L., & Nachman, G. (2009). Dummy caterpillars as a simple method to assess predation rates on invertebrates in a tropical agroecosystem. *Entomologia Experimentalis et Applicata*, *131*(3), 325–329. https://doi.org/10.1111/j.1570-7458.2009.00860.x

Hunter, A. F., & Lechowicz, M. J. (1992). Foliage quality changes during canopy development of some northern hardwood trees. *Oecologia*, *89*(3), 316–323. https://doi.org/10.1007/BF00317408

Iverson, L. R., Prasad, A. M., Matthews, S. N., & Peters, M. (2008). Estimating potential habitat for 134 eastern US tree species under six climate scenarios. *Forest Ecology and Management*, *254*(3), 390–406.

Jackson, W. A. (2020). *Physiology and genetic studies of the hard maple group* [PhD Thesis, Rutgers The State University of New Jersey, School of Graduate Studies]. https://search.proquest.com/openview/c7809a544128bd89f29ba4317ff75d3f/1?pq-origsite=gscholar&cbl=51922&diss=y

Jactel, H., & Brockerhoff, E. G. (2007). Tree diversity reduces herbivory by forest insects. *Ecology Letters*, *10*(9), 835–848. https://doi.org/10.1111/j.1461-0248.2007.01073.x

Jaenike, J. (1978). On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology*, *14*(3), 350–356. https://doi.org/10.1016/0040-5809(78)90012-6

Jeffries, M., & Lawton, J. H. (1984). Enemy free space and the structure of ecological communities. *Biological Journal of the Linnean Society*, *23*, 269–286. https://doi.org/10.1111/j.1095-8312.1984.tb00145.x

Johns, R. C., Flaherty, L., Carleton, D., Edwards, S., Morrison, A., & Owens, E. (2016). Population studies of tree-defoliating insects in Canada: A century in review. *The Canadian Entomologist*, *148*(S1), S58–S81. https://doi.org/10.4039/tce.2015.69

Johnson, M. T. J., Bertrand, J. A., & Turcotte, M. M. (2016). Precision and accuracy in quantifying herbivory. *Ecological Entomology*, *41*(1), 112–121. https://doi.org/10.1111/een.12280

Johnson, N. M., & Baucom, R. S. (2023). *The double life of trichomes: Understanding their dual role in herbivory and herbicide resistance* (p. 2023.04.28.538721). bioRxiv. https://doi.org/10.1101/2023.04.28.538721

Keena, M. A., & Richards, J. Y. (2020). Comparison of Survival and Development of Gypsy Moth Lymantria dispar L. (Lepidoptera: Erebidae) Populations from Different Geographic Areas on North American Conifers. *Insects*, *11*(4), 260. https://doi.org/10.3390/insects11040260

Kennedy, C. E. J., & Southwood, T. R. E. (1984). The number of species of insects associated with British trees: A re-analysis. *The Journal of Animal Ecology*, 455–478.

Kitajima, K., Llorens, A., Stefanescu, C., Timchenko, M. V., Lucas, P. W., & Wright, S. J. (2012). How cellulose-based leaf toughness and lamina density contribute to long leaf lifespans of shade-tolerant species. *New Phytologist*, *195*(3), 640–652. https://doi.org/10.1111/j.1469-8137.2012.04203.x

Kriebel, H. B. (1957). *Patterns of genetic variation in sugar maple*. https://kb.osu.edu/bitstream/handle/1811/62575/1/OARDC_research_bulletin_n0791.pdf

Kriebel, H. B. (1989). Genetic improvement of sugar maple for high sap sugar content. I. Clone selection and seed orchard development. *Canadian Journal of Forest Research*, *19*(7), 917–923. https://doi.org/10.1139/x89-139

Landsberg, J. (1989). A comparison of methods for assessing defoliation, tested on eucalypt trees. *Austral Ecology*, *14*(4), 423–440. https://doi.org/10.1111/j.1442-9993.1989.tb01452.x

