The Chemical Ecology and Insect Associates of the Eastern Larch Beetle (*Dendroctonus simplex* LeConte)

A DISSERTATION

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BY

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Dedication

To Sylvia Darr for teaching me to value all creatures great and small

To Trey Michael David Rhodes (1994-2002). I will never take this life for granted.

And to all of the “silly little girls” who dream of being a scientist. May we make this place a better one for you than what we grew up in.

Dissertation Abstract

The eastern larch beetle, *Dendroctonus simplex* LeConte (Coleoptera: Curculionidae: Scolytinae), is a native insect ranging from Alaska through the western provinces of Canada, dipping down into the Great Lakes region, and extending to the northeast United States and Canadian maritime regions. Historically, this beetle exhibited outbreaks of three to five year durations in stands of tamarack, *Larix laricina* (Du Roi) K. Koch, predisposed to attack after experiencing stress such as defoliation, drought, wind throw, fire, or flooding. Recently, 20+ year outbreaks have been observed in portions of the southern transcontinental range of the insect, likely exacerbated by growing seasons that permit more than one generation of eastern larch beetle per year. In Minnesota, an outbreak has affected more than 75% of tamarack covertype in the state since 2001. I worked to expand our knowledge of the beetle's chemical ecology and natural enemy complex. Specifically, I characterized monoterpenes in the phloem of regional host trees, isolated pheromones produced by *D. simplex*, and assessed antennal responses of these beetles to these compounds. I observed beetle walking and flight responses to beetle and host compounds and documented insect biodiversity within the regional tamarack ecosystem. I discovered that α-pinene and Δ-3-carene were the most prominent compounds present in tamarack phloem. I found that unmated female beetles produce seudenol and low quantities of frontalin to which male beetles respond. After mating, female beetles cease to produce seudenol, instead increasing frontalin production. At high dosages (at or over 3 mg/day release rates), male and female beetles show repulsive responses to frontalin. The attraction of natural enemies such as *Thanasimus* spp. is positively correlated with frontalin concentration. Finally, my biodiversity survey chronicled 185 unique taxa captured in Lindgren funnel traps. Biodiversity indices such as Shannon diversity, species evenness, species richness, and

abundance shifted throughout each summer with Shannon diversity and species evenness reaching the lowest level in mid-June and species richness and abundance peaking at these same dates.

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Dissertation Introduction

Many species of *Dendroctonus* bark beetles are commercially important pests of tree species in the genera *Pinus*, *Picea*, *Pseudotsuga*, and *Larix* when under outbreak conditions (Six and Bracewell 2015). To colonize hosts, in many species, female beetles respond to host volatiles, assess host suitability, and tunnel into the phloem (Wood 1982). As females tunnel, they may emit pheromone cues and, in response, additional beetles aggregate on the tree, including male beetles searching for a mate (Bloomquist et al. 2010). The females lay eggs in niches along the gallery walls and the larvae continue to feed on the phloem until pupation and emergence (Coulson 1979, Wood, 1982, Sauvard 2004, McKee 2022). This feeding activity under the bark can kill otherwise vigorous, large diameter trees if population size is sufficient to allow hundreds of attacks.

Although many species of *Dendroctonus* beetles can reach outbreak levels, in recent years, outbreaks have become more pronounced as alterations in temperature, precipitation, and increased disturbances have led to higher environmental stress for hosts and opportunities for beetles to expand their ranges (Gandhi and Hofstetter 2022). In western North America, the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) caused billions of tree losses spanning more than 27 million hectares from 2000-2020. This outbreak has largely been driven by a lack of inclimate weather allowing the beetle to expand its range over the Rocky Mountains (Cooke and Carroll 2017, Fettig et al. 2022). Simultaneously, the southern pine beetle (*Dendroctonus frontalis* Zimmermann) has been expanding its range from Central America and the southern United States into New York and Maine, likely due to changes in warming winters (Dodds et al. 2018, Kanaskie et al. 2023). Even systems with historically low tree mortality rates

are increasingly impacted by bark beetles. For example, *Dendroctonus pseudotsugae* Hopkins infestations typically have caused 1-5% annual tree mortality rates (Bible 2001), but mortality has risen to 30%-50% mortality in some areas, potentially driven by abiotic stressors such as wind damage and drought (Freeman, 2017, Mafra-Neto et al. 2022). Additionally, tree mortality rates due to spruce beetle, *Dendroctonus rufipennis* Kirby, have jumped from an annual 20-30% to nearly 90% in some regions in response to increased temperatures and drought (Mafra-Neto et al. 2022).

Minnesota is in the midst of its own climate-driven *Dendroctonus* outbreak. The eastern larch beetle, *Dendroctonus simplex* LeConte, has shifted from an intermittent disturbance agent exhibiting short-term outbreaks following periods of stress to an insect experiencing a prolonged outbreak that has lasted more than 20 years (McKee and Aukema 2015, Ward and Aukema 2019). As of 2023, the eastern larch beetle outbreak has impacted 433,863 cumulative hectares, or 75% of tamarack covertype within the state since 2001 (MN DNR 2024). Eastern larch beetle activity has also been noted throughout other portions of the Great Lakes region (WI DNR 2021, MI DNR 2024, WI DNR 2024).

Due to the duration and severity of the outbreak, eastern larch beetle has become a growing concern among state land managers (Snyder et al. 2022) and conservationists (Grinde et al. 2022) as tamarack is a dominant tree in boreal northern forests (Duncan 1954, Johnson 1990, McKee et al. 2022). Prior to European settlement, tamarack was the most abundant tree in the state (MN DNR 2013). These tamarack and peatland systems are critical to climate change mitigation, water storage, and water quality improvement (Euliss et al. 2008). Within peatland habitats, tamarack trees play a critical role in carbon sequestration, hydrology, prevention of soil

erosion, and habitat provision (Burns and Honkala 1990). Thus, insight into refined monitoring and management for eastern larch beetle is valued by stakeholders in the region.

Bark beetle management is a multi-pronged approach in many systems. Silvicultural approaches may include pruning, thinning, prescribed burns or altering stand structure and composition. However, implementation of silvicultural techniques may take time and are typically used in a proactive rather than reactive approach. Additionally, systematic insecticide applications and trunk sprays are used in some instances, but these can be economically impractical for large, natural forests (Coops et al. 2008, Gitau et al. 2013, Clarke et al. 2016). Thus, the usage of semiochemicals in monitoring or management schemes provides a less time-intensive, lower-cost alternative or supplement to long-term strategies.

Beetle pheromones and host volatiles are widely utilized in monitoring and management regimes. Analysis of over 863 experiments by Afzal et al. 2023 has shown these eco-friendly tools to be effective in reducing bark beetle capture rates. Monitoring of *Dendroctonus frontalis*, for example, is conducted using frontalin combined with *endo*-brevicomin and turpentine lures. Ratios of beetle to natural enemy capture rates can be utilized to make management decisions for the upcoming summer (Sullivan et al. 2021). For pheromone-based management strategies, repellent compounds may be attached to high value trees or high-value stands to prevent beetle aggregation. Verbenone is used to protect high value trees from *Dendroctonus ponderosae* (Huber and Borden 2003, Fettig et al. 2015) and MCH (3-methyl-2-cyclohexen-1-one) is used for *Dendroctonus rufipennis* and *Dendroctonus pseudotsugae* (Foote et al. 2020, Audley et al. 2022). Before any of these strategies can be developed, an in-depth understanding of an insect's chemical ecology is necessary.

For my dissertation, I studied the chemical ecology of the eastern larch beetle and its natural enemies. Eastern larch beetles utilize host monoterpenes to locate and select host material, (Langor and Raske 1987, McKee and Aukema 2015). Prior work indicated that α-pinene and β-pinene were the most prevalent monoterpenes within tamarack, but the phloem of tamarack in the Great Lakes region had not been assessed (Stairs 1967, Von Rudloff 1987). Previous work had also indicated that female larch beetles produce frontalin, although it remained unclear whether paired or solitary beetles were utilized during pheromone isolation (Francke et al. 1995, Barkawi et al. 2003). Moreover, field trapping studies have varied in their observed beetle responses to frontalin with some showing attraction while others displayed repulsion (Baker et al. 1977, Werner 1981, Prendergast 1991, Graham and Storer 2011). Although *Thanasimus* spp. are generalist predators of bark beetles that use α-pinene and frontalin as cues to locate prey, *Thanasimus* responses had not been thoroughly assessed in tamarack forests (Aukema and Raffa 2005, Staeben et al. 2014). Moreover, few studies have quantified and identified arthropod biodiversity in tamarack stands (Blackman and Stage 1918, Wolcott and Montgomery 1933, Langor and Raske 1988, Langor 1991). Thus, my work was designed to gain a more comprehensive understanding of eastern larch beetle and natural enemy chemical ecology in tamarack ecosystems within the Great Lakes region.

In Chapter 1, I determined the primary host monoterpene compounds from *Larix laricina* from Wisconsin and Minnesota. This analysis was conducted to determine potential host-selection cues that the eastern larch beetle may be utilizing to locate suitable hosts. My findings that Δ-3-carene is a major component of tamarack phloem in the Great Lakes region prompted lure choices in Chapter 3.

In Chapter 2, I isolated pheromones from single and paired beetles. I then assessed beetle antennal responses through gas chromatography-electroantennographic detection. Our findings that paired female beetles produce higher concentrations of frontalin prompted our dose-response study in Chapter 3. Isolation of seudenol from single female eastern larch beetles is the first time that seudenol has been identified from the species.

In Chapter 3, I conducted two field experiments determining beetle and natural enemy attractancy or repellency to seudenol in combination with 1) a variety of host compounds (2022) and 2) a series of varying dosages of frontalin (2023). Next, I observed beetle walking behavior with exposure to female-produced compounds, seudenol and frontalin. My findings of variation in attractancy of frontalin when paired with seudenol depending on concentration, sex, and modality (i.e., walking vs. flying) could inform future management and tree protection schemes.

In Chapter 4, I identified target taxa from bycatches from field assays in Chapter 3. While arthropod biodiversity in tamarack systems has been assessed in the northeast, community composition in the Great Lakes has not been documented. This work will serve as a checklist. It is my hope that this work can inform future research and conservation efforts in the Great Lakes tamarack ecosystems.

Chapters within my dissertation are written with the intention of publication in peer reviewed journals. Chapter 1 has been published in *Forests*. Chapter 2 was prepared for submission to the *Journal of Chemical Ecology*, Chapter 3 has been prepared for submission to *Environmental Entomology*, and Chapter 4 is being prepared for submission to *ZooKeys*. A small degree of redundancy may exist between chapters to maintain their integrity as stand-alone units. While I led the teams conducting the following work, it would have been impossible to complete without my coauthors: Brian Aukema, Kelly Aukema, Mike Gates, Ian Grossenbacher-

McGlamery, Luke Kresslein, Brian Sullivan, Thomas O'Loughlin, and David Wakarchuk. As such, I utilize the plural voice throughout the remainder of my dissertation.

Chapter 1. Monoterpene composition of phloem of *Larix laricina* in the Great Lakes Region: with what must eastern larch beetle (*Dendroctonus simplex*) contend?

Abstract

The eastern larch beetle (*Dendroctonus simplex* LeConte) is the only tree-killing bark beetle that colonizes tamarack, or eastern larch, (*Larix laricina* (Du Roi) K. Koch) in the Great Lakes region. Historically, outbreaks have been intermittent and of short duration, frequently following predisposing factors such as drought or defoliation. However, over the past two decades, this insect has been in a perpetual state of outbreak in parts of the Great Lakes region, a deviation from historic norms. From 2001-2021, it impacted 300,000 ha, or 60% of the tamarack forests in Minnesota. This activity has prompted renewed interest in the beetle's chemical ecology, including aspects of host semiochemistry. While foliar chemistry has been well documented in *L. laricina*, characterization of the monoterpene composition of the phloem has been lacking. We collected phloem samples from 56 tamarack trees across 14 locations in Wisconsin and Minnesota and assessed monoterpene composition using GCMS. Individual terpenoid components identified included α-pinene (39.4%) and Δ-3-carene (30.0%) followed by several other components in small (<8%) amounts. This knowledge provides a basis for testing monoterpene synergists or antagonists in pheromone lures targeting eastern larch beetle and/or its natural enemies.

Keywords: α-pinene; Δ-3-carene; host defense; kairomones; semiochemicals

Introduction

The eastern larch beetle, *Dendroctonus simplex* LeConte, is a bark beetle (Coleoptera: Curculionidae: Scolytinae) native to North America, ranging from Alaska to Newfoundland. The southern portion of its range extends to the Great Lakes region. This range is sympatric with the insect's host, tamarack or eastern larch, *Larix laricina* (Du Roi) K. Koch, which is an important component of peatlands and lowland conifer forests (Burns and Honkala 1990). Adult beetles emerge in the spring from hibernal galleries or the duff at the bases of trees. After boring through the bark, females oviposit into the phloem where eclosion, larval development, and pupation occur (Langor and Raske 1987). Historically, eastern larch beetle has colonized trees weakened by wind, flooding, drought, harvesting damage, or large-scale herbivory. Sporadic but short-term outbreaks sometimes spill over to vigorous trees for three to five years (Langor and Raske 1989).

Over the past 20 years, the Great Lakes region has been experiencing a large-scale outbreak of eastern larch beetle that represents a duration outside of historic norms. As of 2021, the eastern larch beetle had affected approximately 60% of all tamarack cover type in the state of Minnesota, or approximately 300,000 ha (MN DNR 2021). This outbreak has been associated with multiple factors, such as predisposing defoliation by the non-native defoliator larch casebearer (*Coleophora laricella* (Hübner)) and warming temperatures that may be altering the demography of eastern larch beetle, larch casebearer, and the larch casebearer's biological control complex (McKee and Aukema 2015, Ward and Aukema 2019, Ward et al. 2020). The severity and duration of the current outbreak have prompted renewed interest in the chemical ecology of eastern larch beetle and its host.

This study aims to characterize the chemical composition of tamarack phloem in the Great Lakes region. While several studies have helped characterize pheromone production and response of eastern larch beetle (e.g. Werner et al. 1981, Prendergast 1991, Franke et al. 1995, Graham and Storer 2011) knowledge of the chemical composition of the phloem tissues of *L. laricina* has been lacking especially in the midcontinental range of this tree. The chemical composition of *L. laricina* twig and leaf oil and resin have been characterized in northwestern Canada and New York, but with small sample sizes that limit wider inference (Stairs 1967, Von Rudloff 1987). The phloem's monoterpene composition is important to understand as bark beetles respond to these compounds during host selection and gallery excavation (Raffa et al. 2005). Moreover, some bark beetles exhibit geographic differences in responses to terpenes (Sun et al. 2004). Knowledge of potential regional differences in host monoterpene composition could help optimize lure blends to enhance sampling of bark beetles and their natural enemies or attempt tree protection schemes.

Materials and Methods

Field Collection

Phloem samples were acquired from four trees at 14 sites (Figure 1). An arc punch (2.54 cm diameter) was hammered into the tree at breast height (1.4 m) and phloem was carefully removed with a scalpel. Phloem core samples were placed in 20 mL scintillation vials and kept in a cooler with dry ice before transfer to a -80 °C freezer for 24-48 hours. Sampling was conducted 15-16 July 2021.

Chemical Analysis

Phloem samples were ground in a ceramic mortar and pestle (Coors) after freezing with liquid nitrogen. Then, samples were transferred to a 20 mL beaker. From there, the approximate 1 g sample was covered in hexane (0.3 mL hexane/ 0.6 g phloem 1:5-1:10 phloem to solvent ratio). The sample was left for 15-20 minutes to thaw, and the contents were then filtered through a small cotton batting plug.

GC-FID analysis was conducted using an Agilent DB-1 column (length: 30 m, i.d.: 0.32 mm, column film thickness: 1.00 µm, nonpolar) with a hydrogen carrier gas. Operating conditions were as follows: Injection size per sample was 2 µl with a split ratio of 20:1. The inlet temperature was 250°C and FID temperature was 325°C. The column started at 40°C and was held at this temperature for 1 minute. Temperature was ramped up to 90°C at a rate of 20°C/min and held for 5 minutes. From there, the temperature was ramped at a rate of 25°C/min up to 325°C and held for 3 minutes before finishing the run. The peaks were analyzed using Agilent OpenLab Magic GC. In this study, limonene and -phellandrene were not separated as peaks were too close to differentiate.

Statistical Analysis

We analyze and report proportions of each monoterpene component from the phloem samples instead of amount per gram dry phloem, as phloem dry weight information was not retained due to a data curation error. To calculate mean proportions of each monoterpene in the samples, we fit a cell means model of the component proportion (response variable, asin(sqrt(y)) transformed) as a function of compound type (a categorical variable; each of eight monoterpenes and an ‘unidentified’ category) in a mixed-effects ANOVA. We fit the term for compound type

as a fixed effect and terms for tree nested within sampling site as random effects to contend with geographic variation. This analysis yielded the mean proportion of each compound with an associated standard error. The standard error was multiplied by $Z = \pm 1.96$ and then added/subtracted from the mean estimate to obtain 95% confidence intervals about each mean once back-transformed.

Results

Our results show that α-pinene is the highest concentration monoterpene in phloem of *L. laricina* followed by Δ-3-carene, although there was a high amount of variability across our 56 samples (Figure 2). The percentage of α-pinene in the terpenoid blend within the phloem samples ranged from 12.8% to 75.5%, for example, and the percentage of Δ-3-carene likewise varied from 1.5% to 67.4% (Figure 2). Despite this variability, the median percentages were almost identical at 34.5% for α-pinene and 33.8% for Δ-3-carene. Overall, the estimate of the population mean was approximately 10% higher for α-pinene than Δ-3-carene, at 39.4% vs. 30.0% respectively (Table 1). All other identifiable compounds, such as camphene, sabinene, α-pinene, myrcene, and limonene + -phellandrene, were present in low amounts collectively under 8% (Table 1, Figure 2). Less than 10% of compounds were left unidentified.

Discussion

Our finding that two monoterpenes comprise more than two thirds of the monoterpene complex in phloem samples from *L. laricina* in Minnesota and Wisconsin are similar to previous results across the range of tamarack (Stairs 1967, Von Rudloff 1987). Yet, the proportions of

these compounds are highly variable between studies and there is geographic variation between which compounds make up this two-thirds majority. In New York, for example, Stairs (1967) found that α-pinene dominated the terpene blend (58.9%) with Δ-3-carene at only 9.1%, thus comprising 68.0% of the samples. Our study found a more even mixture, on average, between the two chemicals with 39.4% α-pinene and 30.0% Δ-3-carene (Table 1, Figure 2). Von Rudloff (1987) found a similarly even mixture of α-pinene (23.0%) and Δ-3-carene (22.2%) in twigs of trees across the Yukon Territory, Alberta, and Saskatchewan, although his overall percentages were lower than those we noted. Our finding of β-pinene at only 6.5% of the terpene blend of phloem, on average, was four times lower than noted by Stairs (1967) and half that noted by Von Rudloff (1987). Collectively, results across these studies provide evidence of high variation in constitutive monoterpenes with which beetles must contend across the range of *L. laricina* in North America.

