The role of climate in outbreaks of larch casebearer

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Dedication

This dissertation is dedicated to my brother, Cole Allen Fahrner (1985-1999). Thank you for teaching me to persevere, to always keep things in perspective, and not to take life for granted.

Dissertation Abstract

Larch casebearer, *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae), is an invasive insect in North America that was introduced from Europe in the late 1800s and defoliates larch trees. Once combatted successfully with an importation biological control program, it has resurged in several parts of its invaded range. This dissertation describes several laboratory, field, and landscape studies to examine the effects of photoperiod and temperature on survival and development of larch casebearer. First, I characterized patterns in defoliation. From 1997-2016, defoliation fluctuated synchronously between forests of western larch (*Larix occidentalis*) in Oregon/Washington and eastern larch (*Larix laricina*) in Minnesota, suggesting that climate acting across large spatial scales may drive similar patterns among allopatric casebearer populations. In Minnesota, I found that warmer minimum winter temperatures appeared to have contributed to the resurgence of larch casebearer in Minnesota. Spring phenological synchrony between bud break by eastern larch and larval activation did not appear to drive the resurgence; all larvae were estimated to activate several days after the average day of bud break. Field studies suggested that, in cooler growing seasons, some larvae may not accumulate sufficient degree-days to reach the overwintering stage. I applied degree-day models to historical climate data to elucidate changes in 1) spring phenological synchrony (i.e., the number of days between bud break by eastern larch and subsequent spring activation by larch casebearer larvae) and 2) the proportion of larvae estimated to reach the overwintering stage. I found that larch casebearer consistently activates several days after eastern larch has broken bud and that higher estimated proportions of larvae have reached the overwintering stage over the previous ~50 years.

The importance of degree-day accumulation for outbreaks of larch casebearer was corroborated by a spatiotemporal autologistic model developed on defoliation data from aerial surveys. The analysis of landscape patterns also demonstrated that feeding by larch casebearer may predispose eastern larch to mortality from eastern larch beetle *Dendroctonus simplex* LeConte (Coleoptera: Curculionidae). In summary, the resurgence of larch casebearer in Minnesota appears to have been facilitated by increasingly warmer winters and growing seasons.

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Dissertation introduction

Understanding how climate moderates forest insect herbivores, many of which can cause widespread biotic disturbances, and their interactions with host plants will aid in forest management (Ayres & Lombardero, 2000; Dale *et al.*, 2001). Insects rely on environmental cues, such as those from temperature and photoperiod, to time their seasonal activity (Tauber & Tauber, 1976). As poikilothermic organisms, insect developmental rates and seasonality are driven, in part, by ambient temperatures (Bale *et al.*, 2002). Thus, climate is an important determinant in the distributions, abundances, and impacts of insects (Bale *et al.*, 2002; Weed *et al.*, 2013) and global climate change has been implicated in driving significant deviations from historical patterns of abundance by biotic disturbance agents such as native and non-native insects (Dale *et al.*, 2001). For example, climatic change alter precipitation patterns and render host trees more susceptible to attack from herbivorous insects (Mattson & Haack, 1987; Jamieson *et al.*, 2012), increase insect survival due to warmer minimum winter temperatures (Bale & Hayward, 2010), and/or change the timing of activity by herbivorous insects relative to development and growth of host plants (DeLucia *et al.*, 2012).

Impacts from invasive insects present a global challenge (Mack *et al.*, 2000; Liebhold & Tobin, 2008). Invasions by insects in forest ecosystems can drive changes in ecosystem structure and function (Kenis *et al.*, 2009; Clark *et al.*, 2010) and cause severe economic impacts (Pimentel *et al.*, 2005; Aukema *et al.*, 2011). Larch casebearer is an invasive defoliator in North America first detected in 1886 on European larch, *Larix decidua* Mill. (Pinaceae), planted near Northampton, MA (Hagen, 1886). The insect is native to mountainous regions of central Europe that contain European larch *L. decidua*.

After its detection in North America, it spread throughout most of the range of eastern larch *Larix laricina* (Du Roi) Koch. In 1957, larch casebearer was detected on western larch, *Larix occidentalis* Nutt., an allopatric congener of eastern larch in the northwestern United States and southwestern Canada, and spread throughout most western larch's range (Tunnock & Ryan, 1985).