Levesque, K. R., Fortin, M., & Mauffette, Y. (2002). Temperature and food quality effects on growth, consumption and post-ingestive utilization ef.ciencies of the forest tent caterpillar *Malacosoma disstria* (Lepidoptera: Lasiocampidae). *Bulletin of Entomological Research*, *92*(2), 127–136. https://doi.org/10.1079/BER2002153

Leyva, K. J., Clancy, K. M., & Price, P. W. (2000). Oviposition Preference and Larval Performance of the Western Spruce Budworm (Lepidoptera: Tortricidae). *Environmental Entomology*, *29*(2), 281–289. https://doi.org/10.1093/ee/29.2.281

Libra, M., Tulai, S., Novotny, V., & Hrcek, J. (2019). Elevational contrast in predation and parasitism risk to caterpillars in a tropical rainforest. *Entomologia Experimentalis et Applicata*, *167*(11), 922–931. https://doi.org/10.1111/eea.12851

Lill, J. T., & Marquis, R. J. (2001). The effects of leaf quality on herbivore performance and attack from natural enemies. *Oecologia*, *126*(3), 418–428. https://doi.org/10.1007/s004420000557

López-Carretero, A., Boege, K., Díaz-Castelazo, C., Domínguez, Z., & Rico-Gray, V. (2016). Influence of plant resistance traits in selectiveness and species strength in a tropical plant-herbivore network. *American Journal of Botany*, *103*(8), 1436–1448. https://doi.org/10.3732/ajb.1600045

Low, P. A., Sam, K., McArthur, C., Posa, M. R. C., & Hochuli, D. F. (2014). Determining predator identity from attack marks left in model caterpillars: Guidelines for best practice. *Entomologia Experimentalis et Applicata*, *152*(2), 120–126. https://doi.org/10.1111/eea.12207

Lowman, M. D. (1992). Herbivory in Australian Rain Forests, with Particular Reference to the Canopies of Doryphora sassafras (Monimiaceae). *Biotropica*, *24*(2), 263. https://doi.org/10.2307/2388521

Maguire, D. Y., Nicole, T., Buddle, C. M., & Bennett, E. M. (2015). Effect of fragmentation on predation pressure of insect herbivores in a north temperate deciduous forest ecosystem. *Ecological Entomology*, *40*(2), 182–186. https://doi.org/10.1111/een.12166

Maguire, D. Y., Robert, K., Brochu, K., Larrivée, M., Buddle, C. M., & Wheeler, T. A. (2014). Vertical Stratification of Beetles (Coleoptera) and Flies (Diptera) in Temperate Forest Canopies. *Environmental Entomology*, *43*(1), 9–17. https://doi.org/10.1603/EN13056

Marques, E. S. D. A., Price, P. W., & Cobb, N. S. (2000). Resource Abundance and Insect Herbivore Diversity on Woody Fabaceous Desert Plants. *Environmental Entomology*, *29*(4), 696–703. https://doi.org/10.1603/0046-225X-29.4.696

Mauffette, Y., Lechowicz, M. J., & Jobin, L. (1983). Host preferences of the gypsy moth, *Lymantria dispar* (L.), in southern Quebec. *Canadian Journal of Forest Research*, *13*(1), 53–60. https://doi.org/10.1139/x83-008

Mayhew, P. J. (1997). Adaptive Patterns of Host-Plant Selection by Phytophagous Insects. *Oikos*, *79*(3), 417–428. https://doi.org/10.2307/3546884

Meier, U., Bleiholder, H., Buhr, L., Feller, C., Hack, H., Heß, M., Lancashire, P. D., Schnock, U., Stauß, R., Van Den Boom, T., Weber, E., & Zwerger, P. (2009). The BBCH system to coding the phenological growth stages of plants – history and publications –. *Journal of Cultivated Plants*, 41-52 Pages. https://doi.org/10.5073/JFK.2009.02.01