Within the Great Lakes Region, the phloem chemistry of *L. laricina* appears to be similar to that found in the foliage, although important variation exists. Ward et al. (2019), for example, found similarly high levels of α-pinene and Δ-3-carene followed by lower concentrations of α-pinene in needles of *L. laricina,* mirroring our findings in phloem monoterpenes (Ward et al. 2019). Work done in Wisconsin, however, found that concentrations of α-pinene were approximately double that of Δ-3-carene in short shoot foliage of *L. laricina* and between three and four times higher in the long shoot foliage (mean concentrations between 0.67-2.61mg/g; (Powell and Raffa 1999). The composition of these monoterpenes within the host can vary seasonally as well as in response to resource availability (Powell and Raffa 1999).

Understanding the phloem monoterpene composition of *L. laricina* can inform the development of better monitoring lures or anti-aggregation blends to protect high-value trees. Moreover, predators and other natural enemies may exhibit different responses to host semiochemicals than their bark beetle prey (Aukema et al. 2000). Work is currently ongoing assessing the attractancy of these compounds in field conditions within the Great Lakes region. Future work is necessary to explore concentrations causing attraction or repulsion in concert with pheromone components of *D. simplex*.

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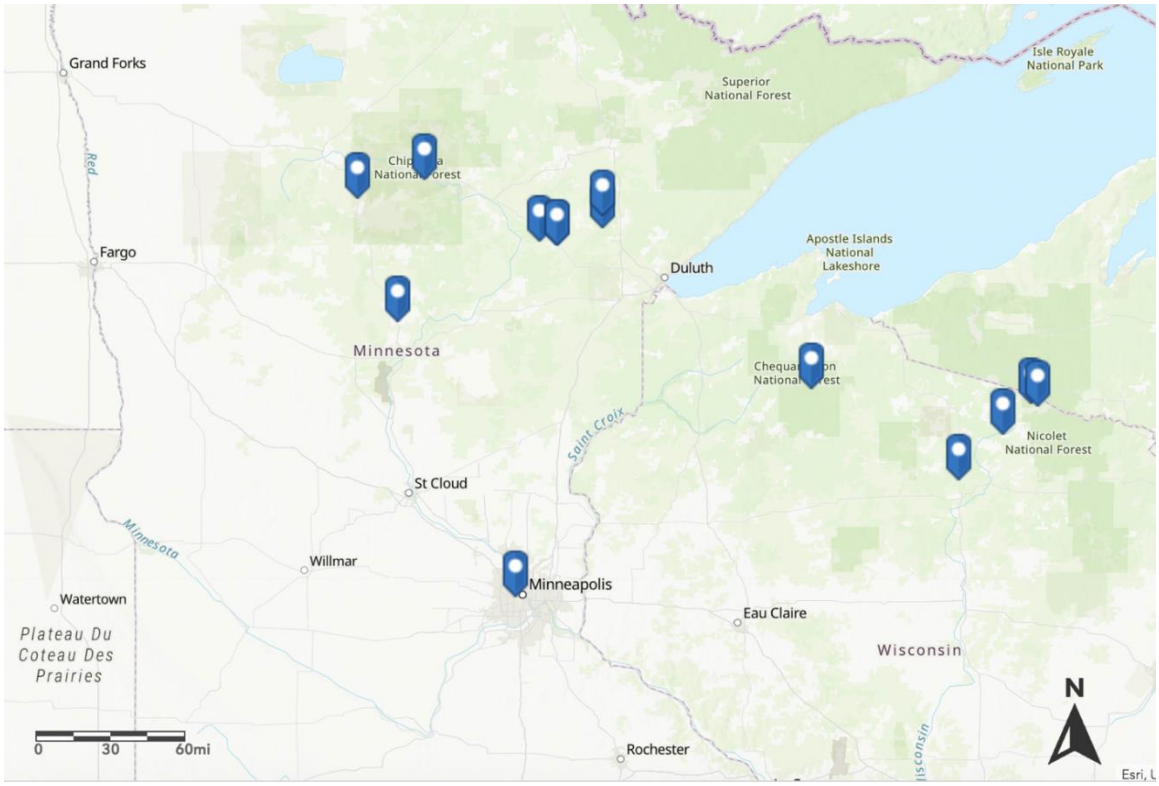
Table 1. Population estimates of mean percent compositions of various monoterpenes within 56 *Larix laricina* (Du Roi) K Koch sampled from Minnesota and Wisconsin, 15–16 July 2021, with 95% CI.

Compound	**Mean Composition (%)**	**95% CI**
α-pinene	39.4	36.7, 42.1
Camphene	0.7	0.3, 1.2
Sabinene	1.2	0.7, 1.9
β-pinene	6.5	5.2, 8.0
Myrcene	1.7	1.1, 2.5
Δ-3-carene	30.0	27.5, 32.6
Limonene + β-phellandrene	3.6	2.7, 4.8
Terpinolene	2.4	1.6, 3.3
Unidentified Compounds	10.0	8.4, 11.8

Figure 1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Fargo
Superior
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Isle Royale
National Park
Apostle Islands
National
Lakeshore
Duluth
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Saint Croix
Nicolet
National Forest
St Cloud
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Watertown
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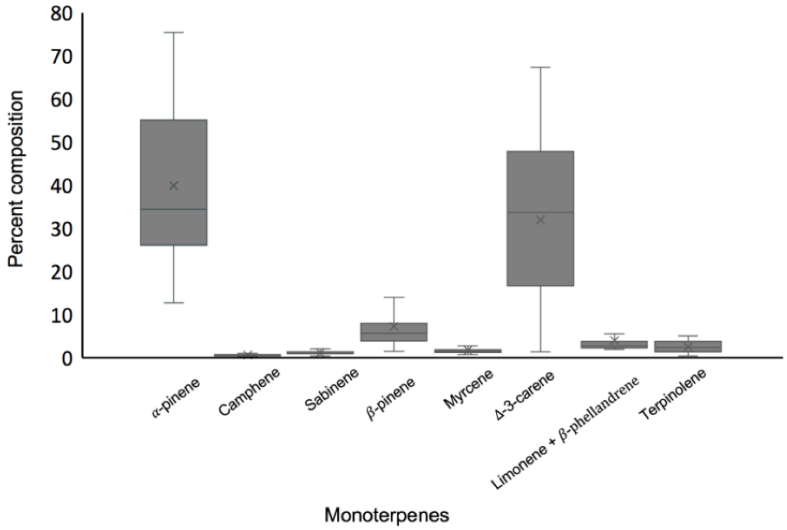
Figure 2


80
70
60
50
40
30
20
10
0
Percent composition
α-pinene
Camphene
Sabinene
β-pinene
Myrcene
Δ-3-carene
Limonene + β-phellandrene
Terpinolene
Monoterpenes

Chapter 2. Pheromone production and olfactory sensitivities in the eastern larch beetle, *Dendroctonus simplex* LeConte (Coleoptera: Curculionidae)

Abstract

The eastern larch beetle, *Dendroctonus simplex* LeConte, colonizes tamarack *Larix laricina* (Du Roi) K. Koch, throughout its temperate North American range but its chemical ecology has not been fully elucidated. Here, we expand our knowledge of pheromone communication for this bark beetle. We collected volatiles from female beetles tunneling solo, and both males and females tunneling as presumably mated pairs in tamarack phloem. Males were also assessed. Compounds identified from the volatiles were screened for antennal detection using gas chromatography-electroantennographic detection (GC-EAD). Then, we added juvenile hormone III (JH III) to the femoral joints of male and female beetles to determine if identified pheromone components were produced by the mevalonate pathway. Solitary female beetles produced seudenol and frontalin when tunneling into host tissue. Within 24 hours of joining a male, female production of seudenol ceased while production of frontalin continued. Both female and male beetles exhibited antennal responses to seudenol and frontalin. Application of JH III induced production of frontalin in solitary female beetles, but not seudenol, indicating that the latter is regulated by a distinct mechanism. Our results provide insight into the mechanisms by which *D. simplex* attracts mates and colonizes trees and may inform future work on monitoring and management strategies exploiting beetle pheromones for management of high-value trees.

Keywords: bark beetles, electroantennogram, frontalin, seudenol, volatiles

INTRODUCTION

Dendroctonus simplex LeConte (eastern larch beetle) is the primary bark beetle associated with *Larix laricina* (Du Roi) (eastern larch or tamarack), a tree with which it has coexisted for at least 12,000 years (Ashworth et al. 1981). In lowland temperate conifer forests of North America, tamarack serves critical environmental roles in hydrology, prevention of soil erosion, carbon sequestration, and habitat provision (Burns and Honkala 1990). These trees also hold cultural significance to indigenous peoples. In Ojibwe cultures, tamarack trees can be utilized for medicinal purposes and are called *muckiwatig* or "swamp tree" (Prindle 2021). The name tamarack is derived from the Abenaki word for "wood used for snowshoes," *hackmatack* (Erichson-Brown 1979). These lowland ecosystems also contain culturally important foods such as wild blueberry (*Vaccinium angustifolium* Aiton) and cranberry (*Vaccinium* spp.) (Kozich et al. 2002, Windmuller-Campione et al. 2023). Thus, these forests serve a variety of roles in ecology and culture.

For much of their recorded history, the eastern larch beetle and tamarack have coexisted with short-term beetle outbreaks occurring only when predisposing factors such as fire, flooding, drought, windthrow, or defoliator damage were present (Hopkins 1909, Baker 1972, Bright 1976, Langor and Raske 1987). However, in the case in southern portions of its transcontinental range, such as the Great Lakes region, 433,863 cumulative hectares of tamarack have been affected by eastern larch beetle in Minnesota alone (MN DNR 2024). The outbreak is likely driven by expanded growing seasons facilitating a shift from univoltine to bi-voltine life cycles and increasingly mild winters allowing increased adult survival (Walters and Venette 2012, McKee and Aukema 2015).

The shift from a non-aggressive disturbance agent to one capable of large-scale outbreaks has occurred rapidly, refocusing attention on the insect's ecology and behavior. In *D. simplex*, as in most *Dendroctonus*, females are the host-selecting sex (McKee et al. 2015). Female eastern larch beetles produce a bicyclic ketal known as frontalin (1,5-dimethyl-6,8–dioxabicyclo[3.2.1.]octane) as their primary pheromone as well as three structurally related terpenoid ketones: 6-methyl-6-hepten-2-one, 6-methyl-5-hepten-2-one, and 6-methyl-3(E),5-heptadien-2-one (Francke et al. 1995, Barkawi et al. 2003). At present, however, it is unknown whether production of these compounds changes with mating status, which could alter behaviors in cooperative, mass attack strategies in host procurement. Historically, most work has focused on frontalin, although behavioral responses to frontalin in the field have been mixed (Werner 1981, Prendergast 1991, Graham and Storer 2011). Current monitoring lures contain a known attractant, seudenol (3-methyl-2-cyclohexen-one-ol), although this compound has not been isolated from eastern larch beetle (Baker 1977, Werner 1981, Prendergast 1991, Seybold et al. 2002, Graham and Storer 2011).

This study aims to elucidate pheromone production and detection in the eastern larch beetle system. Specifically, we tested aerated solitary beetles and paired (i.e., female with male) beetles to identify compounds that may function as aggregation or anti-aggregation pheromones. Our intent was to test unmated (i.e., solitary) vs. mated (i.e., paired) beetles, but cannot preclude the possibility of sibling mating under the bark prior to emergence from a host or reemerged parent adults and collection in Lindgren funnel traps in the field for our assays. Such pre-emergence mating has not been observed in *D. simplex* but is known to occur infrequently in other *Dendroctonus* spp. (Six and Bracewell 2015). Here, we hypothesize that there is variation in the quantity of frontalin produced by paired female beetles in comparison to solitary female

beetles. We also conducted electroantennogram studies using volatiles isolated from solitary and paired beetles to determine which compounds elicited responses. Finally, we topically applied juvenile hormone III (JH III) to allow inferences on pheromone production pathways. While prior work elucidated that frontalin is produced *de novo* through the mevalonate pathway in *D. simplex* (Barkawi et al. 2003), it is likely that additional aerated compounds may be produced by a separate pathway. A more detailed understanding of the pheromones produced by paired and solitary beetles will broaden our understanding of mating and host procurement as well as refine potential pheromone management strategies.

Methods and Materials

Host Material and Insects. Uninfested *Larix laricina* trees of approximately 25.4 cm DBH (diameter at breast height; 1.4 m) were felled and cut to 25.4 cm lengths at Rush Lake Wildlife Management Area, MN (19 August 2021) and at Carlos Avery Wildlife Management Area, MN (2 June 2022) (45.40948 °N, -92.92568 °W). Beetles were captured from Lindgren funnel traps baited with two-component lures (seudenol release rate 7.5 mg/day at 30°C; Synergy Semiochemicals Corp. Delta, BC) and α-pinene ((1S, 5S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene((-)α-Pinene)) (release rate 2 g/day at 23°C; Synergy Semiochemicals Corp. Delta, BC) and collected approximately every other day. In 2021, 17 August – 22 August, insects were collected from Lindgren funnel traps set at Rush Lake Wildlife Management Area, MN (45.66009 °N, -93.02289 °W); 2022 (1 June – 6 June), from Washburn Lake Wildlife Management Area, MN (46.57261 °N, 93.26197 °W); 2023 (3 July – 8 July), from Warren Woessner Bog Boardwalk, MN (47.07559 °N, -92.6616 °W) and Winterberry Bog, MN (47.17072 °N, -92.53954 °W). To improve beetle longevity and reduce predation from natural enemies, moistened paper towels and sphagnum moss were placed in the plastic ml trap collection cups. Male and female beetles

were distinguished by stridulation produced by male beetles (Rosenberger et al. 2016) and stored at approximately 4°C.

Pheromone Production Assay. Beetles for this assay were captured in methods discussed for 2022 and 2023. We placed 40 female beetles into individual gelatin capsule halves over 2 mm diameter holes drilled into the logs to phloem depth. These entrance points were spaced approximately 4 cm apart to reduce possible interference among mining beetles. Female beetles were allowed to tunnel into the logs for 24 h at room temperature (19-31 °C). After 24 h, male beetles were added singly to 20 of the gel capsules, allowing them to enter the nuptial galleries under the bark and mate. Nineteen females were left unpaired. After an additional 24 h, all beetles were excavated from logs. One paired female was mortally injured during debarking, leaving 19 females. We also collected volatiles from trap-captured male (n = 24) and female (n = 14) beetles that were not placed into logs approximately seven days after capture.

All beetles were placed abdomen-first into 100 µl glass conical vials containing HaysepQ chemical adsorbent (polydivinylbenzene; 2-3 mg; 80–100 mesh; Hayes Separations Inc., Bandera, Texas, USA) and held in place with Teflon batting. Methodology is consistent with Sullivan 2005. Conical vials were capped by lids with a 2 mm diameter hole for air circulation and were placed into a file box with a charcoal fabric-lined top pressed against the caps. These samples were incubated at 22°C for 18-24 h at elevated humidity. Beetles were then removed from the conical vials and preserved in 95% ethanol vials for later sex confirmation by dissection.

The HaysepQ adsorbent was extracted with 50 µl hexane containing 3.5 ng/µl heptyl acetate and 3.8 ng/ml cycloheptanone as internal quantitative standards (Sigma-Aldrich Inc. St. Louis, Missouri, USA). Extracts were transferred to a 100 µl autosampler vial insert, the conical

vial was then rinsed with 50 µl redistilled pentane, and the rinsate was added to the autosampler vial. Samples were analyzed on a GC-MS (Hewlett-Packard 6890-5973, Palo Alto, California, USA) using an HP INNOWax column (60 m x 0.25 mm diam. x 0.25 µm film thickness; polyethylene glycol phase; Agilent, Santa Clara, California, USA) at a helium carrier gas flow rate of 1.0 ml/min. Samples were analyzed in splitless mode (inlet 200°C) with the mass spectral detector in electron ionization mode with an ion source temperature of 230°C. The quadrupole temperature was set to 150°C and the repeller voltage was set to 29.5 V. The oven program was set for 40°C for 1 min then ramping 16°C/minute until reaching 80°C, and then 7°C/minute until reaching 230°C before being held for seven minutes. We utilized matches of mass spectra and retention times to identify beetle-produced compounds (Sources: Sigma-Aldrich; Synergy Semiochemicals Inc, Delta, British Columbia, Canada; Bedoukian Research Inc., Danbury, Connecticut, USA; Phero Tech Inc., Delta, British Columbia). Compounds were quantified against a standard curve made from sequential dilutions of compounds of interest each containing the internal standards in the samples.

Olfactory Responses. Coupled gas chromatography-electroantennographic detection (GC-EAD) was used to assess antennal responses of male and female beetles using conical vial samples from single females, paired females, and paired males. Preparatory methodology is described in Sullivan et al. (2021). In brief, electrodes were a pair of tapered glass capillaries filled with Beadle-Ephrussi Ringer solution and with an $Ag/AgCl_2$ wire inserted into the interior. They made contact with the base of the beetle's excised head and one of the antennal clubs. Between three to six insects were analyzed depending on the assay. Thes GC inlet was in splitless mode, and the injection volume of the sample was 2 µl. Data were recorded using an SRI Instruments (Torrence, California, USA) model 202 computer interface with Peak simple

software (SRI Instruments, Torrence, California, USA). As a check at the beginning and the end of each GC run, antennae were exposed to a two second duration puff of air through a Pasteur pipette holding a piece of filter paper with 10 µl mineral oil containing 0.2-2 µg/µl *α*-pinene, myrcene, frontalin, and *exo*- and *endo*-brevicomin.

Following the assay, insects were dissected to confirm sex identifications that had been previously based on gallery position upon extraction from the logs. Unfortunately, we found that the sexes of individuals within five pairs may have been misidentified previous to mixing the material to which the insect antennae were exposed, and thus the "paired female" odors were not all females and the "paired male" odors were not all males. Despite this discrepancy, we still are able to present odors of both paired males and females to conspecific antennae and document odors that produced an olfactory response.

JH Application. To determine whether JH III regulates production of pheromone components, we applied 10 µl of a JH III solution to the femoral joints of solitary male (n = 27) and female (n = 26) beetles using a blunt tipped 10 µl Hamilton syringe. JH III for application was prepared by dissolving 3.8 ng/µg of JHIII in 0.5 µl acetone. Solitary male (n = 10) and female (n = 10) beetles received 0.5 µl acetone applied to the femoral joint as controls. Beetles were collected via trapping in 2023 as described previously.