Eastern larch is a deciduous conifer with a distribution extending from the northeastern United states, across the Great Lakes region and boreal forest of Canada, northwest to Alaska (Burns & Honkala, 1990). Eastern larch is a shade intolerant, early successional species and is an important component of lowland conifer ecosystems in the Great Lakes region (Duncan, 1954). In Minnesota, which encompasses the southwestern edge of eastern larch's distribution, eastern larch often co-occurs with black spruce, *Picea mariana* (Mill.) BSP, or northern white-cedar, *Thuja occidentalis* L., and inhabits bogs and, less frequently in Minnesota, upland sites (Duncan, 1954). Needles are flushed in early spring, a process driven by chilling during winter and heat accumulation across winter and spring (Nanninga *et al.*, 2017), and dropped in late autumn, which may be driven by photoperiod and/or temperature (Worrall, 1993; Migliavacca *et al.*, 2008; Moser *et al.*, 2009).

Larch casebearer damages eastern larch trees by mining out the inner tissues of needles. Defoliation is most damaging in spring, when fourth instar larch casebearers that reside in cases constructed from hollowed out larch needles feed on several needles (Herrick, 1911; Thorpe, 1933; Denton, 1979). At high densities, larval feeding render trees with a burnt appearance and can decrease growth of host trees (Tunnock *et al.*, 1969), predispose trees to attack from fungi or secondary agents (Tunnock *et al.*, 1969;

Benoit & Blais, 1988), and/or kill trees when repeated annually (Craighead, 1950; Dowden, 1957). To combat larch casebearer and reduce damages to North American larches, an importation biological control program was established in the 1930s in eastern larch forests and 1960s in western larch forests and was eventually considered successful in both regions. Control has been attributed to two introduced parasitoids: *Agathis pumila* Ratzeburg (Hymenoptera: Braconidae) and *Chrysocharis laricinellae* Ratzeburg (Hymenoptera: Eulophidae) (Graham, 1948; Webb & Quednau, 1971; Otvos & Quednau, 1981; Ryan, 1990, 1997). For example, during aerial detection surveys via fixed-wing aircrafts in which sketch mappers document landscape signatures of disturbance by native and non-native insects, defoliation by larch casebearer was non-existent in eastern larch forests of Minnesota until 2000 and mapped once from 1979-1996 in western larch forests or Oregon/Washington.

Since the late 1990s and early 2000s, larch casebearer populations have resurged at several locations throughout its invasive range in North America. Moreover, during the outbreak of larch casebearer, a concomitant outbreak of a native, tree-killing bark beetle, eastern larch beetle (*Dendroctonus simplex* Leconte; Coleoptera: Curculionidae), has occurred on tamarack, likely facilitated by climate (Mckee, 2015). The role of climate in outbreaks of larch casebearer, and its interactions with eastern larch beetle, are not well understood. Previous work demonstrated that larch casebearer is responsive to photoperiod and has an obligate diapause (Ryan, 1975). Most research concerning responses of larch casebearer to environmental cues have been directed at maximizing production of rearing material for the imported biological control agents (Ryan, 1979). However, there is a lack of understanding of casebearer developmental across a range of ecologically-relevant temperatures and photoperiods, and how such development progresses in relation to bud break and needle senescence by eastern larch. To that end, this dissertation reports experiments designed to understand if and how changes in climate facilitate outbreaks of larch casebearer in Minnesota and, finally, if those outbreaks facilitate outbreaks of eastern larch beetle.

In Chapter 1, I describe patterns in defoliation by larch casebearer within and between eastern larch forests of Minnesota and western larch forests of Oregon/Washington. I analyze rasterized aerial survey data using non-parametric spatial covariance functions to understand if defoliation fluctuates synchronously within and among larch forests of North America. Analyses in this chapter were conducted, in part, because large scale synchrony in insect populations may indicate that climate, which is often synchronous across large geographic scales, drives similar patterns of abundance in allopatric insect populations. Thus, findings of synchrony in defoliation at large spatial scales may suggest that casebearer populations are highly sensitive to climate.

In Chapter 2, I estimate the effects of simulated spring precipitation and defoliation on monoterpene production, growth, and survival of three-year old potted eastern larch trees. Monoterpene concentrations are also quantified across the first six weeks of the growing season to provide insight on how defensive capacity changes with needle age. I quantify the survival of casebearer larvae to adulthood in response to water-stress treatments and monoterpene concentrations.

In Chapter 3, I characterize the cold hardiness of larch casebearer larvae. I quantify temperatures required to kill 50% of larvae and mean supercooling points from autumn through spring. Because both overwintering (i.e., diapausing) and active larvae occur simultaneously on trees in autumn and spring, I also compare cold hardiness between these two groups. Finally, supercooling point data are linked to historical climate data to provide insight on temporal patterns of estimated overwintering survival across the past ~50 years.

In Chapter 4, I attempt to disentangle the relative contributions of degree-day accumulation, chilling unit accumulation, and photoperiod to bud break by eastern larch and spring activation of larch casebearer larvae. Moreover, I investigate how the responses of each species to select cues changes across dormancy. I provide degree-day models of bud break by eastern and spring activation by larch casebearer larvae. I also investigate the effects of phenological synchrony, defined as the days between bud break and subsequent activation of casebearer larvae, on casebearer performance.