Messier, J., McGill, B. J., Enquist, B. J., & Lechowicz, M. J. (2017). Trait variation and integration across scales: Is the leaf economic spectrum present at local scales? *Ecography*, *40*(6), 685–697. https://doi.org/10.1111/ecog.02006

Mina, M., Messier, C., Duveneck, M. J., Fortin, M., & Aquilué, N. (2022). Managing for the unexpected: Building resilient forest landscapes to cope with global change. *Global Change Biology*, *28*(14), 4323–4341. https://doi.org/10.1111/gcb.16197

Mooney, K. A., Pratt, R. T., & Singer, M. S. (2012). The Tri-Trophic Interactions Hypothesis: Interactive Effects of Host Plant Quality, Diet Breadth and Natural Enemies on Herbivores. *PLOS ONE*, *7*(4), e34403. https://doi.org/10.1371/journal.pone.0034403

Morrow, C. J., Jaeger, S. J., & Lindroth, R. L. (2022). Intraspecific variation in plant economic traits predicts trembling aspen resistance to a generalist insect herbivore. *Oecologia*, *199*(1), 119–128. https://doi.org/10.1007/s00442-022-05158-z

Muiruri, E. W., Barantal, S., Iason, G. R., Salminen, J., Perez-Fernandez, E., & Koricheva, J. (2019). Forest diversity effects on insect herbivores: Do leaf traits matter? *New Phytologist*, *221*(4), 2250–2260. https://doi.org/10.1111/nph.15558

Murakami, M., & Wada, N. (1997). *Difference in leaf quality between canopy trees and seedlings affects migration and survival of spring-feeding moth larvae*. *27*, 6.

Murakami, M., Yoshida, K., Hara, H., & Toda, M. J. (2005). Spatio-temporal variation in Lepidopteran larval assemblages associated with oak, Quercus crispula: The importance of leaf quality. *Ecological Entomology*, *30*(5), 521–531. https://doi.org/10.1111/j.0307-6946.2005.00724.x

Murphy, S. M., & Loewy, K. J. (2015). Trade-offs in host choice of an herbivorous insect based on parasitism and larval performance. *Oecologia*, *179*(3), 741–751. https://doi.org/10.1007/s00442-015-3373-8

Nakadai, R., Murakami, M., & Hirao, T. (2014). Effects of phylogeny, leaf traits, and the altitudinal distribution of host plants on herbivore assemblages on congeneric Acer species. *Oecologia*, *175*(4), 1237–1245. https://doi.org/10.1007/s00442-014-2964-0

Narango, D. L., Tallamy, D. W., & Shropshire, K. J. (2020). Few keystone plant genera support the majority of Lepidoptera species. *Nature Communications*, *11*(1), 5751. https://doi.org/10.1038/s41467-020-19565-4

Nobel, P. S., Zaragoza, L. J., & Smith, W. K. (1975). *Relation between Mesophyll Surface Area, Photosynthetic Rate, and Illumination Level during Development for Leaves of Plectranthus parviforus Henckel'. 55.*

Panzuto, M., Lorenzetti, F., Mauffette, Y., & Albert, P. J. (2001). Perception of Aspen and Sun/Shade Sugar Maple Leaf Soluble Extracts by Larvae of Malacosoma disstria. *Journal of Chemical Ecology*.

Parry, M. L. (2007). *Climate change 2007-impacts, adaptation and vulnerability: Working group II contribution to the fourth assessment report of the IPCC* (Vol. 4). Cambridge University Press. https://books.google.com/books?hl=en&lr=&id=TNo-SeGpn7wC&oi=fnd&pg=PA135&dq=Parry,+2007&ots=vS3Bqg1qkC&sig=q95em66ioB4CnPGqj2D26TTbHMU

Payette, S., Morneau, C., & Fortin, M. J. (1996). The recent sugar maple decline in southern Quebec: Probable causes deduced from tree rings. *Canadian Journal of Forest Research*, *26*(6), 1069–1078.