Statistical Analysis. To assess pheromone production across treatments (sex and mating status), we conducted an ANOVA model. A ln(y +1) transformation was utilized for pheromone concentrations to satisfy assumptions of homoscedasticity and normal distribution of residuals, examined visually. Additionally, an ANOVA test comparing solely paired vs. solitary female beetle production of frontalin was conducted. Again, a ln(y +1) transformation was utilized to satisfy assumptions of homoscedasticity and normal distribution.

Response amplitudes by EAD were normalized against linear regressions constructed using the antennal response data from control stimulus where *y* was the response amplitude of the puffs and *x* was the retention times of the first and second puffs stimuli. These calculations allowed accommodation for loss of antennal vigor was testing is completed, as antennal sensitivity declines through time after decapitation. We used regressions to account for these changes when analyzing compounds eluted at various times.

RESULTS

Pheromone Production. Solitary, feeding female eastern larch beetle produced an average of 433.62 ± 105.35 SE ng/beetle of frontalin, 96.62 ± 34.71 ng/beetle of seudenol, and 28.19 ± 6.42 ng/beetle of 3-methyl-2-cyclohexan-1-one (MCH). Production of both seudenol and MCH by females dropped to almost zero after females were paired with males, although production of frontalin, verbenone, and trans-verbenol continued. The amount of frontalin produced by solitary vs. paired females was similar ($F_{1, 38}$= 2.59, P = 0.1160). Male beetles did not produce any pheromone components in quantities greater than 2.65 ng/beetle. Oxygenated monoterpenes such as myrtenol and hydrocarbon monoterpenes such as myrcene and limonene commonly present in hindguts of *Dendroctonus* beetles were released by most female beetles but few males.

Olfactory Responses. Antennae of male beetles responded to the seudenol and frontalin from aerations of single and paired female beetles as well as trace amounts of frontalin from paired males beetle (Fig 1). Antennae of female beetles responded to frontalin from aerations of single and paired female beetles as well as seudenol from aerations of single female beetles, but did not respond to any compounds in paired male aerations (Fig 2).

JH III Application. After topical application of JH III, we observed low production of frontalin (< 1.00 ng/µl) by ten females. No other compounds were stimulated in either females or males.

DISCUSSION

Our use of single beetle aerations rather than hindgut extraction (Francke et al. 1995, Barkawi et al. 2003) indicates production of seudenol by female beetles. It thus appears that solitary, pioneering females produce seudenol to attract male beetles to their galleries. After mating, seudenol production decreases, but frontalin production is retained, potentially serving an anti-aggregation role indicating that the tree is full when large quantities of beetles have colonized the host (Raffa 2001). This increase in frontalin production after mating also occurs in adult female spruce beetles, *D. pseudotsugae* Hopkins (Isitt et al. 2020). The trace amounts of frontalin found in male beetle aerations (Table 1) is likely due to proximity to female beetles in galleries.

Electroantennograms indicated that male beetles exhibit higher voltage responses to seudenol and frontalin than female beetles. Male beetles may have higher selection pressure to respond to seudenol and frontalin than female beetles. In the genus *Dendroctonus*, female beetles frequently utilize host compounds to locate hosts (Pearson 1931, Wood 1982, Raffa et al. 2015). Females are the pioneering sex and chew into the bark of the tree to "taste" its profile (Langor and Raske 1987, McKee 2015). After deeming a host suitable, female beetles tunnel into the tree, working to overcome host defenses in tandem with additional pioneering beetles (Franceschi et al. 2005, Fettig et al. 2022). The male beetles thus utilize pheromones, such as seudenol and frontalin produced by female beetles after feeding, to locate mates while females utilize the

pheromone cue in flight to identify potential hosts (Werner 1986, Prendergast 1991, Seybold et al. 2002).

Pheromone production within *Dendroctonus* spp. occurs via one or more pathways such as structural modification of host compounds or *de novo* biosynthesis (Seybold et al. 2000). The lack of seudenol production after topical application of JH III indicates that seudenol is regulated by a separate pathway from the JH III that regulates *de novo* production of frontalin (Barkawi et al. 2003). One possibility is that seudenol follows a pathway similar to that of *trans*-verbenol (4,7,7-trimethylbicyclo[3.1.1]hept-3-en-2-ol), which is produced under similar behavioral conditions in bark beetles. In some species, production of *trans*-verbenol ends after mating occurs (Pureswaran et al. 2000, Blomquist et al. 2010, Keeling et al. 2021). Production of *trans*-verbenol most likely occurs through cytochrome P450 (CYP6DEI)-mediated hydroxylation of *α*-pinene from the host (Pureswaran 2000, Blomquist et al. 2021). However, as there is not a similar known precursor for seudenol or MCH, it is more likely that seudenol is produced *de novo* by a currently unknown pathway. We found that seudenol production began after feeding and ceased after mating as well.

Our work suggests two avenues for future research. First, future work could integrate pheromone production in eastern larch beetle with P450 gene expression in antennal regions as opposed to the midgut. Studies comparing P450 gene expression in *D. ponderosae* display abundant expression in each of these regions, indicating the interlacing of roles in olfaction, detoxification, and pheromone biosynthesis (Chiu et al. 2019). Additionally, the expression of P450 genes shifts during host colonization (Cano-Ramírez et al. 2013, Dai et al. 2015, Obregón-Molina et al. 2015, Nadeau et al. 2017). A more comprehensive understanding of linkage

between P450 functions could elucidate drivers behind response behaviors expanding management opportunities.

Second, more work could be conducted on eastern larch beetles from disparate regions to assess potential variation in pheromone blends or chiralities between regions. Congeners such as *D. rufipennis* display a range of concentrations and products (Isitt et al. 2020), and *D. frontalis* displays geographic variation in attraction to aggregation pheromones (Berisford et al. 1990). This variation in geographic response is seen broadly within the scolytines, as *Ips pini* also varies in its attraction to lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-l-one), a male-produced aggregation pheromone (Teale et al. 1991, Miller et al. 1997). As eastern larch beetle ranges with tamarack transcontinentally from Alaska to the Great Lakes and northeastern US, geographic variation in pheromone response or production is likely.

Many pheromones and host-produced compounds are utilized in "push-pull" management schemes using tactics including conspecific compounds, host repellents, heterospecific compounds, and combinations of these treatments (Afzal et al. 2023). A meta-analysis of 506 *Dendroctonus* studies using such schemes showcased significant population reductions of *D. valens*, *D. rufipennis*, *D. pseudotsugae*, *D. ponderosae*, *D. jeffreyi*, *D. frontalis*, and *D. brevicomis* (Afzal et al. 2023). The most closely related congeners to *D. simplex* are *Dendroctonus rufipennis* and *D. pseudotsugae* (Six and Bracewell 2015, Ramírez-Reyes et al. 2023). *Dendroctonus rufipennis* produces frontalin, seudenol, MCOL (1-methylcyclohex-2-en-1-ol), and verbenone (4-methylene-6, 6-dimethylbicyclo [3.1.1] hept-2-ene) (Furniss et al. 1976, Gries et al. 1988, Gries et al. 1992, Borden et al. 1996) while *D. pseudotsugae* produces frontalin, seudenol, *trans*-pent-3-en-2-ol, and MCOL (Kinzer et al. 1969, Pitman and Vité et al. 1970, Furniss and Schmitz 1971, Vité et al. 1972, Ryker et al. 1979, Dickens et al. 1983,

Lindgren et al. 1992). Both species respond to α-pinene, limonene, and ethanol as host volatiles (Dickens et al. 1983, Gray et al. 1990, Werner 1995). While *Dendroctonus* pheromone components are randomly distributed in most clades of the phylogeny, the clade containing *D. rufipennis*, *D. pseudotsugae*, and *D. simplex* appear to be relatively conserved, potentially because their hosts are not species of *Pinus* (Symonds and Elgar 2004). As these pheromones have been utilized successfully in management for these sister groups, gleaning a better understanding of pheromone production and responses in eastern larch beetle could inform contemporary management practices.

Ultimately, this work will allow field studies to build upon knowledge of pheromone production to demonstrate potential monitoring and management strategies for high-value trees. Currently, management options are limited as tamarack grow in wetland ecosystems where silvicultural methods such as thinning or harvesting must wait until the winter when the ground has frozen sufficiently to hold equipment (Vincent et al. 2018, McKee et al. 2022). Thus, a wider range of more cost-effective methods are needed for outbreak management, especially if outbreaks of eastern larch beetle increase with a changing climate of reduced snowfall, increased drought, and warmer temperatures (Hodges and Lorio 1975, Lombardero et al. 2000, Gaylord et al. 2007, Kolb et al. 2013).

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Table 1: Compounds identified from volatiles collected from male and female eastern larch beetles, with means in ng/beetle and SE listed in parentheses below each listed mean. F is indicative of female beetles while M is indicative of male beetles. Trap-captured beetles were taken directly from Lindgren funnel traps. Data collected summers 2021 and 2022.

Compound	Paired		Single	Trap Captured		$F_{4,96}$	*P*
	F (19)	M (20)	F (19)	F (14)	M (24)		
Myrcene	4.29 (1.17)	1.82 (0.34)	18.47 (7.36)	0.02 (0.02)	0.00 (0.00)	31.61	<0.0001
Limonene	5.18 (0.89)	2.43 (0.37)	10.24 (3.58)	1.25 (0.10)	1.05 (0.10)	15.92	<0.0001
Frontalin	626.72 (105.35)	2.65 (1.36)	433.62 (105.67)	2.74 (2.31)	0.00 (0.00)	128.64	<0.0001
3-me-3-cyclohexen-1-ol[1]	0.00 (0.00)	0.00 (0.00)	21.29 (7.53)	0.00 (0.00)	0.00 (0.00)	26.24	<0.0001
Sulcatol	8.38 (3.02)	0.73 (0.29)	13.44 (3.44)	0.00 (0.00)	0.00 (0.00)	21.08	<0.0001
Seudenol	0.31 (0.31)	0.00 (0.00)	96.62 (34.71)	0.00 (0.00)	0.00 (0.00)	21.68	<0.0001
MCH	0.45 (0.26)	0.00 (0.00)	28.19 (6.42)	0.00 (0.00)	0.00 (0.00)	44.96	<0.0001
Myrtenal	10.64 (1.96)	11.4 (4.39)	2.26 (0.80)	0.46 (0.22)	0.19 (0.19)	23.46	<0.0001
Unknown[1]	24.59 (5.57)	0.00 (0.00)	11.69 (4.01)	2.91 (1.22)	0.14 (0.14)	30.51	<0.0001
trans-Verbenol	10.33 (2.63)	0.29 (0.17)	4.01 (1.14)	0.00 (0.00)	0.00 (0.00)	16.31	<0.0001
Borneol	3.11 (0.46)	2.22 (0.43)	3.22 (0.56)	0.10 (0.06)	0.02 (0.02)	50.31	<0.0001
Verbenone	7.24 (1.48)	1.98 (0.40)	6.26 (1.83)	0.00 (0.00)	0.00 (0.00)	36.61	<0.0001
3-Caren-10-al[1]	42.40 (8.91)	64.88 (22.53)	12.32 (2.13)	5.16 (2.06)	1.60 (1.20)	51.10	<0.0001
Myrtenol	100.16 (15.77)	51.52 (9.11)	40.31 (8.82)	3.75 (0.79)	0.85 (0.39)	91.74	<0.0001
Methylheptanedione[1]	56.56 (15.63)	0.14 (0.10)	34.72 (10.11)	0.10 (0.10)	0.00 (0.00)	46.04	<0.0001
Unknown[1]	74.08 (11.18)	50.13 (7.83)	77.16 (15.05)	7.50 (1.73)	1.42 (0.83)	103.95	<0.0001

[1] Indicates likely identification, but has not been confirmed with matches to an identified standard.

Table 2: Female and male *Dendroctonus simplex* electroantennogram responses to aerations from single (i.e., putatively unmated) and paired beetles (i.e., putatively mated). Responses are adjusted for loss of antennal vigor over time as described in methods. NR indicates no response to the listed compound (i.e., paired female beetles did not produce seudenol and thus beetles did not respond to seudenol in those treatments). Please note: the sexes of individuals within five pairs may have been misidentified.

Responder	Exposure	Compound	Adjusted Response Mean (mV)	*n*	SE
Female	Paired Female	Frontalin	0.462	5	0.156
Female	Paired Female	Seudenol	NR	5	NR
Female	Single Female	Frontalin	0.465	5	0.299
Female	Single Female	Seudenol	0.619	5	0.086
Female	Paired Male	Frontalin	NR	2	NR
Female	Paired Male	Seudenol	NR	2	NR
Male	Paired Female	Frontalin	0.401	4	0.098
Male	Paired Female	Seudenol	1.156	4	0.749
Male	Single Female	Frontalin	0.539	5	0.096
Male	Single Female	Seudenol	0.507	5	0.094
Male	Paired Male	Frontalin	0.314	4	0.053
Male	Paired Male	Seudenol	NR	4	NR

Figure Captions

Fig. 1. Male *D. simplex* composite antennal responses recorded with GC-EAD to odors produced by a) paired females, b) single females, and c) paired males aerations with five, five, and two GC-EAD runs respectively. Please note: the sexes of individuals within five pairs may have been misidentified.

Fig. 2. Female *D. simplex* composite antennal responses recorded with GC-EAD to odors produced by a) paired females, b) single females, and c) paired males aerations with five, five, and two GC-EAD runs, respectively. Please note: the sexes of individuals within five pairs may have been misidentified.

Figure 1

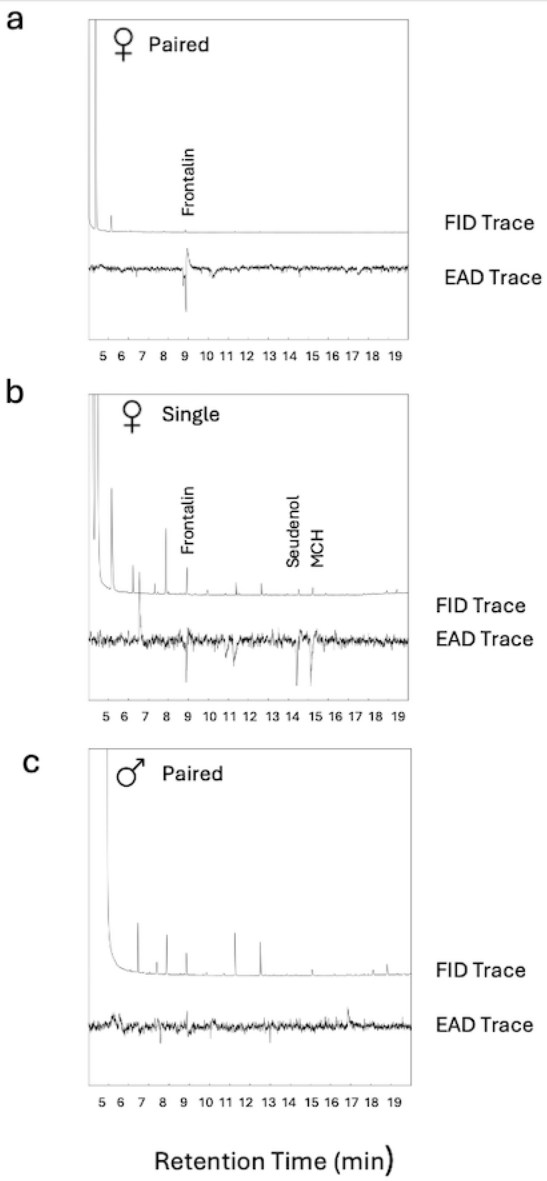
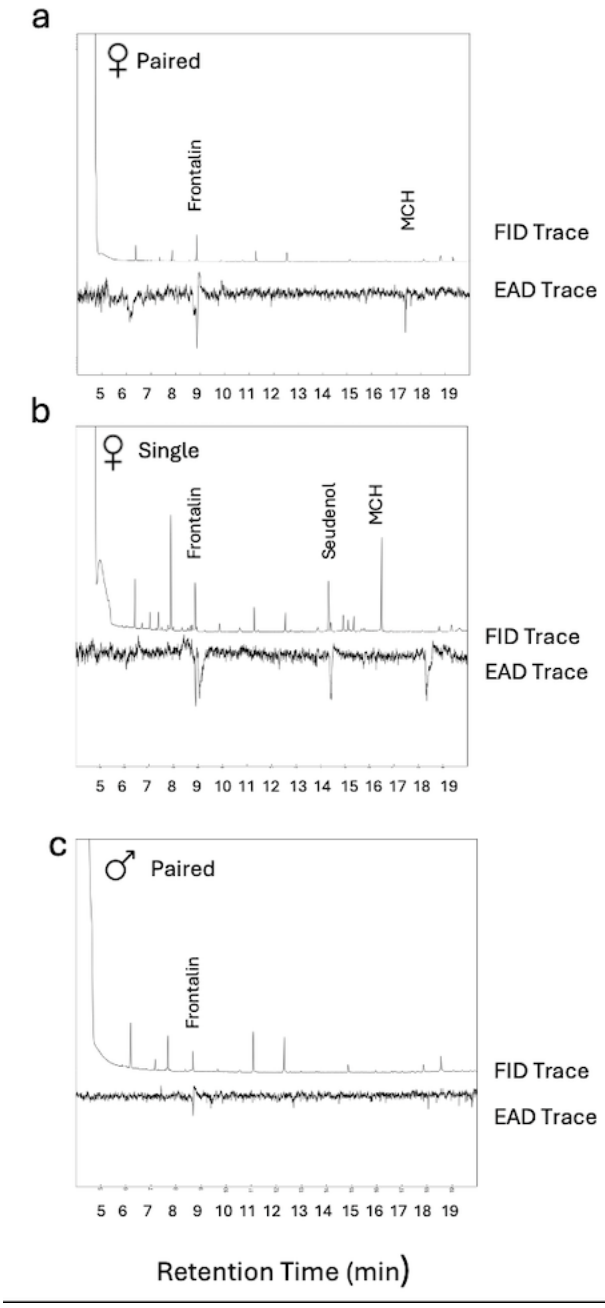

a
♀ Paired
Frontalin
FID Trace
EAD Trace
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
b
♀ Single
Frontalin
Seudenol
MCH
FID Trace
EAD Trace
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
c
♂ Paired
FID Trace
EAD Trace
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Retention Time (min)

Figure 2


a
♀ Paired
Frontalin
MCH
FID Trace
EAD Trace
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
b
♀ Single
Frontalin
Seudenol
MCH
FID Trace
EAD Trace
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
c
♂ Paired
Frontalin
FID Trace
EAD Trace
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Retention Time (min)

Chapter 3. Behavioral responses of eastern larch beetle, *Dendroctonus simplex* LeConte (Coleoptera: Scolytinae) to pheromone components and selected host-produced monoterpenes in the Great Lakes region

Abstract

The eastern larch beetle, *Dendroctonus simplex* LeConte, is a North American species of bark beetle with a distribution sympatric to that of its host, *Larix laricina* (Du Roi) K. Koch. A recent outbreak in the Great Lakes region has prompted renewed interest in the chemical ecology of eastern larch beetle and its natural enemies. Here, we describe field assays elucidating flight responses to host monoterpenes and pheromone components in Minnesota in the summers of 2022-2023 and laboratory olfactometer assays elucidating walking responses to pheromone components produced by female beetles. In field trials, the addition of host monoterpenes Δ-3-carene and α-pinene to seudenol lures increased capture rates of eastern larch beetles vs. traps baited solely with seudenol. Similarly, seudenol-baited funnel traps amended with lures containing α-pinene increased capture rates of *Thanasimus* spp. in comparison to those without α-pinene. Frontalin release rates between 0.02-3 mg/day exhibited high bark beetle attractancy when paired with seudenol in comparison to higher concentrations of frontalin (24 mg/day), which decreased attraction of eastern larch beetle to seudenol. In contrast, higher frontalin release increased attraction to *Thanasimus* spp. when paired with seudenol. In a laboratory olfactometer assay, a combination of frontalin and seudenol released at rates comparable to those of a solitary, mining female arrested walking male beetles whereas either compound alone did

not. Insights into the eastern larch beetle's chemical ecology will help us understand cues in host and mate location that may be exploited for management in the future.