Pearse, I. S., & Hipp, A. L. (2009). Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proceedings of the National Academy of Sciences*, *106*(43), 18097–18102. https://doi.org/10.1073/pnas.0904867106

Pearse, I. S., & Hipp, A. L. (2012). GLOBAL PATTERNS OF LEAF DEFENSES IN OAK SPECIES. *Evolution*, *66*(7), 2272–2286. https://doi.org/10.1111/j.1558-5646.2012.01591.x

Potter, K. M., & Hargrove, W. W. (2013). Quantitative assessment of predicted climate change pressure on North American tree species. *Mathematical and Computational Forestry & Natural-Resource Sciences (MCFNS)*, *5*(2), 151–169.

Pugeaut, A., Postaire, B., & Surget-Groba, Y. (2024). Genomics for species delineation and conservation status determination: The Black Maple as a case study. *Authorea*, *902*, 90211437.

Quebec 4-H clubs. (2019). La Chenille Espionne. https://www.chenilles-espionnes.com/le-projet

Quebec Ministry of Forests. (2023). *Forest Domain Map of Quebec, Retrieved from https://www.forestsquebec.gouv.qc.ca/domaines-forestiers/carte.jsp*. https://www.forestsquebec.gouv.qc.ca/domaines-forestiers/carte.jsp

Rasmann, S., & Agrawal, A. A. (2011). Evolution of specialization: A phylogenetic study of host range in the red milkweed beetle (Tetraopes tetraophthalmus). *The American Naturalist*, *177*(6), 728–737. https://doi.org/10.1086/659948

Reich, P. B. (2014). The world-wide 'fast-slow' plant economics spectrum: A traits manifesto. *Journal of Ecology*, *102*(2), 275–301. https://doi.org/10.1111/1365-2745.12211

Robinson, S. K., & Holmes, R. T. (1982). Foraging Behavior of Forest Birds: The Relationships Among Search Tactics, Diet, and Habitat Structure. *Ecology*, *63*(6), 1918–1931. https://doi.org/10.2307/1940130

Roland, J. (1993). Large-scale forest fragmentation increases the duration of tent caterpillar outbreak. *Oecologia*, *93*(1), 25–30. https://doi.org/10.1007/BF00321186

Rowe, W. J., & Potter, D. A. (1996). Vertical stratification of feeding by Japanese beetles within linden tree canopies: Selective foraging or height per se? *Oecologia*, *108*(3), 459–466. https://doi.org/10.1007/BF00333722

Salgado-Luarte, C., González-Teuber, M., Madriaza, K., & Gianoli, E. (2023). Trade-off between plant resistance and tolerance to herbivory: Mechanical defenses outweigh chemical defenses. *Ecology*, *104*(1), e3860. https://doi.org/10.1002/ecy.3860

Schoonhoven, L. M., Loon, J. J. A. van, & Dicke, and M. (2006). *Insect-Plant Biology* (Second Edition, Second Edition). Oxford University Press.

Schowalter, T. D. (2006). *Insect ecology: An ecosystem approach*. Academic press.

Schowalter, T. D., & Ganio, L. M. (1998). Vertical and seasonal variation in canopy arthropod communities in an old-growth conifer forest in southwestern Washington, USA. *Bulletin of Entomological Research*, *88*(6), 633–640.

Schowalter, T. D., Hargrove, W. W., & Crossley, D. A. (1986). Herbivory in Forested Ecosystems. *Annual Review of Entomology*, *31*(1), 177–196. https://doi.org/10.1146/annurev.en.31.010186.001141

Schultz, J. C., Nothnagle, P. J., & Baldwin, I. T. (1982). *Seasonal and Individual Variation in Leaf Quality of Two Northern Hardwoods Tree Species*. 8.

Seifert, C. L., Jorge, L. R., Volf, M., Wagner, D. L., Lamarre, G. P. A., Miller, S. E., Gonzalez-Akre, E., Anderson-Teixeira, K. J., & Novotný, V. (2021). Seasonality affects specialisation of a temperate forest herbivore community. *Oikos*, *130*(9), 1450–1461. https://doi.org/10.1111/oik.08265

Seifert, C. L., Lamarre, G. P. A., Volf, M., Jorge, L. R., Miller, S. E., Wagner, D. L., Anderson-Teixeira, K. J., & Novotný, V. (2020). Vertical stratification of a temperate forest caterpillar community in eastern North America. *Oecologia*, *192*(2), 501–514. https://doi.org/10.1007/s00442-019-04584-w

Seiwa, K. (1998). Advantages of early germination for growth and survival of seedlings of *Acer mono* under different overstorey phenologies in deciduous broad-leaved forests. *Journal of Ecology*, *86*(2), 219–228. https://doi.org/10.1046/j.1365-2745.1998.00245.x

Sigut, M., Šigutová, H., Šipoš, J., Pyszko, P., Kotásková, N., & Drozd, P. (2018). Vertical canopy gradient shaping the stratification of leaf-chewer–parasitoid interactions in a temperate forest. *Ecology and Evolution*, *8*(15), 7297–7311. https://doi.org/10.1002/ece3.4194

Singer, M. S., & Stireman, J. O. (2005). The tri-trophic niche concept and adaptive radiation of phytophagous insects. *Ecology Letters*, *8*(12), 1247–1255. https://doi.org/10.1111/j.1461-0248.2005.00835.x

Skepner, A. P. (1997). cpDNA of Acer saccharum and Acer nigrum are Very Similar. *The Ohio Journal of Science*, *97*(4), 90–93.

Southwood, T. R. E., Wint, G. W., Kennedy, C. E., & Greenwood, S. R. (2005). The composition of the arthropod fauna of the canopies of some species of oak (Quercus). *European Journal of Entomology*, *102*(1), 65–72.

Stemmelen, A., Paquette, A., Benot, M.-L., Kadiri, Y., Jactel, H., & Castagneyrol, B. (2022). Insect herbivory on urban trees: Complementary effects of tree neighbours and predation. *Peer Community Journal*, *2*, e22. https://doi.org/10.24072/pcjournal.106

Stireman, J. O., Cerretti, P., Whitmore, D., Hardersen, S., & Gianelle, D. (2012). Composition and stratification of a tachinid (Diptera: Tachinidae) parasitoid community in a European temperate plain forest: Tachinid parasitoid community stratification. *Insect Conservation and Diversity*, *5*(5), 346–357. https://doi.org/10.1111/j.1752-4598.2011.00168.x

Stoepler, T. M., & Lill, J. T. (2013). Direct and indirect effects of light environment generate ecological trade-offs in herbivore performance and parasitism. *Ecology*, *94*(10), 2299–2310. https://doi.org/10.1890/12-2068.1

Strong, A. M., Sherry, T. W., & Holmes, R. T. (2000). Bird predation on herbivorous insects: Indirect effects on sugar maple saplings. *Oecologia*, *125*(3), 370–379. https://doi.org/10.1007/s004420000467

Taylor, K. M., & Aarssen, L. W. (1989). NEIGHBOR EFFECTS IN MAST YEAR SEEDLINGS OF ACER SACCHARUM. *American Journal of Botany*, *76*(4), 546–554. https://doi.org/10.1002/j.1537-2197.1989.tb11346.x

Thomas, S. C., Sztaba, A. J., & Smith, S. M. (2010). Herbivory patterns in mature sugar maple: Variation with vertical canopy strata and tree ontogeny. *Ecological Entomology*, *35*(1), 1–8. https://doi.org/10.1111/j.1365-2311.2009.01133.x

Thompson, J. N. (1988). Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata*, *47*(1), 3–14. https://doi.org/10.1111/j.1570-7458.1988.tb02275.x

Turcotte, M. M., Thomsen, C. J. M., Broadhead, G. T., Fine, P. V. A., Godfrey, R. M., Lamarre, G. P. A., Meyer, S. T., Richards, L. A., & Johnson, M. T. J. (2014). Percentage leaf herbivory across vascular plant species: *Ecological Archives* E095-065. *Ecology*, *95*(3), 788–788. https://doi.org/10.1890/13-1741.1

Turgeon, J. (2019). *The Effect of Vertical Stratification and Forest Management on Beetle (Insecta: Coleoptera) and Spider (Arachnida: Araneae) Communities in a Temperate Forest*. McGill University.