Keywords

α-pinene, Δ-3-carene, *Thanasimus*, frontalin, seudenol

Introduction

The genus *Dendroctonus* contains the most aggressive as well as economically and ecologically important species of bark beetles (Coleoptera: Curculionidae: Scolytinae) in North America (Six and Bracewell 2015). The Latin translation of the genus, "tree-destroyer," is apt given the outbreak behavior occasionally displayed by these beetles (Raffa 2008). An outbreak of southern pine beetle (*Dendroctonus frontalis* Zimmerman), for example, caused approximately 1.2 billion US dollars in damage in South Carolina from 1977-2004 (Pye et al. 2011). At the peak of an outbreak of spruce beetle (*D. rufipennis* (Kirby)) between 1989-2004 in Alaska, 30 million trees were lost annually (Werner et al. 2006, Grégoire et al. 2015). On a more pronounced scale, a "hyperepidemic" of mountain pine beetle (*D. ponderosae* Hopkins) from 2000-2020 in British Columbia released approximately 270 megatons of carbon from the landscape (Kurz et al. 2008).

Bark beetles within the *Dendroctonus* genus colonize conifers across several genera of trees: *Pinus*, *Picea*, *Pseudotsuga*, *Abies*, and *Larix* (Six and Bracewell 2015). Some species of *Dendroctonus* utilize a pheromone-induced mass attack of the tree bole to colonize vigorous hosts, especially when at outbreak levels (Raffa et al. 2008). At endemic levels, other species utilize single or paired smaller attacks on the bole that facilitate tree colonization (Reeve et al. 2012, Six and Bracewell 2015). Some *Dendroctonus* species utilize host compounds to initially locate and select suitable host trees (Pearson 1931, Wood 1982, Raffa et al. 2015), chewing a gallery through the phloem in an attempt to overcome host defenses (Franceschi et al. 2005, Fettig et al. 2022). Attack initiating females and, in some species, subsequently arriving males, produce pheromones that attract additional beetles thereby inducing a mass aggregation that overcomes resin defenses of vigorous trees (Bloomquist et al. 2010). After mating, the female

deposits eggs in a gallery, with some females reemerging and laying additional broods (Coulson 1979, Wood 1982, Sauvard 2004, McKee et al. 2022). While prior work is indicative of monogamy within this species, additional research may be necessary to confirm this pattern (Langor and Raske 1987, McKee and Aukema 2015). After mating occurs, many species produce antiaggregation compounds, indicating the tree is at optimal capacity as larvae develop in the restricted habitat under the bark, consuming the phloem tissue (Coulson 1979, Raffa 2001). Development of the larvae to the pupal stage and adulthood is regulated by thermal accumulation (Danks 2007, Bentz and Jönsson 2015, Hofmann et al. 2024).

The range of eastern larch beetle (*D. simplex* LeConte) is sympatric with its host, eastern larch or tamarack (*Larix laricina* (Du Roi) K. Koch) that spans the Canadian Maritime region, the Great Lakes region, the northeastern United States, British Columbia, and Alaska (Simpson 1929, Langor and Raske 1989, Burns and Honkala 1990, Seybold et al. 2002). Historically, eastern larch beetle has not been deemed aggressive or of economic concern, with sporadic outbreaks occurring in mature tamarack stands following predisposing factors such as fire, flooding, drought, wind throw, or defoliation (Hopkins 1909, Baker 1972, Bright 1976, Langor and Raske 1987). This historical pattern has shifted in recent decades in the southern transcontinental portion of the range, potentially due to a change in voltinism in some regions (McKee and Aukema 2015). For example, since 2001, Minnesota has experienced a large outbreak of eastern larch beetle with approximately 433,863 cumulative hectares or 75% of tamarack covertype in the state affected (MN DNR 2024). Additional outbreaks have recently occurred in Wisconsin and Michigan (WI DNR 2021, MI DNR 2024, WI DNR 2024). Previous work has suggested that extended growing seasons concomitant with a warming climate now

allow the beetle to develop two generations in a growing season, which has exacerbated the outbreak (McKee and Aukema 2015, Ward and Aukema 2019).

While many species of *Dendroctonus* and their natural enemies are well studied, some aspects of the behavior and chemical ecology of the eastern larch beetle require further investigation. Female beetles have been shown to produce frontalin and methylcyclohexanone (MCH), but the role of each is poorly understood (Francke 1995, Barkawi et al. 2003). Previous studies have suggested that frontalin may either attract or repel flying eastern larch beetles (Baker et al. 1977, Prendergast 1991, Francke 1995, Werner 1995, Graham and Storer 2015), perhaps depending upon concentration. Graham and Storer (2015) found that frontalin eluting at 2.5 mg/day combined with seudenol decreased the number of beetles captured in Lindgren funnel traps compared to seudenol alone. Werner et al. (1981) reported similar inhibitory effects, although the release rate of frontalin was unreported. In contrast, Francke et al. (1995) reported that traps baited with solely frontalin were attractive to flying beetles, but the release rate was not given. Eastern larch beetle is attracted to seudenol (Baker et al. 1977, Graham and Storer 2015), and the compound was recently isolated from solitary female beetles after tunneling into host material (Chapter 2).

Likewise, the role that host compounds play in attraction of eastern larch beetles is not fully understood. Most work in this area has centered on α-pinene, as it is the predominant component of the volatile blend produced by tamarack (Stairs 1967, Von Rudloff 1987, Ward et al. 2019, Althoff et al. 2023). Less work has addressed other components of the host volatile profile. Within the Great Lakes region, Δ-3-carene is also a predominant host monoterpene in the phloem of *L. laricina* (Althoff et al. 2023). Eastern larch beetle is highly attracted to α-pinene in combination with seudenol (Baker et al. 1977, Werner et al. 1981, Werner 1995, Graham and

Storer 2015) with potential attractancy to Δ-3-carene in combination with seudenol (Prendergast 1991). Additionally, 4-allylanisole, a phenylpropanoid also present in the phloem tissues of *L. laricina* (Althoff et al. 2023), is exceptionally toxic among host compounds to *Dendroctonus simplex* and may function as a repellent (Werner 1995).

Variation in airborne pheromone and host volatile concentrations can mediate intra- and interspecific resource partitioning as well as host colonization and mating behaviors in bark beetles. Here, we describe laboratory and field bioassays studying the attraction of eastern larch beetle and *Thanasimus* spp. to seudenol, frontalin, and selected host compounds. *Thanasimus* spp. are natural enemies of bark beetles that use prey pheromones as kairomones (Erbilgin and Raffa 2001). The adults feed on bark beetles arriving to the surface of a host tree, while the larvae prey on developing brood within the subcortical habitat (Aukema and Raffa 2002). In the field, we tested Δ-3-carene, α-pinene, and 4-allylanisole in combination with the known attractant seudenol for attractant and/or repellent properties using baited funnel traps. Second, we conducted a trapping experiment where we tested responses of eastern larch beetles and *Thanasimus* spp. to lures of seudenol in combination with varying concentrations of frontalin. We hypothesized that (1) low concentrations of frontalin might be attractive to conspecifics, indicative of early stages of host attack and resource availability on a tree whereas (2) high concentrations of frontalin might be repellent to conspecifics, indicative of limited resources due to higher densities of eastern larch beetles. Finally, we conducted a laboratory walking bioassay where we tested responses of eastern larch beetles to combinations of frontalin and seudenol in a 2 x 2 factorial design. We hypothesized that walking eastern larch beetles may exhibit sex-specific responses to these compounds different than noted in flight assays as similar shifts in behavior occur in other Scolytine species (Camacho et al. 1998, Sullivan 2005, Niño-Domínguez

et al. 2015, and Niño-Domínguez et al. 2016). We hypothesized that female-produced compounds, such as seudenol and frontalin, would elicit longer engagement times from walking male bark beetles than female beetles.

Methods

Field Experiments

Sites were selected in mature tamarack forests with active outbreaks of eastern larch beetle in central Minnesota in the summers of 2022 and 2023. These outbreak locations were identified from aerial survey data and ground truthing (Table 1). In 2022, we conducted a study assessing the attraction of eastern larch beetle and *Thanasimus* spp., predators of the eastern larch beetle, to seudenol and selected host compounds. At each of eight sites, five ten-unit Lindgren funnel traps (Synergy Semiochemicals Corp. Delta, BC) were set in a line at 50 m spacing and then baited with one of five treatments 1) seudenol 2) seudenol + 4-allylanisole 3) seudenol + α-pinene 4) seudenol + Δ-3-carene 5) seudenol + α-pinene + Δ-3-carene. Lures for 4-allylanisole were created by adding 250 µl to a 400 µl capacity low density polyethylene microcentrifuge tube (Fisher Scientific, Hampton, NH) and released 6 mg/d. Lures of α-pinene, Δ-3-carene, and seudenol (3-methylcyclohex-2-en-l-ol) had elution rates of 2 g/day at 23°C, 150-200 mg/day at 23°C, and 7.5 mg/day at 30°C and were sourced from Synergy Semiochemicals (Delta, BC). A piece of approximately 5 cm by 2.54 cm No-Pest ® Strip (18% Dichlorvos (2,2-dichlorovinyl dimethyl phosphate)) (Spectrum Brands Inc., Middleton, WI) was placed in each trap's collection cup to prevent captured predators from consuming bark beetles. Trap contents were collected once per week for six weeks from 2 June to 6 July 2022. Lure positions were rerandomized each week within a site. Collected trap contents were stored at -20°C until

processing when all bark beetles and clerid predators were identified, counted, and stored in ethanol. Bycatch was also identified and is reported in a separate biodiversity study (Chapter 4).

In 2023, we conducted a study to examine the behavioral responses of eastern larch beetles and *Thanasimus* spp., predators of the eastern larch beetle, to seudenol lures when paired with five different concentrations of frontalin: none (i.e., seudenol control), and an approximate release rate of 0.02 mg/day, 0.1 mg/day, 0.5 mg/day, 3 mg/day, and 24 mg/day at 25°C. As in the previous experiment, we used eight sites with six traps at 50 m spacing. Lures of seudenol (3-methylcyclohex-2-en-l-ol) were sourced as before. The smallest release rates (0.02, 0.1, and 0.5 mg/day) of frontalin were produced with glass capillary devices (internal diameters: 0.2 mm, 0.45 mm, and 1.05 mm; length: 45 mm, Drummond, Broomall, PA) that were heat sealed at one end. The closed ends of the capillaries were inserted into half of a silicone rubber GC inlet septum (Agilent, Santa Clara, CA) and the capillaries were secured by this means open-end up in an inverted 4 ml glass vial to protect them from rain. Capillary tubes were secured to traps by securing the external vial to 7.62 cm aluminum carabiners. The second highest frontalin-releasing device (3 mg/day) was a low-density polyethylene microcentrifuge tube (400 µl capacity, Fisher Scientific, Hampton, NH), and the highest release rate lures (24 mg/day) were heat sealed, 4 ml capacity polyethylene transfer pipets (Samco, San Fernando CA) containing ~1 ml frontalin. Racemic frontalin was approximately 99% purity (BASF, Florham Park, NJ). An aluminum foil barrier between the device and the trap preventing compound transfer between the plastic funnels and the lure which was secured to the funnel lip using a binder clip. Trap contents were collected weekly (14 June through 20 July 2023) for six weeks and procedures were otherwise the same (including the re-randomization of lure positions) as in the previous trapping experiment.

In 2023, we analyzed whether the sex ratios of eastern larch beetles changed with changing concentrations of frontalin. Ten beetles from each treatment for two sites over each of our six collection dates were sampled at random and sexed for a total of 720 beetles. We utilized the presence of two medial teeth that serve as stridulation structures on the 7th abdominal tergite of male beetles to distinguish male beetles from female beetles (Armendáriz-Toledano and Zúñiga 2017).

Laboratory Olfactometer Assay

Live eastern larch beetles were collected from Lindgren funnel traps baited with seudenol at Warren Woessner Bog Boardwalk, MN (47.07559 °N, -92.6616 °W) and Winterberry Bog, MN (47.17072 °N, -92.53954 °W). Traps were checked twice per week. Beetles were stored at room temperature after collection and held for 3-5 days. Male vs. female beetles were distinguished by noting the stridulation produced by male beetles when handled (Rosenberger et al. 2016).

We constructed an arena for a four-way choice test for walking beetles. The four treatments were 1) seudenol released at 0.10 µg/hr 2) frontalin released at 0.29 µg/hr 3) both seudenol and frontalin combined 4) blank control. The arena was made of a 10 cm x 10 cm x 1 cm acrylic sheet with four 3 mm diameter holes penetrating through the sheet and arranged in a square, centered in the arena, with sides 1.7 cm. The arena was approximately level and elevated above the bench top with 3 cm tall rubber stoppers beneath each corner. A circle of 10 cm diameter filter paper, centered over the square, was placed on the arena as a walking surface for the beetle, and 50 µL disposable polypropylene pipettor tips were inserted through the filter paper and into the holes. Prior to this step, the pipettor tips had been cut with a razor blade so their upper openings were flush with the plexiglass surface when inserted into the holes. The

pipettor tips protected the arena from contamination by the pheromone release devices (described below) that were inserted into the pipettes. The release devices, consisting of 6.4 mm long, 2 mm diameter pieces of silicone cord treated with the pheromone components were inserted into each pipettor tip so that volatiles would waft out of the top opening into the arena. The silicone pieces had been treated with pheromone components by steeping them in a hexane solution of the compound overnight in a capped glass vial and then allowing the solvent to evaporate completely from the silicone. Afterwards, silicone pieces were stored in a capped vial and held in a freezer.

Release rates of each compound from the silicone were chosen to reflect the pheromone output of a hypothetical colonizing, solitary female beetle, with release rates modeled after those of female beetles in conical vial aerations (Chapter 2). Release rates were measured through aerations of the silicone cord/pipettor tip assemblies placed into Teflon tubing with air drawn into a cartridge of Porapak adsorbent at 30 mL/ min for one hour. Pheromones in extracts (1.5 mL hexane) of the cartridges were quantified by GC-MS. The concentration of pheromones in the hexane in which the silicone cord was steeped was adjusted until the desired rates were achieved.

For each trial, a single beetle was placed in the center of the arena and allowed to walk for five minutes while its position was monitored. The total time spent within a 6.4 mm radius of each release device was then recorded for each five-minute assay. Silicone tubing/pipettor tip assemblies were replaced every five trials and treatment assignments to holes was rerandomized. Treatment placements were randomized between runs. Beetles were not reused. All assays were conducted sequentially on a single day under fluorescent lighting (60 Hz, 4100K, 25 W, T8 bulb) at room temperature (approximately 21°C). The assay was completed with n = 19 female and n = 20 male beetles.

Statistical Analysis

Field Assay with Seudenol and Host Volatiles

All statistical analyses were performed using R version 1.3.1073 using the multcomp, lmerTest, nlme, and lme4 packages (R Core team 2020). To assess insect responses to host compounds in combination with seudenol, we conducted an ANOVA in a mixed effects model framework. We examined how beetle capture varied in response to lure treatment with a random effect of site. A ln(y +1) transformation was utilized on beetle count data to satisfy homoscedasticity and normal distribution of residuals, assessed using visual inspections of residual plots. Where significant treatment effects existed, means were compared using least squares differences (LSD). To isolate the main effects of α-pinene and Δ-3-carene in this experiment, we also examined linear contrasts for each of these effects and their interaction.

Field Assay with Seudenol and Frontalin Dose Response

To assess how varying concentrations of frontalin when paired with seudenol affected beetle capture, we conducted a mixed effects model analysis of variance (ANOVA) with a fixed effect of lure treatment and a random effect term of site to assess how beetle capture varied in response to lure treatment. A square root transformation was utilized on beetle count data to satisfy assumptions of equal variance and normal distribution of residuals. Where significant treatment differences existed, means comparisons were conducted using LSD as above.

A generalized linear mixed-effects model (GLMM) was used to determine if beetle sex ratios varied in response to treatment as a fixed effect with a term for site as a random effect. The model used a binomial distribution for the binary the response variable, male vs. female.

Laboratory Olfactometer Assay

We fit a model examining the total time a beetle spent at each treatment within the five-minute assay using a 2 x 2 factorial design with terms for frontalin, seudenol, and their interactions as factors. Male beetles and female beetles were analyzed separately. A ln(y +1) transformation of the response variable, time in seconds, was utilized to satisfy test requirements of normal distributions of residuals for both male and female beetle models.

Results

Select Host Compounds Paired with Seudenol

In total, we captured 140,532 eastern larch beetles, 2,829 *T. dubius*, and 439 *T. undulatus* (Say) in 2022. The number of eastern larch beetle captured in the traps varied between treatments ($F_{4, 220}$ = 3.92, P = 0.0042). Traps baited with seudenol captured an average of 58.5 ± 145.2 eastern larch beetles/trap/week, a number similar to traps baited with seudenol and 4-allylanisole, indicating that 4-allylanisole did not have a repellent effect at the concentration tested (Fig. 1A). Of the three host compounds tested, Δ-3-carene appeared to enhance attraction to seudenol the most for eastern larch beetles (Fig. 1A). Traps baited with seudenol and Δ-3-carene captured the most eastern larch beetles with a median of 265.5 ± 154.0 beetles/trap/week ($F_{4, 225}$= 11.60, P < 0.0001). The addition of α-pinene to this blend did not increase capture rates. Those traps captured 238 ± 272.0 eastern larch beetles/trap/week.