Ulyshen, M. D. (2011). Arthropod vertical stratification in temperate deciduous forests: Implications for conservation-oriented management. *Forest Ecology and Management*, *261*(9), 1479–1489. https://doi.org/10.1016/j.foreco.2011.01.033

Van Bael, S. A., Brawn, J. D., & Robinson, S. K. (2003). Birds defend trees from herbivores in a Neotropical forest canopy. *Proceedings of the National Academy of Sciences*, *100*(14), 8304–8307. https://doi.org/10.1073/pnas.1431621100

van Ginkel, J. R. (2023). Handling Missing Data in Principal Component Analysis Using Multiple Imputation. In L. A. van der Ark, W. H. M. Emons, & R. R. Meijer (Eds.), *Essays on Contemporary Psychometrics* (pp. 141–161). Springer International Publishing. https://doi.org/10.1007/978-3-031-10370-4_8

Vance, C. C., Smith, S. M., Malcolm, J. R., Huber, J., & Bellocq, M. I. (2007). Differences Between Forest Type and Vertical Strata in the Diversity and Composition of Hymenpteran Families and Mymarid Genera in Northeastern Temperate Forests. *Environmental Entomology*, *36*(5), 1073–1083. https://doi.org/10.1603/0046-225X(2007)36[1073:DBFTAV]2.0.CO;2

Vet, L. E. M., & Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, *37*, 141–172.

Vidal, M. C., & Murphy, S. M. (2018). Bottom-up vs. top-down effects on terrestrial insect herbivores: A meta-analysis. *Ecology Letters*, *21*(1), 138–150. https://doi.org/10.1111/ele.12874

Ville de Montreal. (2023, March 25). *Ville de Montréal*. Ville de Montréal. http://ville.montreal.qc.ca/pls/portal/docs/page/conseil_patrimoine_mtl_fr/media/documents/ecoterritoire2fr.pdf

Volf, M., Klimeš, P., Lamarre, G. P. A., Redmond, C. M., Seifert, C. L., Abe, T., Auga, J., Anderson-Teixeira, K., Basset, Y., Beckett, S., Butterill, P. T., Drozd, P., Gonzalez-Akre, E., Kaman, O., Kamata, N., Laird-Hopkins, B., Libra, M., Manumbor, M., Miller, S. E., … Novotny, V. (2019). Quantitative assessment of plant-arthropod interactions in forest canopies: A plot-based approach. *PLoS ONE*, *14*(10), 20.

Volf, M., Pyszko, P., Abe, T., Libra, M., Kotásková, N., Šigut, M., Kumar, R., Kaman, O., Butterill, P. T., Šipoš, J., Abe, H., Fukushima, H., Drozd, P., Kamata, N., Murakami, M., & Novotny, V. (2017). Phylogenetic composition of host plant communities drives plant-herbivore food web structure. *Journal of Animal Ecology*, *86*(3), 556–565. https://doi.org/10.1111/1365-2656.12646

Welch, B. L. (1938). The Significance of the Difference Between Two Means when the Population Variances are Unequal. *Biometrika*, *29*(3/4), 350–362. https://doi.org/10.2307/2332010

Whitfeld, T. J. S., Novotny, V., Miller, S. E., Hrcek, J., Klimes, P., & Weiblen, G. D. (2012). Predicting tropical insect herbivore abundance from host plant traits and phylogeny. *Ecology*, *93*(sp8), S211–S222. https://doi.org/10.1890/11-0503.1

Wickham, H. (2016). *Ggplot2*. Springer International Publishing. https://doi.org/10.1007/978-3-319-24277-4

Wilkinson, D. M., & Sherratt, T. N. (2016). Why is the world green? The interactions of top–down and bottom–up processes in terrestrial vegetation ecology. *Plant Ecology & Diversity*, *9*(2), 127–140. https://doi.org/10.1080/17550874.2016.1178353

Wint, G. W. (1983). Leaf damage in tropical rain forest canopies. In *Tropical rain forest: Ecology and management* (pp. 229–239).