Thanasimus dubius and *T. undulatus* exhibited similar responses to lure compounds: traps baited with α-pinene (with or without Δ-3-carene) captured the most *Thanasimus* spp. ($F_{4,220}$ =28.29, P < 0.0001 and $F_{4,220}$ = 19.24, P < 0.0001, respectively for each species). Traps baited with seudenol and α-pinene had average capture rates of 25 *T. dubius* and five *T. undulatus*. Traps baited with lures where Δ-3-carene replaced the α-pinene did not capture as many

Thanasimus of either species but were still more effective than controls with seudenol and no host volatile (Fig. 1B, C).

Frontalin when paired with seudenol

In total, we captured 68,125 eastern larch beetles, 2,872 *T. dubius*, and 252 *T. undulatus* in 2023. Overall, there was a steep drop off in eastern larch beetle capture after 0.5 mg/day release rates. Traps baited with the highest dose of frontalin, 24 mg/day, captured the fewest eastern larch beetles with a median capture rate of 23 ± 20.99 beetles/trap/week (Fig. 2A). This amount was 67% less than the control lure of seudenol alone (Fig. 2A).

Natural enemies such as *T. dubius* and *T. undulatus* also demonstrated variation in trap capture between treatments ($F_{5, 288}$=19.99, $P < 0.0001$ and $F_{5, 288} = 8.59$, $P < 0.0001$, respectively; Fig. 2B, C). In contrast to the pattern exhibited by eastern larch beetle, more *Thanasimus* spp. predators were captured at higher release rates of frontalin when paired with seudenol.

The sex ratio of male to female eastern larch beetles arriving to the traps was 1.19. This pattern did not change with increasing concentrations of frontalin when paired with seudenol ($\chi^2_1 = 0.0119$, $P = 0.91$) (Fig. 3).

Laboratory Olfactometer Assay

Within our olfactometer assay, we found that male eastern larch beetles preferred the combination of seudenol and frontalin (Fig. 4A; $F_{3, 19} = 7.18$, $P = 0.0091$). On average, they spent just over one minute of each five-minute assay in close proximity to the hole in the arena floor from which these compounds were emanating. In contrast, neither of the compounds on their own or the control garnered attention other than that due to random chance. Female beetles had no preference when exposed to the same compounds, with seudenol eliciting no response

($F_{1, 19}$ = 3.196, P = 0.08), frontalin eliciting no response ($F_{3, 19}$ = 0.61, P = 0.44), and no interaction observed as well (Fig. 4B; $F_{3, 19}$ = 0.13, P = 0.72).

Discussion

Our results demonstrating variation in attraction of flying eastern larch beetles to varying concentrations of frontalin, when paired with seudenol, begins to explain the mechanisms by which eastern larch beetles colonize new hosts while managing arrival rates of conspecifics that increase intraspecific competition. Prior work has indicated that female eastern larch beetles produce frontalin and seudenol (Franke et al. 1995, Chapter 2). Thus, low numbers of female beetles beginning to pioneer attacks into a tree would be attractive to male beetles searching for a mate as well as additional females looking to colonize a host tree, but increasing concentrations of frontalin produced by increasing densities of arriving females would begin repelling additional conspecifics from the colonized host. High concentrations of frontalin thus indicate limited resources due to intraspecific competition. Additionally, the cease in seudenol production after mating likely allows male eastern larch beetles to locate susceptible mates more efficiently.

The repellency of eastern larch beetles to high concentrations of frontalin likely reflects the costs associated with pioneering behavior and responding late. While pioneering individuals often face higher mortality rates due to tree defenses and resinosis, lower probability of recruitment, and lower reproductive success than later-arriving individuals (Raffa and Berryman 1983, Pureswaran et al. 2006, Latty and Reid 2009a. Latty and Reid 2009b), individuals arriving late may be faced with increased intraspecific competition and predation risk as high concentrations of frontalin attract higher quantities of predators (Fig. 3B, C) (Aukema and Raffa 2004). Thus, behaviorally, this pattern is consistent with previous models indicating tradeoffs between group attack and sharing the host within bark beetle systems (Berryman et al. 1985).

Our olfactometer results are also consistent with our hypothesis that low to medium release rates of frontalin in combination with seudenol are most attractive to eastern larch beetles, with one important caveat. Female eastern larch beetles are attracted to frontalin and seudenol when in flight (Fig. 2A, 3) but not when walking (Fig. 4). Prior work on Scolytines has indicated differences between flight and walking behavior in multiple species (Comancho et al. 1998, Sullivan 2005, Niño-Domínguez et al. 2015, and Niño-Domínguez et al. 2016), and dosage of pheromone components can alter walking responses within the *Dendroctonus* genus (Rudinsky et al. 1974, Sullivan et al. 2011, Niño-Domínguez et al. 2015). As female beetles are the host-selecting sex in *D. simplex*, it is possible that female beetles use pheromone cues while flying to locate suitable hosts and, after landing upon a tree and beginning to walk, rely more heavily on host compounds to determine host suitability. Work by McKee (2015) showed that eastern larch beetle will frequently engage in "tasting" behavior that leaves marks on the surface of the tree prior to determining a suitable place to bore. Male beetles may primarily pursue female-produced compounds as they are searching for mates (Langor and Raske 1987, Chapter 2). However, it should be noted that this work was conducted within the laboratory and further studies should be developed assessing walking behavior in the eastern larch beetle's natural environment.

Attraction to Δ-3-carene by eastern larch beetle could reflect evolutionary resource partitioning distinguishing eastern larch beetle from Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins. The range of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco (Pinales: Pinaceae)), overlaps with eastern and western larch (*Larix occidentalis* Nutt. (Pinales: Pinaceae)) (Reed et al. 1986). *Dendroctonus pseudotsugae* specializes on Douglas-fir, in which Δ-3-carene is not abundant. However, it is the second most prevalent compound in eastern larch

and is present in high concentrations in western larch (Stairs 1967, Von Rudloff 1987, Althoff et al. 2023). In contrast, Douglas-fir beetles are repelled by Δ-3-carene (Ross et al. 2022). Additionally, there is the potential for this divide between Δ-3-carene producing hosts to provide enemy free space with fewer predators and competitors, as our results indicated fewer *Thanasimus* spp. in the presence of Δ-3-carene in comparison to traps containing α-pinene (Fig 1B, C).

The increasing attraction of predators such as *Thanasimus dubius* to higher concentrations of frontalin instead of repellency as noted with eastern larch beetle (Fig. 2) suggests that these predator responses are not being limited by competition. *Thanasimus* spp. are generalist predators that specialize on bark beetles with relatively high dispersal capacities, which could alleviate competition (Cronin et al. 2000). Environmental conditions, prey abundance, and longevity likely constrain these insects' population growth (Aukema and Raffa 2002, Munro et al. 2021).

Thanasimus dubius utilizes α-pinene in combination with frontalin and other bark beetle pheromones to locate prey (Aukema and Raffa 2005, Staeben et al. 2014). Here, we demonstrated heightened attractancy to α-pinene (with or without Δ-3-carene), with lower attractancy responses to seudenol and Δ-3-carene. The heightened attractancy of *T. dubius* to α-pinene is not surprising due to its generalist nature. The conifer hosts of many bark beetle species emit α-pinene (Gitau et al. 2013). Further exploration of predator choice should be conducted to elucidate if preference for hosts without Δ-3-carene may occur in regions where *Dendroctonus* spp. overlap. Assessing preference is especially important as geographic variation in pheromone preference and a pattern consistent with optimal foraging theory (i.e., shifts in prey preference in

response to host abundance) exists in populations of *T. dubius* (Murdoch 1969, Stephens and Krebs 1986, Reeve et al. 2009). Additionally, as proportions of α-pinene and Δ-3-carene vary among tamarack trees, responses of *Thanasimus dubius* to a range of ratios and dosages should be assessed (Althoff et al. 2023).

We did not observe a decrease in response of flying eastern larch beetle to seudenol in combination with 4-allylanisole noted by Werner (1995). Historically, this compound has been noted as an inhibitor of bark beetle attacks (Hayes and Strom 1994, Emerick et al. 2008). However, more recent studies have found 4-allylanisole to be an attractant synergist for *D. frontalis*, *D. terebrans* (Olivier), and *T. dubius* at lower population densities of southern pine beetle (Sullivan et al. 2022). Further exploration could explore eastern larch beetle populations at varying dosages and population levels.

The repellency of high doses of frontalin to eastern larch beetles as a deterrent from "full" trees suggests behavioral properties that could be exploited in pest management schemes. It is unknown, however, whether such high doses may simply serve as attractants as the compounds diffuse and reach lower concentrations at the margins of infestations, thus functioning as medium-range aggregation pheromones. More research is needed to assess other compounds and the effectiveness of tree protection schemes in the absence of seudenol.

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Table 1: Locations of eight sites in Minnesota utilized in the 2022 and 2023 trapping studies of eastern larch beetle and *Thanasimus* spp. With selected host compounds (2022) and frontalin dosage (2023) in combination with seudenol over the course of six weeks (2-VI-22 through 6-VII-22, 14-VI-23 through 20-VII-23).

Year	Site	County	Coordinates
2022	1	Aitkin	46.95735 °N, -93.4388 °W
2022	2	Aitkin	46.95875 °N, -93.4443 °W
2022	3	Aitkin	46.97418 °N, -93.4470 °W
2022	4	Aitkin	46.99788 °N, -93.3592 °W
2022	5	Aitkin	46.58568 °N, -93.4653 °W
2022	6	Aitkin	46.58018 °N, -93.4654 °W
2022	7	Aitkin	46.58534 °N, -93.4626 °W
2022	8	Aitkin	46.67992 °N, -93.6741 °W
2023	1	Aitkin	46.58199 °N, -93.4637 °W
2023	2	Aitkin	46.95767 °N, -93.4397 °W
2023	3	Aitkin	46.97371 °N, -93.4473 °W
2023	4	St. Louis	47.17088 °N, -92.5374 °W
2023	5	St. Louis	47.07532 °N, -92.6619 °W
2023	6	St. Louis	47.03439 °N, -92.6713 °W
2023	7	St. Louis	47.02786 °N, -92.6619 °W
2023	8	Carlton	46.59246 °N, -92.9242 °W

Figure Captions

Fig. 1. Box and whisker plots of A. eastern larch beetle, B. *Thanasimus dubius*, and C. *Thanasimus* undulatus responses to five lures containing seudenol plus various host compounds. This experiment was conducted at eight sites over six collection periods from June 2, 2022 to July 6, 2022 in Minnesota. The middle line of each box represents the median number of beetles, while the box boundaries represent the first and third quantiles. Letters indicate statistically significant differences between treatments.

Fig. 2. Box and whisker plots of A. eastern larch beetle, B. *Thanasimus dubius*, and C. *Thanasimus undulatus* responses to varying frontalin release rates in combination with seudenol. This experiment was conducted at eight sites over six collection periods from June 14, 2023 to July 20, 2023 in Minnesota. The middle line of each box represents the median within each treatment, while the box boundaries represent the first and third quartiles. Letters indicate statistically significant differences between treatments.

Fig. 3. Box and whisker plots of eastern larch beetle male beetle percentages in subsamples of 10 individuals per trap in response to varying release rates of frontalin in combination with seudenol. This experiment was conducted at eight sites over six collection periods from June 14, 2023 to July 20, 2023 in Minnesota. The middle line of each box represents the median within each treatment, while the box boundaries represent the first and third quantiles. Letters indicate statistically significant differences between treatments.

Fig. 4: Box and whisker plots of A. male (*n* = 20) and B. female (*n* = 19) eastern larch beetle responses to seudenol, frontalin, seudenol + frontalin, and control treatments in a walking olfactometer bioassay (*n* = 20). The *y*-axis represents the total time spent within the 6.4 mm radius of each treatment. Letters indicate statistically significant differences between treatments.

Figure 1

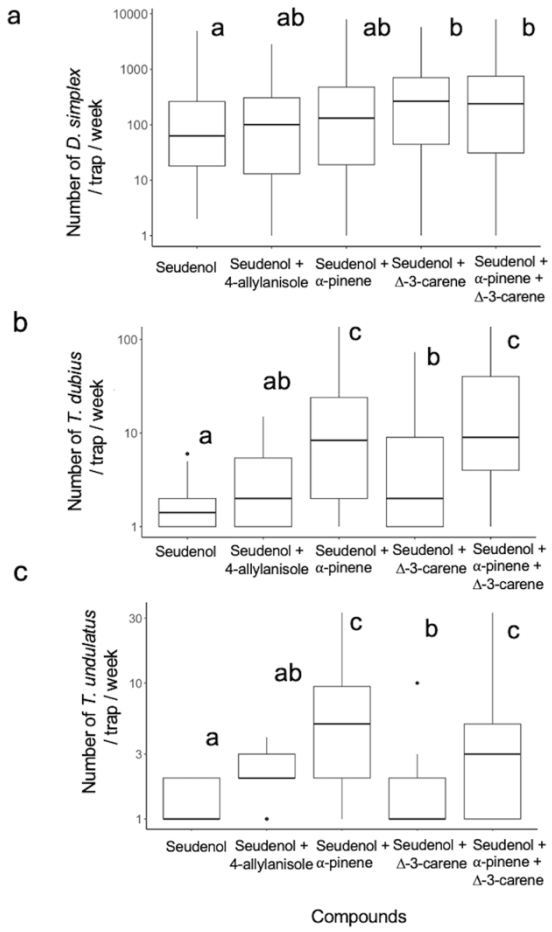

a
b
c
Number of D. simplex / trap / week
Number of T. dubius / trap / week
Number of T. undulatus / trap / week
10000
1000
100
10
1
30
3
a
ab
ab
b
b
c
Seudenol
Seudenol + 4-allylanisole
Seudenol + α-pinene
Seudenol + Δ-3-carene
Seudenol + α-pinene + Δ-3-carene
Compounds

Figure 2

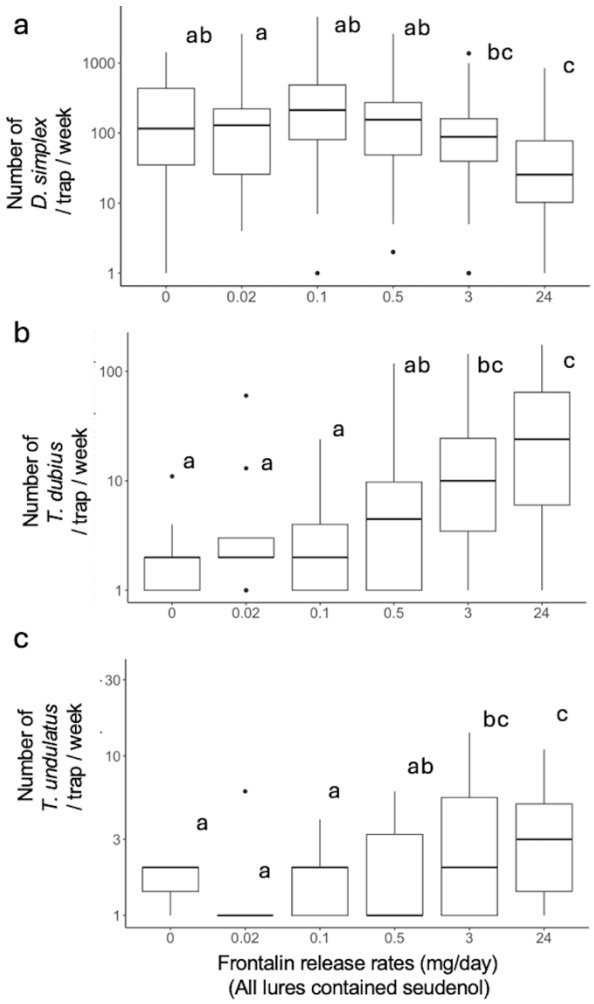

a
ab
a
ab
ab
bc
c
1000
100
10
1
Number of
D. simplex
/ trap / week
0
0.02
0.1
0.5
3
24
b
a
a
a
ab
bc
c
100
10
1
Number of
T. dubius
/ trap / week
c
a
a
a
ab
bc
c
30
10
3
1
Number of
T. undulatus
/ trap / week
Frontalin release rates (mg/day)
(All lures contained seudenol)

Figure 3

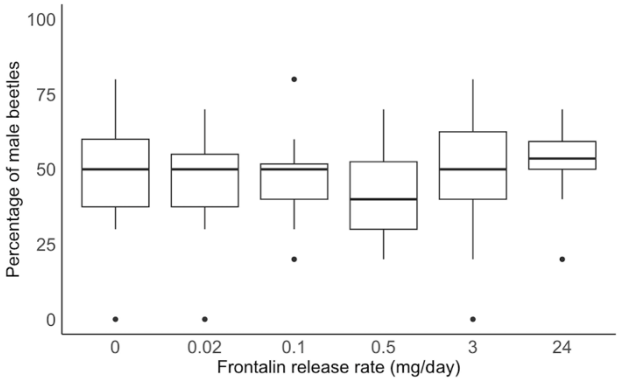
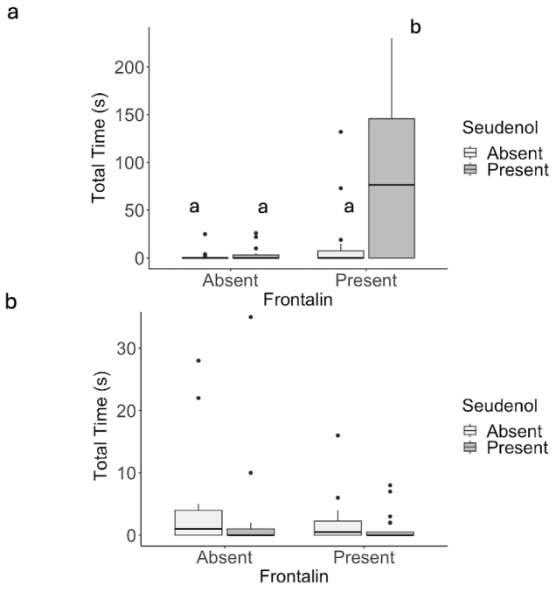

100
75
50
25
0
Percentage of male beetles
0
0.02
0.1
0.5
3
24
Frontalin release rate (mg/day)

Figure 4


a
b
200
150
100
50
0
Total Time (s)
a
a
a
Absent
Present
Frontalin
Seudenol
Absent
Present
b
30
20
10
0
Total Time (s)
Absent
Present
Frontalin
Seudenol
Absent
Present

Chapter 4. A checklist of insects sampled with lures for the eastern larch beetle, *Dendroctonus simplex* LeConte (Coleoptera: Curculionidae) within tamarack and black spruce stands in the Great Lakes region

Abstract:

Tamarack (*Larix laricina* (Du Roi) K. Koch) forests and peatlands provide many ecosystem services from flooding protection to carbon sequestration. An understudied ecosystem function is habitat provision for arthropods. The biodiversity of arthropods in tamarack stands has primarily been documented in New York, Newfoundland, and Indiana and to a lesser degree in Alaska and Michigan. Broadening our understanding of the arthropod community within lowland conifer forests is essential for studying shifts in biodiversity over time, especially when impacted by disturbances. At present, more than 75% of the tamarack in Minnesota has been affected by a 23-year outbreak of the eastern larch beetle, *Dendroctonus simplex* LeConte. In this work, we characterized potential predators, competitors, and parasitoids of eastern larch beetle within the orders Coleoptera and Hymenoptera that we captured in Lindgren funnel traps baited with eastern larch beetle lures weekly for six weeks in the summers of 2022 and 2023 and natural enemies reared from tamarack bolts. Our work identified 135 taxa and five new state records for Minnesota. Documenting current diversity will inform future work quantifying and cataloging biodiversity in this ecosystem, as well as assessing legacy impacts of eastern larch beetle on broader biodiversity in these forests.

Keywords: biodiversity, conifer, Hymenoptera, peatlands, phenology

Introduction:

Insects are the most abundant of all described animals on earth with estimates of the total number of species ranging from 5.5-7 million species, most of which have yet to be discovered (Stork 2017). Some scientists hypothesize that this number could be as high as 30 million (Erwin 1982). Insects provide ecosystem services such as pollination, decomposition, and regulation of components of the food web (Bianchi et al. 2006, Losey and Vaughan 2006, Yang and Gratton 2014, Noriega et al. 2018). In recent years, studies have suggested that some insects are in decline due to anthropogenic destruction of habitat and climate change (Hallmann et al. 2017, Wagner 2020, Wagner et al. 2020).

Not all groups of insects are impacted equally, with specialists declining more rapidly than generalist insect species (Kotiaho et al. 2005, Warren et al. 2020, Wagner 2020). Insect behavior such as migration and phenology are also affected by changes in climate, and there is evidence that extended growing seasons lead to increases in voltinism for species previously constrained by temperatures (Bale and Hayward 2010, Forrest 2016). As many habitat and climatic shifts are occurring rapidly, it is critical to understand how changes in community composition and phenology might impact long-term natural enemy assemblages and changes in competition (Damien and Tougeron 2019, Rudolf 2019, Tougeron et al. 2020). Yet, to conduct these long-term assessments, we must first determine what insects reside in various habitats.

The target habitat for our study was the northern tamarack woodlands of Minnesota where the eastern larch beetle, *Dendroctonus simplex* LeConte, has experienced shifts in population phases from endemic to outbreak in portions of its southern transcontinental range since 2001 (McKee and Aukema 2015, MN DNR 2024a). Historically, the eastern larch beetle has only exhibited outbreaks following events such as fire, drought, flooding, or defoliation that

predispose forests to increased activity from bark beetles (Hopkins 1909, Baker 1972, Bright 1976, Langor and Raske 1987). This population shift is likely due to a change in voltinism from one to two generations per year in some areas (McKee and Aukema 2015, McKee et al. 2022). In Minnesota, the outbreak of eastern larch beetle has resulted in a loss of over one million acres of tamarack covertype (Minnesota DNR 2024a). The peatlands in which tamarack grows cover over six million acres in the state (Anderson et al. 2008).

Tamarack serves many important cultural and ecological roles. In Ojibwe culture, tamarack bark is used as a laxative (Densmore 1974, Prindle 2001). Peatland landscapes have also served as sources for indigenous foods such as wild blueberry (*Vaccinium angustifolium* Aiton) and cranberry (*Vaccinium* spp.) (Kozich et al. 2002, Windmuller-Campione et al. 2023). Ecologically, tamarack forests are critical to carbon sequestration as a single acre can absorb approximately 750 metric tons of carbon dioxide with Minnesota peatlands containing 4,250 megatons of carbon (Anderson et al. 2008). Tamarack also plays a critical role in hydrology, prevention of erosion, and habitat provision (Burns and Honkala 1990).

While populations of larger and more charismatic animals such as birds (Zlonis et al. 2017, Bednar 2017) as well as plants (Locky and Bayley 2006, Warner and Asada 2006, McPartland et al. 2019) have been assessed in tamarack ecosystems, the understanding of insect diversity within tamarack forests is limited. Moreover, while studies of insect diversity have been conducted in the Maritime regions (Langor and Raske 1988, Langor 1991), New York (Blackman and Stage 1918), and Indiana (Wolcott and Montgomery 1933), the diversity of insects in mixed tamarack stands has not been assessed in the Great Lakes region.

From June through July of 2022 and 2023, we placed Lindgren funnel traps baited with eastern larch beetle attractants in tamarack stands throughout north-central Minnesota for studies

of eastern larch beetle chemical ecology (experimental design described in Chapter 3). In the present study, we identified potential competitors, predators, and parasitoids of the eastern larch beetle in the bycatch from these traps. As information regarding natural enemies of the eastern larch beetle is still limited, we remained broad in our identification scheme but primarily targeted taxa within the orders Hymenoptera and Coleoptera. We hope this information will inform future conservation and research efforts.

Methods

Experimental Design

Our sites consisted of tamarack stands with some black spruce (*Picea mariana* (Mill.)) interspersed and an understory composed of speckled alder (*Alnus incana* (L.) Moench), Labrador tea *(Rhododendron groenlandicum* (Oeder) Kron & Judd)), leatherleaf (*Chamaedaphne calyculata* (L.) Moench), starflower (*Lysimachia borealis* (Raf.)), Canada mayflower (*Maianthemum canadense* Desf.), spinulos shield fern (*Dryopteris carthusiana* (Vill.) H.P. Fuchs), small cranberry (*Vaccinium oxycoccos* L.), bunchberry (*Cornus canadensis* L.), dwarf raspberry (*Rubus pubescens* Richardson), swamp marigolds (*Caltha palustris* L.), pink lady slippers (*Cypripedium acaule* Aiton), pitcher plants (*Sarracenia purpurea* L.), *Sphagnum* moss, horsetails (*Equisetum sp.),* and other plant fauna identified in Northern Rich Tamarack Swamp types FPn72 and FPn82 (MN DNR 2024b, MN DNR 2024c). Insects were collected in two ways: using flight traps and rearing from tamarack logs. Insects identified in this study were primarily bycatch from five (2022) and six (2023) baited Lindgren funnel traps at each of eight sites in central Minnesota (Table 1). In brief, traps were baited with seudenol, frontalin, and various tamarack compounds, 4-allylanisole, Δ-3-carene, and α-pinene (Althoff et al. 2023). These experiments were conducted in the summers of 2022 (2 June -7 July) and 2023 (14 June -

20 July) over six weekly collection intervals. We also felled a tamarack of approximately 25 cm DBH (diameter at breast height; 1.4 m) that was naturally infested with eastern larch beetles within the Carlos Avery Wildlife Management Area (45.40948 °N, 92.92568 °W) in March 2022. Trees were sectioned into 61 cm lengths and melted paraffin wax was applied to each end to slow desiccation. Logs were left outdoors to maintain field conditions until April 2022 when they were brought inside. Then, logs were placed into separate emergence tubes (McKee and Aukema 2015) at room temperature (24 ± 0.5°C) with 24 hr light.

Insects were removed from funnel traps weekly and frozen before being sorted into higher taxonomic groups. Insects that emerged into rearing jars, which tended to be parasitoids, were collected daily from 3 May – 16 May 2022. Insects were pinned, labeled, and curated for identification to lower taxonomic groups. Data for eastern larch beetle and *Thanasimus* spp. are already included in Chapter 3 (which focused on their semiochemical responses), but *Thanasimus* spp. are included again in the present biodiversity survey as they are important elements of the community of eastern larch beetle associates. All specimens have been accessioned into the University of Minnesota Insect Collection as vouchers.

Identification Methods

We utilized a variety of taxonomic keys for identifications. Bark beetle identification used Wood (1982). Identification of buprestids used Hallinen et al. (2021). Clerids were identified to genus using Barr (1962) and compared to identified species in the University of Minnesota Insect Collection and SCAN database specimens. Cerambycidae identification used Lindsley and Chemsak (1961). Staphylinidae identification utilized Brunke et al. (2011), Kovarik and Caterino (2000), prior literature on Staphylinidae in this ecosystem type (Blackburn and Stage 1918, Langor 1991), and comparison to identified species at the University of Minnesota Insect

Collection. Histeridae identification was conducted using Kovarik and Caterino (2000) and comparisons to specimens at the University of Minnesota Insect Collection. Insects in the families Ichneumonidae, Braconidae, Diapriidae, and Pteromalidae were identified with assistance from Dr. John Luhman, research associate at the University of Minnesota Insect Collection. Siricidae were identified utilizing Schiff et al. (2006). Orussidae were identified using Vilhelmsen 2003, Agridae were identified using Smith (1969), and Pompillidae identification used Townes (1957) and comparisons to specimens in the University of Minnesota Collection.

State Record Confirmation

State records were confirmed through comparisons with 1) Symbiota Collections of Arthropods Network (SCAN) database (https://scan-bugs.org/portal/), 2) Minnesota Biodiversity Atlas-Bell Museum (https://bellatlas.umn.edu/collections/index.php), 3) iNaturalist 'Research Grade' identifications (https://www.inaturalist.org), and 4) University of Minnesota Entomology Collection. For cases in which names have changed, contemporary names were confirmed using the Global Biodiversity Information Facility (GBIF) (https://www.gbif.org).

Statistical Analysis

Data analysis was conducted using the vegan package in R to assess Shannon diversity indices temporally between sites (R Core Team 2020, Version 3.5.0, Vienna Austria). Species abundance, richness, and evenness are also reported utilizing Pielou's evenness index (H/log(number of species)) in which H represents the Shannon diversity index.

Results

In total, we counted and identified 8,001 insects from bycatch material in the Lindgren funnel traps. We found 135 unique taxa (Table 2) in addition to *Dendroctonus simplex* with five new

state records: (*Ichneumon uncinatu*s Cresson (Hymenoptera: Ichneumonidae), *Ichneumon heterodon* Heinrich (Hymenoptera: Ichneumonidae), *Syspasis trauma* Heinrich (Hymenoptera: Ichneumonidae), *Quedionuchus plagiatus* Mannerheim (Coleoptera: Staphylinidae), and *Quedionuchus glaber* (O. F. Müeller) (Coleoptera: Staphylinidae)). In total, we identified 23 families, 93 genera, and 135 species. Within Coleoptera, we identified 12 families, 41 genera, and 51 species. Within Hymenoptera, we identified 11 families, 52 genera, and 84 species. The most abundant insects identified within our bycatch included *Thanasimus dubius* (Fabricius) (Coleoptera: Cleridae) (5,702), *Thanasimus undulatus* (Say) (Coleoptera: Cleridae) (691), *Phymatodes dimidiatus* (Kirby) (Coleoptera: Cerambycidae) (398), *Madoniella dislocata* (Say) (Coleoptera: Cleridae) (290), *Asemum striatum* (L.) (Coleoptera: Cerambycidae) (115), *Xylotrechus undulatus* Casey (Coleoptera: Cerambycidae) (71), *Philonthus caerulepennis* (Mannerheim) (Coleoptera: Staphylinidae) (64), *Drycoetes autographus* (Ratzeburg) (Coleoptera: Curculionidae) (53), *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae) (51), *Polygraphus rufipennis* (Kirby) (Coleoptera: Curculionidae) (48), *Platysoma cylindricum* (Paykull) (Coleoptera: Histeridae) (47), *Quedionuchus plagiatus* Mannerheim (Coleoptera: Staphylinidae) (27), *Dicerca tenebrica* (Kirby) (Coleoptera: Buprestidae) (21).

Discussion

Our results identifying 135 unique taxa showcase the insect biodiversity of tamarack peatland ecosystems in Minnesota. Visualizing our diversity data over time indicated shifts in Shannon diversity, species evenness, species richness, and abundance throughout the collection periods (Figures 1, 2). Shannon diversity and species evenness dipped to the lowest in mid-June while species richness and abundance peaked at these same dates (Tables 3,4, Figures 1, 2). The decrease in species evenness seemed to follow the increase in *Thanasimus* spp. at this same

collection period. Here, we break down our findings into groups of known natural enemies and known competitors of eastern larch beetle and new state records.

Known natural enemies:

Although we cataloged many previously described natural enemies, our results suggest populations may shift regionally. As indicated in Chapter 3, *Thanasimus dubius* and *undulatus* were the predators captured at the highest abundance. These species are predators of the eastern larch beetle at both the adult and larval stage (Barker et al. 1977, Werner 1981, Langor 1991, Seybold et al. 2002). These species have been previously documented at low population densities within Newfoundland sites infested with eastern larch beetle (Langor 1991). Thus, while prior work has indicated that predation occurs at low rates throughout the eastern larch beetle lifecycle, predation rates could be region-specific and merit broader investigation (Langor and Raske 1988, Langor 1991).

Additional proposed natural enemies in the families Histeridae and Staphylinidae were also captured, but their behavior is still poorly understood. *Platysoma cylindricum* (Paykull), *Platysoma leconti* Marseul, and *Platysoma gracile* Leconte were also captured in this study. *Platysoma spp*. are known to broadly predate upon bark beetle species at both adult and larval stages (Aukema and Raffa 2004), and prior studies have indicated that *Platysoma* species may prey upon *D. simplex* (McKee 2015). Additionally, *Athleta sp.* was captured but it remains unclear if this insect is truly a predator. Prior literature states that it has been observed feeding on eggs of eastern larch beetle, but the viability of the eggs was uncertain (Langor 1991).

Intraguild predation, a combination of predation and competition, of Cerambycidae species on bark beetles has been well documented (Polis et al. 1989). While *Stictoleptura canadensis* are known competitors, larvae may additionally be predators of *D. simplex* larvae

(Langor 1991). We identified 11 species of Cerambycids in this study, with *Phymatodes dimidiatus* captured in the highest quantity (398) followed by *Asemum striatum* (115), and *Monochamus scutellatus* (51). Of these, *Monochamus scutellatus* has been confirmed to colonize *Larix laricina* and are known to prey facultatively on bark beetle larvae (Dodds et al. 2001).

Natural enemies in orders such as Diptera were collected, but not identified as these taxa were outside the bounds of the author's expertise. Thus, further work should be conducted to determine the presence and populations of *Medetera* spp. (Diptera: Dolichopodidae) and *Zabrachia* spp. (Diptera: Stratiomyidae) as each is known to predate upon eastern larch beetle (Langor 1991).

In eastern populations, approximately 30% of eastern larch beetles experienced mortality from parasitism past the peak of the outbreak in the Maritimes region (Langor and Raske 1988). Thus, parasitoid abundance and diversity was of interest in this study. However, only one known ectoparasitoid of the eastern larch beetle was recovered from traps (*Spathius canadensis* Ashmead). In future studies, sampling for parasitoids in flight could be enhanced by setting yellow sticky traps in the upper bole of trees where parasitism is more active (Langor and Raske 1988). Parasitoids reared from tamarack bolts in the lab included *Spathius canadensis*, *Rhopalicus tutela* (Walker), *Coeloides rufovariegatus* (Provancher), *Ropterocerus xylophagorum* (Ratzeburg), and a *Eurytoma* species. We suspect that the *Eurytoma* sp. previously found in work by Blackman and Stage 1918, Langor 1991, and Haack et al. 2024 was also found in our studies given manuscript descriptions. This species is currently undergoing DNA barcoding analysis. It will be assessed as a potential new species if more individuals are captured, as it has only been described to genus (Althoff, Aukema, Kresslein, and Gates unpublished). Ultimately,

more work is necessary to bolster our understanding of species' behavior and function within this system.

Known competitors:

Drycoetes autographus (Ratzeburg 1837) (Coleoptera: Curculionidae), *Polygraphus rufipennis* (Kirby 1837) (Coleoptera: Curculionidae), and *Strictoleptura canadensi*s (Olivier 1795) (Coleoptera: Cerambycidae) have all been listed as competitors with eastern larch beetle in prior literature (Langor 1991). Additional wood-boring beetles utilizing *L. laricina* include *Dicerca tenebrica* (Kirby 1837) (Coleoptera: Buprestidae), *Chrysobothris rotundicollis* (Gory and Laporte 1837) (Coleoptera: Cerambycidae), and *Anthaxia inornata* (Randall, 1838) (Coleoptera: Buprestidae) (Hallinen et al. 2021). Moreover, these competitors may be hosts of additional parasitoids and predators within the tree. Although *Orussus* spp. are not known to parasitize *D. simplex*, they frequently parasitize beetles in the family Buprestidae (Middlekauff 1983). Thus, parasitism rates in Buprestid-colonized *L. laricina* could influence competition dynamics.

New state records:

This work has expanded the known ranges of five insect species, with new state records for Minnesota. These species include *Ichneumon uncinatu*s Cresson, *Ichneumon heterodon* Heinrich, *Syspasis trauma* Heinrich, *Quedionuchus plagiatus* Mannerheim, and *Quedionuchus glaber* (O. F. Müeller).

Conclusion:

This work provides insight into biodiversity within an understudied peatland ecosystem. In Europe, work has highlighted the variation between peatland systems in bog plateaus as opposed to lagg zones due to variations in host availability (Främbs 1994, Desrochers and van Duinen 2006). Future work is necessary on Dipteran parasitoids of the eastern larch beetle and

pollinators in the orders Diptera, Lepidoptera, and Hymenoptera. Additional sampling methods will also recover additional species richness and diversity within this ecosystem. Future work, for parasitoid sampling, for example, should include yellow sticky traps high in the boles of trees and Malaise traps (Darling and Parker 1988, Langor and Raske 1988). Similarly, panel traps and wet cup collection can be more successful at sampling woodboring beetles and associates (Allison and Redak 2017) than the multiple funnel traps we deployed as standard for capturing eastern larch beetles (Baker et al. 1977, Werner 1981, Prendergast 1991, Graham and Storer 2015). Nevertheless, our work addresses gaps in our current understanding of regional variation in this system, and we hope that this research can be used as a tool for future conservation and biodiversity assessments over time within the region.

Acknowledgements

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Experiment Station Project MIN 17-117, as well as a University of Minnesota Department of Entomology Excellence in Entomology Fellowship, a University of Minnesota Doctoral Dissertation Fellowship, and a University of Minnesota Department of Entomology Beneficial Insect Award to ERA.