Witkowski, E. T. F., & Lamont, B. B. (1991). Leaf specific mass confounds leaf density and thickness. *Oecologia*, *88*(4), 486–493. https://doi.org/10.1007/BF00317710

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., … Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, *428*(6985), 821–827. https://doi.org/10.1038/nature02403

Yang, B., Li, B., He, Y., Zhang, L., Bruelheide, H., & Schuldt, A. (2018). Tree diversity has contrasting effects on predation rates by birds and arthropods on three broadleaved, subtropical tree species. *Ecological Research*, *33*(1), 205–212. https://doi.org/10.1007/s11284-017-1531-7

Yang, L. H. (2014). Insects as drivers of ecosystem processes. *Current Opinion in Insect Science*, 7.

Yataco, A. P., Noor, S., Girona, M. M., Work, T., & Despland, E. (2024). Limited Differences in Insect Herbivory on Young White Spruce Growing in Small Open Plantations and under Natural Canopies in Boreal Mixed Forests. *Insects*, *15*(3), 196. https://doi.org/10.3390/insects15030196

Zalucki, M. P., Clarke, A. R., & Malcolm, S. B. (2002). Ecology and Behavior of First Instar Larval Lepidoptera. *Annual Review of Entomology*, *47*(Volume 47, 2002), 361–393. https://doi.org/10.1146/annurev.ento.47.091201.145220

Zalucki, M. P., Malcolm, S. B., Paine, T. D., Hanlon, C. C., Brower, L. P., & Clarke, A. R. (2001). It's the first bites that count: Survival of first-instar monarchs on milkweeds. *Austral Ecology*, *26*(5), 547–555. https://doi.org/10.1046/j.1442-9993.2001.01132.x

Zehnder, C. B., Stodola, K. W., Joyce, B. L., Egetter, D., Cooper, R. J., & Hunter, M. D. (2009). Elevational and Seasonal Variation in the Foliar Quality and Arthropod Community of *Acer pensylvanicum*. *Environmental Entomology*, *38*(4), 1161–1167. https://doi.org/10.1603/022.038.0424

Zhang, S., Xu, G., Zhang, Y., Zhang, W., & Cao, M. (2023). Canopy height, rather than neighborhood effects, shapes leaf herbivory in a tropical rainforest. *Ecology*, *104*(5), e4028. https://doi.org/10.1002/ecy.4028

Zhong-Tao, P., Guang-Ze, J. I. N., & Zhi-Li, L. I. U. (2024). Leaf trait variations and relationships of three Acer species in different tree sizes and canopy conditions in Xiao Hinggan Mountains of

Northeast China. *Chinese Journal of Plant Ecology*, *48*(6), 730. https://doi.org/10.17521/cjpe.2023.0369

Zuur, A. F., Ieno, E. N., & Smith, G. M. (Eds.). (2007). Principal component analysis and redundancy analysis. In *Analysing Ecological Data* (pp. 193–224). Springer. https://doi.org/10.1007/978-0-387-45972-1_12

Zvereva, E. L., & Kozlov, M. V. (2023). Predation risk estimated on live and artificial insect prey follows different patterns. *Ecology*, *104*(3), e3943. https://doi.org/10.1002/ecy.3943

Zvereva, E. L., Paolucci, L. N., & Kozlov, M. V. (2020). Top-down factors contribute to differences in insect herbivory between saplings and mature trees in boreal and tropical forests. *Oecologia*, *193*(1), 167–176. https://doi.org/10.1007/s00442-020-04659-z