Table 1: Field site coordinates for eight locations in north-central Minnesota containing 5-6 traps that were checked weekly over the course of six weeks. In 2022, collection dates were 02 June 2022 to 06 July 2022 and traps contained seudenol and a variety of host compound blends. The 2023 collection dates occurred from 14 June 2023 to 20 July 2023 and the traps contained seudenol and varying release rates of frontalin. Canopy cover is categorized as dense if the understory was shaded and had a density of approximately > 5 trees per 3 m^2 and as sparse if the understory was not shaded and density was approximately < 5 trees per 3 m^2. Standing water was deemed absent if it did not exceed 2.54 cm.

Year	Site	County	Coordinates	Cover Type	Canopy Cover	Standing Water
2022	1	Aitkin Co.	46.95735°N, -93.4388°W	Tamarack & Black Spruce	Dense	Absent
2022	2	Aitkin Co.	46.95875°N, -93.4443°W	Tamarack	Sparse	Absent
2022	3	Aitkin Co.	46.97418°N, -93.4470°W	Tamarack & Black Spruce	Dense	Present
2022	4	Aitkin Co.	46.99788°N, -93.3592°W	Tamarack	Sparse	Absent
2022	5	Aitkin Co.	46.58568°N, -93.4653°W	Tamarack & White Cedar	Dense	Absent
2022	6	Aitkin Co.	46.58018°N, -93.4654°W	Tamarack	Sparse	Present
2022	7	Aitkin Co.	46.58534°N, -93.4626°W	Tamarack & White Cedar	Dense	Present
2022	8	Aitkin Co.	46.67992°N, -93.6741°W	Tamarack & White Cedar	Dense	Absent
2023	1	Aitkin Co.	46.58199°N, -93.4637°W	Tamarack & White Cedar	Sparse	Absent
2023	2	Aitkin Co.	46.95767°N, -93.4397°W	Tamarack & Black Spruce	Dense	Absent
2023	3	Aitkin Co.	46.97371°N, -93.4473°W	Tamarack & Black Spruce	Dense	Present
2023	4	St. Louis Co.	47.17088°N, -92.5374°W	Tamarack	Dense	Absent
2023	5	St. Louis Co.	47.07532°N, -92.6617°W	Tamarack	Dense	Absent
2023	6	St. Louis Co.	47.03439°N, -92.6713°W	Tamarack & Birch	Dense	Absent
2023	7	St. Louis Co.	47.02786°N, -92.6619°W	Tamarack	Dense	Absent
2023	8	Carlton Co.	46.59246°N, -92.9242°W	Tamarack & Birch	Dense	Present

Table 2: All identified bycatch from Lindgren funnel traps. Sites are listed in Table 1. Insects were frozen and then stored in ethanol until they could be counted and identified. Insects included in this table were identified to at least genus. Frequency is categorized as follows: Abundant: > 25 specimens; Common: 5-25 specimens; Uncommon: < 5 specimens.

Order	Family	Subfamily	Genus	Species	Authority	Number	Frequency
Coleoptera	Anthribidae	Anthribinae	*Euparius*	*marmoreus*	(Olivier 1795)	1	Uncommon
Coleoptera	Buprestidae	Buprestinae	*Anthaxia*	*inornata*	(Randall 1838)	3	Uncommon
Coleoptera	Buprestidae	Buprestinae	*Chrysobothis*	*sp.*	Eschscholz, 1829	1	Uncommon
Coleoptera	Buprestidae	Buprestinae	*Chrysobothris*	*rotundicollis*	Gory & Laporte 1837	3	Uncommon
Coleoptera	Buprestidae	Chrysochroinae	*Dicerca*	*tenebrica*	(Kirby 1837)	21	Abundant
Coleoptera	Cantharidae	Cantharinae	*Podabrus*	*diadema*	(Fabricius 1798)	1	Uncommon
Coleoptera	Cerambycidae	Cerambycinae	*Clytus*	*ruricola*	(Olivier 1795)	5	Common
Coleoptera	Cerambycidae	Cerambycinae	*Phymatodes*	*dimidiatus*	(Kirby 1837)	398	Abundant
Coleoptera	Cerambycidae	Cerambycinae	*Xylotrechus*	*sagittatus*	(Germar)	11	Common
Coleoptera	Cerambycidae	Cerambycinae	*Xylotrechus*	*undulatus*	Casey 1912	71	Abundant
Coleoptera	Cerambycidae	Laminiiae	*Monochamus*	*scutellatus*	(Say 1824)	51	Abundant
Coleoptera	Cerambycidae	Lepturinae	*Judolia*	*m. montevagus*	Hansen & Osgood 1983	2	Uncommon
Coleoptera	Cerambycidae	Lepturinae	*Pygoleptura*	*nigrella*	(Say 1826)	7	Common
Coleoptera	Cerambycidae	Lepturinae	*Rhagium*	*inquisitor*	Linnaeus 1758	10	Common
Coleoptera	Cerambycidae	Lepturinae	*Stictoleptura*	*canadensis*	(Olivier, 1795)	1	Uncommon
Coleoptera	Cerambycidae	Lepturinae	*Trachysida*	*mutabilis*	*(*Newman *1841)*	9	Common
Coleoptera	Cerambycidae	Lepturinae	*Trachysida*	*aspera brevifrons*	Howden, 1959	11	Common
Coleoptera	Cerambycidae	Spondylidinae	*Asemum*	*striatum*	(Linnaeus 1758)	115	Abundant
Coleoptera	Thanocleridae	.	*Zenodosus*	*sanguineus*	(Say 1835)	17	Common
Coleoptera	Cleridae	Clerinae	*Enoclerus*	*ichneumoneus*	(Fabricius 1776)	2	Uncommon
Coleoptera	Cleridae	Clerinae	*Thanasimus*	*dubius*	(Fabricius 1776)	5,701	Abundant
Coleoptera	Cleridae	Clerinae	*Thanasimus*	*undulatus*	(Say 1835)	691	Abundant
Coleoptera	Cleridae	Epiphloeinae	*Madoniella*	*dislocata*	(Say 1825)	290	Abundant
Coleoptera	Curculionidae	Cryptorhynchinae	*Cryptorhynchus*	*lapathi*	Linnaeus 1758	2	Uncommon
Coleoptera	Curculionidae	Curculioninae	*Curculio*	sp.	Linnaeus 1758	1	Uncommon
Coleoptera	Curculionidae	Molytinae	*Hylobius*	sp	E. F. Germar 1817	71	Abundant

Coleoptera	Curculionidae	Molytinae	*Hylobius*	*transversovittatus*	(J.A.E. Goeze 1777)	2	Uncommon
Coleoptera	Curculionidae	Molytinae	*Pissodes*	*rotundatus*	LeConte 1876	11	Common
Coleoptera	Curculionidae	Molytinae	*Pissodes*	*strobi*	Peck 1817	7	Common
Coleoptera	Curculionidae	Myrmexinae	*Myrmex*	sp.	Sturm 1826	1	Uncommon
Coleoptera	Curculionidae	Scolytinae	*Dryocoetes*	*autographus*	(Ratzeburg 1837)	53	Abundant
Coleoptera	Curculionidae	Scolytinae	*Pityokteines*	*sparsus*	(LeConte 1868)	7	Common
Coleoptera	Curculionidae	Scolytinae	*Polygraphus*	*rufipennis*	(Kirby 1837)	48	Abundant
Coleoptera	Histeridae	Histerinae	*Platysoma*	*cylindricum*	(Paykull 1811)	47	Abundant
Coleoptera	Histeridae	Histerinae	*Platysoma*	*leconti*	Marseul 1853	6	Common
Coleoptera	Histeridae	Histerinae	*Platysoma*	*gracile*	LeConte 1845	1	Uncommon
Coleoptera	Oedemeridae	Calopodinae	*Calopus*	*angustus*	LeConte 1851	1	Uncommon
Coleoptera	Pyrochroidae	Pyrochroinae	*Dendroides*	*canadensis*	Latreille 1817	1	Uncommon
Coleoptera	Scarabaeidae	Melolonthinae	*Dichelonyx*	*linearis*	(Gyllenhal 1817)	2	Uncommon
Coleoptera	Staphylinidae	Aleocharinae	*Atheta*	sp.	Thomson 1858	1	Uncommon
Coleoptera	Staphylinidae	Staphylininae	*Ontholestes*	*cingulatus*	(Gravenhorst 1802)	2	Uncommon
Coleoptera	Staphylinidae	Staphylininae	*Philonthus*	*caeruleipennis*	(Mannerheim 1830)	64	Abundant
Coleoptera	Staphylinidae	Staphylininae	*Philonthus*	*rectangulus*	(Sharp 1874)	25	Common
Coleoptera	Staphylinidae	Staphylininae	*Quedionuchus*	*plagiatus*	Mannerheim 1843	27	Abundant
Coleoptera	Staphylinidae	Staphylininae	*Quedionuchus*	*glaber*	(O.F. Müeller 1776)	3	Uncommon
Coleoptera	Staphylinidae	Xantholininae	*Nudobius*	*cephalus*	(Say 1834)	6	Common
Coleoptera	Tenebrionidae	Stenochiinae	*Upis*	*ceramboides*	(Linnaeus 1758)	2	Uncommon
Coleoptera	Tenebrionidae	Tenebrioninae	*Bolitophagus*	*corticola*	Say 1826	4	Uncommon
Coleoptera	Tenebrionidae	Tenebrioninae	*Bolitotherus*	*cornutus*	(Panzer 1794)	1	Uncommon
Coleoptera	Tenebrionidae	Tenebrioninae	*Eleates*	*depressus*	(Randall 1838)	2	Uncommon
Coleoptera	Tenebrionidae	Diaperinae	*Corticeus*	*parallelus*	(Melsheimer 1846)	9	Common
Hymenoptera	Argidae	.	*Atomacera*	*debilis*	Say 1836	1	Uncommon
Hymenoptera	Braconidae	Alysiinae	*Dapsilarthra*	sp.	Förster 1862	1	Uncommon
Hymenoptera	Braconidae	Alysiinae	*Phaenocarpa*	sp.	Förster 1862	1	Uncommon
Hymenoptera	Braconidae	Aphidiinae	*Ephedrus*	sp.	Haliday 1833	1	Uncommon
Hymenoptera	Braconidae	Brachistinae	*Blacus*	sp.	Nees 1818	1	Uncommon

Hymenoptera	Braconidae	Braconinae	*Digonogastra*	sp. 1	Viereck 1912	1	Uncommon
Hymenoptera	Braconidae	Braconinae	*Digonogastra*	sp. 2	Viereck 1913	9	Common
Hymenoptera	Braconidae	Braconinae	*Digonogastra*	sp. 3	Viereck 1914	3	Uncommon
Hymenoptera	Braconidae	Braconinae	*Digonogastra*	sp. 4	Viereck 1915	2	Uncommon
Hymenoptera	Braconidae	Braconinae	*Digonogastra*	sp. 5	Viereck 1916	1	Uncommon
Hymenoptera	Braconidae	Cenocoeliinae	*Cenocoelius*	sp. 1	Haliday 1840	2	Uncommon
Hymenoptera	Braconidae	Cenocoeliinae	*Cenocoelius*	sp. 1	Haliday 1840	2	Uncommon
Hymenoptera	Braconidae	Cenocoeliinae	*Cenocoelius*	sp. 2	Haliday 1841	1	Uncommon
Hymenoptera	Braconidae	Cenocoeliinae	*Cenocoelius*	sp. 2	Haliday 1841	1	Uncommon
Hymenoptera	Braconidae	Cheloninae	*Chelonus*	sp.	Panzer 1806	1	Uncommon
Hymenoptera	Braconidae	Doryctinae	*Heterospilus*	sp.	Haliday 1836	1	Uncommon
Hymenoptera	Braconidae	Doryctinae	*Spathius*	*laflammei*	Provancher 1880	5	Common
Hymenoptera	Braconidae	Doryctinae	*Spathius*	*canadensis*	Ashmead 1891	1	Uncommon
Hymenoptera	Braconidae	Euphorinae	*Meteorus*	sp.	Haliday 1835	1	Uncommon
Hymenoptera	Braconidae	Microgastrinae	*Cotesia*	sp.	Cameron 1891	1	Uncommon
Hymenoptera	Braconidae	Microgastrinae	*Diolcogaster*	sp.	Ashmead 1900	1	Uncommon
Hymenoptera	Diapriidae	Diapriinae	*Idiotypa*	sp.	Förster 1856	1	Uncommon
Hymenoptera	Diapriidae	Diapriinae	*Trichopria*	sp. 1	Ashmead 1893	1	Uncommon
Hymenoptera	Diapriidae	Diapriinae	*Trichopria*	sp. 2	Ashmead 1893	1	Uncommon
Hymenoptera	Eulophidae	Eulophinae	*Sympiesus*	sp.	Förster 1856	1	Uncommon
Hymenoptera	Ibaliidae	.	*Ibalia*	*leucospoides ensiger*	Norton 1862	1	Uncommon
Hymenoptera	Ichneumonidae	Cryptinae	*Cubocephalus*	*alacris*	(Cresson 1864)	2	Uncommon
Hymenoptera	Ichneumonidae	Cryptinae	*Cubocephalus*	*maurus*	(Cresson 1879)	1	Uncommon
Hymenoptera	Ichneumonidae	Cryptinae	*Cubocephalus*	*nitidus nitidus*	(Provanncher 1874)	2	Uncommon
Hymenoptera	Ichneumonidae	Cryptinae	*Cubocephalus*	*prolixus*	Townes & Gupta 1962	1	Uncommon
Hymenoptera	Ichneumonidae	Cryptinae	*Lysibia*	sp.	Förster 1869	1	Uncommon
Hymenoptera	Ichneumondiae	Cryptinae	*Trychosis*	*albitarsis*	(Cresson 1864)	1	Uncommon
Hymenoptera	Ichneumonidae	Cryptinae	*Trychosis*	*similis*	(Cresson 1864)	2	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Centeterus*	sp. 1	Wesmael 1845	2	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Centeterus*	sp. 2	Wesmael 1846	1	Uncommon

Hymenoptera	Ichneumonidae	Ichneumoninae	*Coelichneumon*	*eximus*	(Stephens 1835)	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Coelichneumon*	*orpheus*	(Cresson 1864)	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Cratichneumon*	*unifasciatorius*	(Say 1825)	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Diadromus*	sp.	Wesmael 1835	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Dicaelotus*	sp.	Wesmael 1845	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Ichneumon*	*devinctor*	Say 1825	5	Common
Hymenoptera	Ichneumonidae	Ichneumoninae	*Ichneumon*	*uncinatus*	Cresson 1877	2	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Ichneumon*	*canadensis*	Cresson 1867	2	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Ichneumon*	*centrator*	Say 1825	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Ichneumon*	*cessatorops*	Heinrich 1956	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Ichneumon*	*heterodon*	Heinrich 1961	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Ichneumon*	*ultimus*	Cresson 1877	2	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Ophion*	sp.	Fabricius 1798	2	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Orgichneumon*	*calcatorins*	(Thunberg 1822)	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumoninae	*Syspasis*	*tauma*	Heinrich 1951	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumonindae	*Dicaelotus*	sp.	Wesmael 1845	2	Uncommon
Hymenoptera	Ichneumonidae	Ichneumonindae	*Phaeogenes*	sp. 1	Wesmael 1845	1	Uncommon
Hymenoptera	Ichneumonidae	Ichneumonindae	*Phaeogenes*	sp. 2	Wesmael 1846	1	Uncommon
Hymenoptera	Ichneumonidae	Metopiinae	*Chorinaeus*	*subcarinatus*	Holmgren 1858	1	Uncommon
Hymenoptera	Ichneumonidae	Orthocentrinae	*Eusterlnx*	sp.	Förster 1850	1	Uncommon
Hymenoptera	Ichneumonidae	Orthocentrinae	*Orthocentrus*	sp. 1	Gravenhorst 1829	1	Uncommon
Hymenoptera	Ichneumonidae	Orthocentrinae	*Orthocentrus*	sp. 2	Gravenhorst 1830	1	Uncommon
Hymenoptera	Ichneumonidae	Phygadeuontinae	*Gelis*	*meabilis*	(Cresson 1872)	1	Uncommon
Hymenoptera	Ichneumonidae	Phygadeuontinae	*Gelis*	sp.	Thunberg 1827	1	Uncommon
Hymenoptera	Ichneumonidae	Phygadeuontinae	*Mesoleptus*	sp.	Gravenhorst 1829	1	Uncommon
Hymenoptera	Ichneumonidae	Phygadeuontinae	*Pygocryptus*	sp.	Roman 1925	1	Uncommon
Hymenoptera	Ichneumonidae	Phygadeuontinae	*Pygocryptus*	sp.	Roman 1925	1	Uncommon
Hymenoptera	Ichneumonidae	Phygadeuontinae	*Theroscopus*	sp.	Förster 1850	2	Uncommon
Hymenoptera	Ichneumonidae	Pimplinae	*Delomerista*	*novita*	(Cresson 1870)	1	Uncommon
Hymenoptera	Ichneumonidae	Pimplinae	*Ephialtes*	*spatulata*	(Townes 1960)	1	Uncommon

Hymenoptera	Ichneumonidae	Pimplinae	*Pimpla*	*pedalis*	(Cresson 1865)	1	Uncommon
Hymenoptera	Ichneumonidae	Xoridinae	*Odontocolon*	*mellipes*	(Say 1829)	1	Uncommon
Hymenoptera	Ichneumonidae	Xoridinae	*Odontocolon*	sp.	Cushman 1942	1	Uncommon
Hymenoptera	Ichneumonidae	Xoridinae	*Xorides*	sp.	Latreille 1809	1	Uncommon
Hymenoptera	Orussidae	.	*Orussus*	*terminalis*	Newman 1838	1	Uncommon
Hymenoptera	Pompilidae	Pepsinae	*Dipogon*	*sayi*	Banks 1941	33	Abundant
Hymenoptera	Pompilidae	Pepsinae	*Dipogon*	*papago*	(Banks 1933)	10	Common
Hymenoptera	Pteromalidae	Pteromalinae	*Brachycaudonia*	*sp.*	Ashmead 1904	1	Uncommon
Hymenoptera	Pteromalidae	Pteromalinae	*Diabrachys*	*cavus*	(Walker 1835)	1	Uncommon
Hymenoptera	Pteromalidae	Pteromalinae	*Diabrachys*	sp. 1	Förster 1856	1	Uncommon
Hymenoptera	Pteromalidae	Pteromalinae	*Diabrachys*	sp. 2	Förster 1857	3	Uncommon
Hymenoptera	Pteromalidae	Pteromalinae	*Diabrachys*	sp. 3	Förster 1858	1	Uncommon
Hymenoptera	Pteromalidae	Pteromalinae	*Diabrachys*	sp. 4	Förster 1859	3	Uncommon
Hymenoptera	Pteromalidae	Pteromalinae	*Epipteromalus*	*algonquinensis*	Ashmead 1904	1	Uncommon
Hymenoptera	Pteromalidae	Pteromalinae	*Pachyneuron*	sp.	Walker 1833	1	Uncommon
Hymenoptera	Siricidae	Siricinae	*Urocerus*	*albicornis*	(Fabricius 1781)	5	Common
Hymenoptera	Siricidae	Siricinae	*Urocerus*	*cressoni*	Norton 1864	4	Uncommon
Hymenoptera	Siricidae	Tremecinae	*Xeris*	sp.	Costa 1894	6	Common
Hymenoptera	Tenthredinidae	Nematinae	*Nematus*	sp.	Panzer 1801	1	Uncommon
					Total Identified	**8,001**	

Table 3: Diversity indices among sites for insects captured in Lindgren funnel traps baited with semiochemicals for eastern larch beetles at eight sites in the summer of 2022. H is the diversity index at each site, J is Pielou's species evenness, S is the number of species, and N is total abundance.

Date	Variable	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
2 June 2022	H	0.64	0.63	0.92	0.95	0.76	1.07	1.07	0.96
8 June 2022	H	1.48	1.36	1.78	0.67	1.08	0.98	1.46	1.92
15 June 2022	H	0.43	0.71	0.96	0.43	0.89	1.23	1.07	0.70
23 June 2022	H	0.75	1.50	0.85	1.01	1.83	1.69	1.81	1.54
30 June 2022	H	1.21	1.43	1.67	1.03	2.15	1.63	1.73	1.8
7 July 2022	H	1.19	1.75	1.42	1.49	1.24	1.39	1.60	0.73
2 June 2022	J	0.77	0.41	0.74	0.53	0.82	0.58	0.71	0.61
8 June 2022	J	0.96	0.77	0.61	0.93	0.91	0.75	0.71	0.77
15 June 2022	J	0.96	0.20	0.22	0.17	0.60	0.26	0.83	0.71
23 June 2022	J	0.97	0.21	0.33	0.24	0.57	0.28	0.28	0.32
30 June 2022	J	0.85	0.21	0.44	0.30	0.61	0.61	0.83	0.71
7 July 2022	J	0.88	0.39	0.48	0.25	0.65	0.59	0.77	0.53
2 June 2022	S	2.00	4.00	6.00	3.00	4.00	4.00	4.00	3.00
8 June 2022	S	6.00	9.00	7.00	7.00	5.00	7.00	7.00	9.00
15 June 2022	S	9.00	9.00	14.00	6.00	7.00	6.00	8.00	7.00
23 June 2022	S	14.00	14.00	13.00	9.00	13.00	8.00	10.00	12.00
30 June 2022	S	10.00	9.00	10.00	11.00	13.00	7.00	8.00	8.00
7 July 2022	S	6.00	6.00	12.00	10.00	4.00	5.00	7.00	3.00
2 June 2022	N	3	30	33	5	18	8	8	7
8 June 2022	N	21	35	25	76	49	47	23	25
15 June 2022	N	288	276	322	420	114	31	69	160
23 June 2022	N	343	167	284	224	44	43	52	116
30 June 2022	N	85	56	45	91	32	19	22	26

7 July 2022	N	49	12	120	28	6	8	19	20

Table 4: Diversity indices among sites for insects captured in Lindgren funnel traps baited with semiochemicals for eastern larch beetles at eight sites in the summer of 2023. H is to the diversity index at each site, J is Pielou's species evenness, S is the number of species, and N is total abundance.

Date	Variable	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
14 June 2023	H	1.78	1.20	2.00	1.26	2.11	1.34	1.97	1.39
21 June 2023	H	2.31	1.69	1.51	2.05	2.11	1.65	1.28	1.61
28 June 2023	H	2.39	0.42	0.53	0.40	1.25	0.54	1.26	1.48
5 July 2023	H	2.62	0.49	0.75	0.42	1.32	0.72	0.58	0.82
12 July 2023	H	1.87	0.53	0.79	0.71	1.10	1.34	1.83	1.63
20 July 2023	H	2.74	0.63	0.86	0.40	0.72	0.95	1.76	0.85
14 June 2023	J	0.77	0.41	0.74	0.53	0.82	0.58	0.71	0.61
21 June 2023	J	0.96	0.77	0.61	0.93	0.91	0.75	0.71	0.77
28 June 2023	J	0.96	0.20	0.22	0.17	0.60	0.26	0.55	0.67
5 July 2023	J	0.97	0.21	0.33	0.24	0.57	0.28	0.323	0.51
12 July 2023	J	0.85	0.21	0.44	0.30	0.61	0.61	0.83	0.71
20 July 2023	J	0.88	0.39	0.48	0.25	0.65	0.59	0.77	0.53
14 June 2023	S	10.00	19.00	15.00	11.00	12.00	10.00	16.00	10.00
21 June 2023	S	11.00	8.00	12.00	9.00	8.00	9.00	6.00	8.00
28 June 2023	S	11.00	8.00	10.00	10.00	7.00	8.00	10.00	9.00
5 July 2023	S	15.00	9.00	10.00	6.00	10.00	13.00	6.00	5.00
12 July 2023	S	9.00	12.00	6.00	10.00	6.00	9.00	9.00	10.00
20 July 2023	S	22.00	5.00	6.00	5.00	3.00	5.00	9.00	5.00
14 June 2023	N	36	276	73	63	37	105	48	102
21 June 2023	N	16	40	119	16	22	39	36	38
28 June 2023	N	16	204	183	220	61	120	54	47
5 July 2023	N	21	263	193	212	121	150	154	39
12 July 2023	N	14	242	82	87	28	29	16	39

20 July 2023	N	43	33	49	89	40	32	25	39

Figure Captions

Figure 1: A) Shannon diversity index, B) species evenness, C) species richness, and D) abundance at each of eight sites in central Minnesota with specimens collected from 02 June 2022 to 06 July 2022. Lindgren funnel traps contained seudenol and a variety of host compound blends.

Figure 2: A) Shannon diversity index in central Minnesota, B) species evenness, C) species richness, and D) abundance at each of eight sites in central Minnesota with specimens collected from 14 June 2023 to 20 July 2023. Lindgren funnel traps contained seudenol and variable release rate of frontalin.

Figure 1

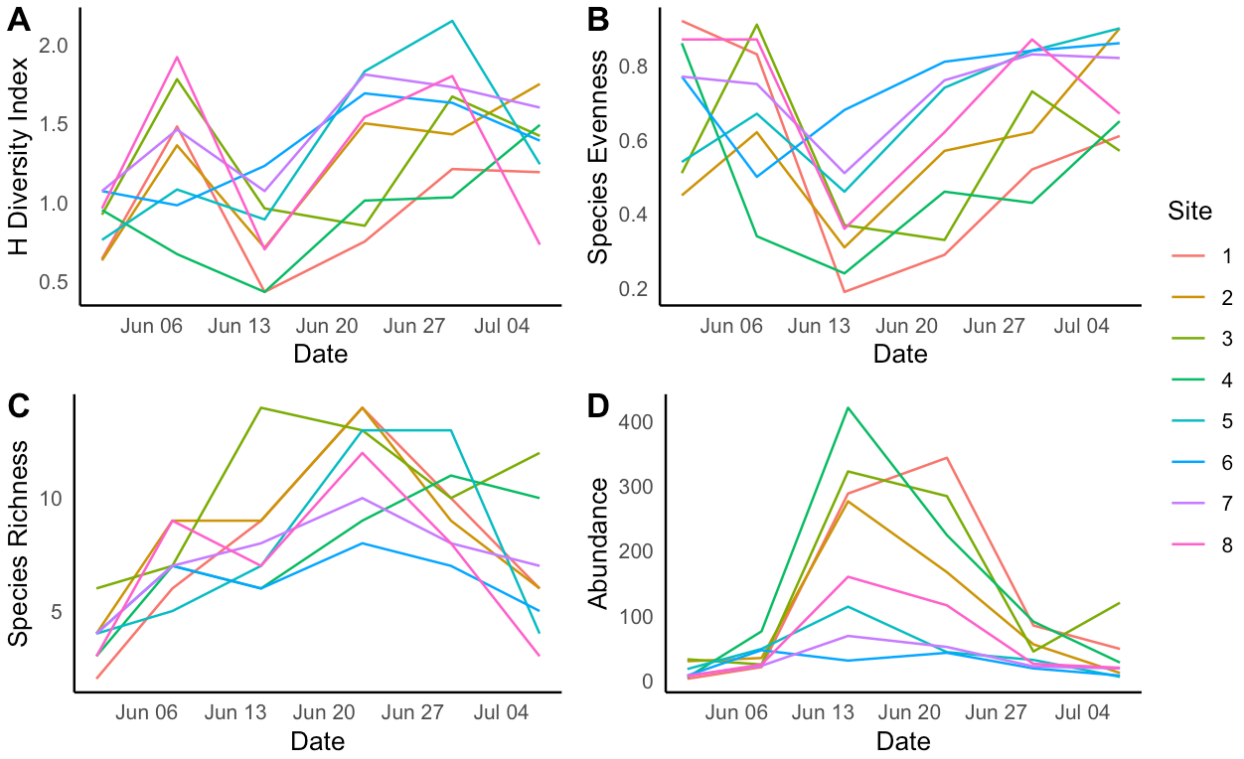
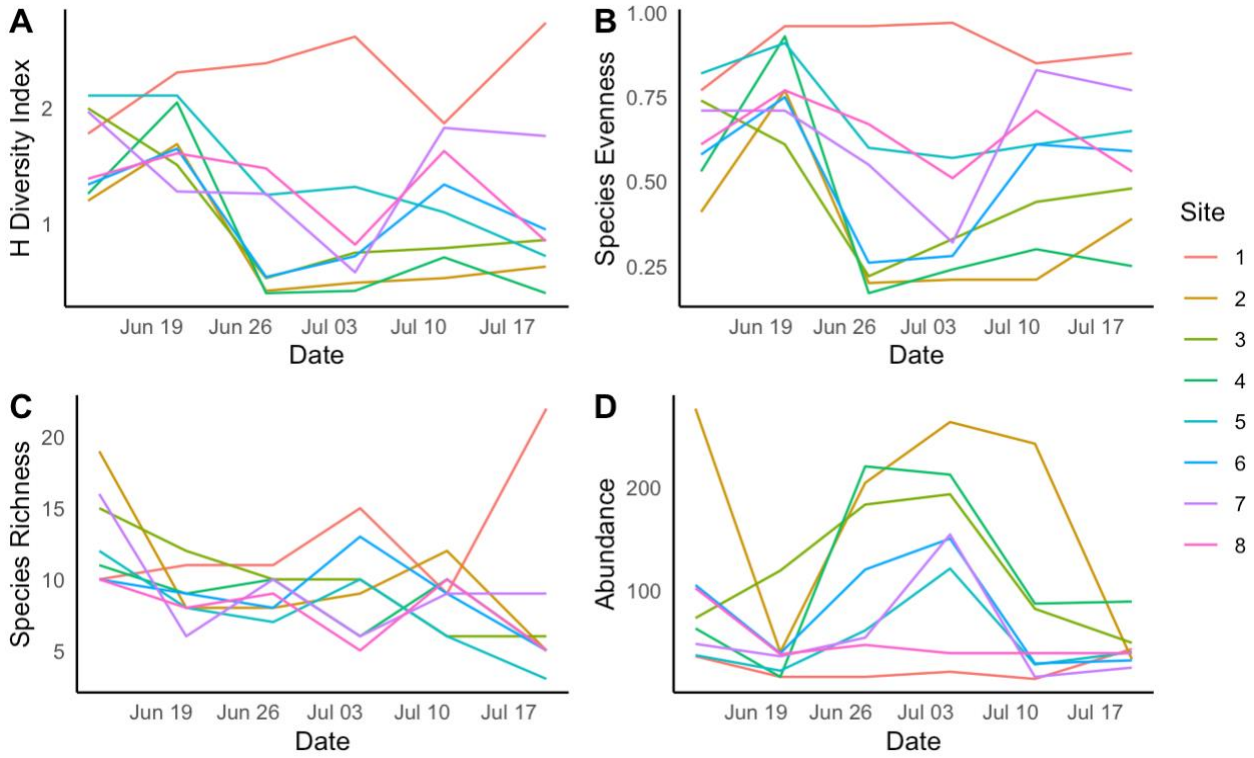

A
H Diversity Index
2.0
1.5
1.0
0.5
Jun 06
Jun 13
Jun 20
Jun 27
Jul 04
Date
B
Species Evenness
0.8
0.6
0.4
0.2
Jun 06
Jun 13
Jun 20
Jun 27
Jul 04
Date
Site
1
2
3
4
5
6
7
8
C
Species Richness
10
5
Jun 06
Jun 13
Jun 20
Jun 27
Jul 04
Date
D
Abundance
400
300
200
100
0
Jun 06
Jun 13
Jun 20
Jun 27
Jul 04
Date

Figure 2


A
H Diversity Index
2
1
Jun 19
Jun 26
Jul 03
Jul 10
Jul 17
Date
B
Species Evenness
1.00
0.75
0.50
0.25
Jun 19
Jun 26
Jul 03
Jul 10
Jul 17
Date
C
Species Richness
20
15
10
5
Jun 19
Jun 26
Jul 03
Jul 10
Jul 17
Date
D
Abundance
200
100
Jun 19
Jun 26
Jul 03
Jul 10
Jul 17
Date
Site
1
2
3
4
5
6
7
8

Dissertation Conclusions

1. **The predominant monoterpenes in the phloem tissues of *Larix laricina* in Minnesota and Wisconsin are α-pinene and Δ-3-carene.** This finding inspired research into the effect of Δ-3-carene on the behavior of eastern larch beetle and its natural enemies responding to semiochemical lures.
2. **Unmated female eastern larch beetles produce both seudenol and frontalin. Frontalin production continues in female beetles after pairing with a male, but seudenol production ceases. Male eastern larch beetles produce no unique pheromone components. Each sex was able to detect seudenol and frontalin.** Female beetles, as the host-selecting sex, play a central role in host procurement and mate attraction, using seudenol and frontalin.
3. **JH III regulates the production and release of frontalin into the hindgut of female eastern larch beetles. The production of seudenol appears to be regulated by a separate pathway.** Seudenol is likely produced after food consumption. It is likely that a physiological change after mating causes production of seudenol to cease. The pathway regulating seudenol production has yet to be determined. However, as none of the pheromone components were produced prior to feeding, it is possible that feeding stimulates the production of JH III, and thus frontalin.
4. **Eastern larch beetles are most attracted to Δ-3-carene with seudenol, while their primary predators, *Thanasimus* spp., are more attracted to lures containing α-pinene in combination with seudenol.** These findings may indicate evolutionary resource partitioning or evidence of evolutionary divergence of *D. simplex* from other

closely related *Dendroctonus* spp. that overlap in range. The attraction of *Thanasimus* to α-pinene, likely coinciding with attraction to bark beetles in other conifers, affords the eastern larch beetle a greater degree of enemy-free space.

5. **The addition of high concentrations of frontalin to seudenol resulted in repellency of eastern larch beetle. However, *Thanasimus* spp. were attracted to higher concentrations of frontalin.** The continued production of frontalin after mating, which would increase with additional arriving females, likely serves to repel additional conspecific females past a certain threshold and thus reduce intraspecific competition. The predators are using these signals as kairomones, responding to higher numbers of potential prey within the tree.
6. **Male eastern larch beetles were attracted to the combination of frontalin and seudenol when engaging in walking behavior while female eastern larch beetles exhibited no response.** Altered responses when eastern larch beetles were engaged in walking vs. flight behavior may be driven by key behavioral differences between sexes. Female eastern larch beetles may shift from in-flight attractive responses to aggregative compounds to a walking behavior focusing on other cues, possibly host compounds that indicate a suitable host. Meanwhile, male beetles are responding to pheromones emitted from female beetles to locate mates while engaging in both flight and walking behavior. Both sexes may also benefit from mass aggregation.
7. **Our biodiversity survey identified 135 unique taxa from tamarack stands within central Minnesota, including five state records.** Here, we noted that Shannon's biodiversity index and species evenness decreased in mid-June while species richness and

abundance increased during this period. These results serve as a baseline for future work on conservation and restoration of tamarack stands.

8. **Through rearing insects in the laboratory, we may have discovered a new species of parasitoid wasp.** This *Eurytoma* sp. is likely the same species noted in prior studies on *D. simplex* that had only been identified to genus. The wasp awaits identification to species.
9. **Finally, our biodiversity work is intended to lay the groundwork for future biological surveys within the state as well as large-scale modeling.** We hope that the data provided is able to inform work investigating long-term impacts of disturbance on tamarack landscapes within Minnesota.

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Appendix 1

Evidence of a potential new species of *Dendroctonus simplex* LeConte parasitoid

A1.1 Introduction

The eastern larch beetle, *Dendroctonus simplex* LeConte is a native bark beetle that utilizes *Larix laricina* (Du Roi) as a host. With recent outbreaks in the Great Lakes region, interest in the eastern larch beetle's ecology and natural enemies has experienced a resurgence (McKee and Aukema 2015, Ward and Aukema 2019).

Langor and Raske (1988), for example, found that parasitism accounted for 30% of eastern larch beetle mortality. Thus, gaining a broader understanding of the potential parasitoids could be crucial in assessments of population dynamics. One such natural enemy is a wasp in the family Eurytomidae. This wasp has been identified to the genus *Eurytoma* in Newfoundland and Michigan (Langor 1991, Haack et al. 2024), but the species has not been identified to date. Here, we reared eastern larch beetle from infested bolts and identified subsequent parasitoid species, with the primary target being the *Eurytoma* sp. (Fig. 1).

A1.2 Methods

Host material was collected from Carlos Avery Wildlife Management Area (45.40948 N, 92.92568 W). Selected trees were approximately 25.4 cm DBH (diameter at breast height; 1.4 m). We applied wax to the ends of each 61 cm long log to retain moisture. Logs were kept outdoors to maintain environmental exposure until March 2022. Then, logs were placed into cardboard emergence tubes with a glass jar attachment on one end. Once parasitoids emerged into the jars, they were placed into ethanol vials until they could be identified. We utilized Bugbee (1966) and Gates (unpublished) to identify the *Eurytoma* sp. to genus. Three specimens were collected (Fig. 1). These specimens were sent to the USDA SEL Laboratory at the

Smithsonian Museum of Natural History to Dr. Michael Gates and Luke Kresslein for DNA barcoding. Gates was able to confirm the species to *Eurytoma morio* species group via taxonomic key, but further identification efforts are ongoing. Currently, we are awaiting comparison to Delvare et al. (2014) matrices.

Figure Caption

Figure 1 Image of unidentified *Eurytoma* sp. This insect was reared from tamarack bolts containing *Dendroctonus simplex* in March of 2022.

Figure 1