In Chapter 5, I catalog the abundance of all life stages of larch casebearer at three field sites in Minnesota. Life stage abundances are then linked to degree-day accumulation to develop degree-day models for all life stages of larch casebearer. These degree-day models are then used to estimate 1) spring activation of larvae relative to bud break and 2) the proportion of larvae that reach the overwintering across the previous ~50 years in north central Minnesota. Such temporal analyses provide insight on any changes in insect population dynamics that may help explain the resurgence of larch casebearer.

In Chapter 6, I compile and rasterize aerial survey data cataloging abundance of larch casebearer and eastern larch beetle and PRISM climate data into 4 ×4 km grid cells. Then I develop a landscape-level model predicting the presence of defoliation by larch casebearer and tree-killing behavior by eastern larch beetle at the grid cell level in Minnesota, USA from 2003-2016 as a function of several climatic predictors. In conjunction with correlates of defoliation identified during model development in this chapter, previous chapters on larch casebearer are used to develop mechanistic hypotheses for the resurgence of larch casebearer. Finally, I evaluate the role of defoliation in facilitating outbreaks of eastern larch beetle.

Chapters comprising this dissertation have been written for future publication in peer-reviewed journals. Thus, there may exist redundancy across chapters to maintain the integrity of each chapter as a standalone piece. I served as the principal investigator for each chapter but invaluable input and contributions were provided by many coauthors and/or committee members: Brian Aukema, George Heimpel, Daniel Herms, Aubree Kees, Mitchell Maddox III, Rebecca Montgomery, Roger Moon, Robert Venette, and Julian Wolfson. Therefore, I present the following research in the plural voice, rather than singular.

Chapter 1. Continental resurgence of an invasive defoliator once controlled by imported natural enemies

Coauthors: Brian H. Aukema

Preface

Larch casebearer is in an invasive defoliator in North America distributed within the regions of two allopatric hosts, eastern larch and western larch. After years of successful importation biological control, larch casebearer has undergone a resurgence in regions of both host plants. We analyzed defoliation data, a proxy for insect abundance, to quantify spatiotemporal synchrony and found that defoliation of larch casebearer was synchronous both within and between eastern and western larch forests. For allopatric populations with positively correlated population dynamics, climate is the most likely driver of synchrony. Thus, we hypothesize that a changing climate has facilitated the resurgence of larch casebearer.

Introduction

Larch casebearer, *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae), is a needle-mining moth native to Europe that was discovered near Northampton, Massachusetts, USA. in the late 1800s. The insect subsequently spread throughout most of the range of eastern larch, *Larix laricina* (Du Roi) K. Koch (Pinaceae) (Figure 1.1). Likely following an introduction from eastern larch forests, larch casebearer was detected in northern Idaho in the 1950s and spread throughout most of the range of western larch *Larix occidentalis* Nutt. (Figure 1.1; Hagen 1886, Tunnock and Ryan 1985). Subsequently, an importation biological control program was established against larch casebearer and considered successful in both regions (Graham, 1948; Webb & Quednau, 1971; Otvos & Quednau, 1981; Ryan, 1990, 1997). Between 1960 and 2000 in eastern larch forests, for example, defoliation by larch casebearer was not detected via aerial surveys conducted by the United States Department of Agriculture Forest Service (USDA-FS). In western larch forests, defoliation was mapped sporadically in five years in each of the 1960s and 1970s but only once from 1979-1996. The apparent resurgence of larch casebearer has been surprising after several years of successful importation biological control.

Since 2000 in Minnesota and 1997 in Oregon/Washington, however, 40 - 32,000 hectares of defoliation of larch have been detected in each year except one. Here, we aimed to quantify the presence and strength of spatiotemporal synchrony in defoliation both within and between regions. Synchrony across continental scales may implicate climate as a driving factor of contemporaneous increases and fluctuations in larch casebearer populations.

Materials and Methods

Data collection and processing: defoliation

We obtained aerial survey data collected by the USDA Forest Service between 1965-2016 for Minnesota, Oregon, and Washington (Figure 1.1). Aerial surveys use fixed wing aircraft to detect and delineate damage to foliage. Assigning causative agents can be challenging when disparate insects or diseases cause similar landscape-level signatures. In eastern larch forests, larch casebearer is reliably mapped as an individual agent due to low densities of fungal competitors. In western larch forests, defoliation is recorded jointly as larch casebearer and *Hypodermella laricis* Tubeuf and *Meria laricis* Peace & Holm (Flowers *et al.*, 2013), which are fungi that cause similar discoloration of the tree crown. We submit, however, that the primary disturbance agent mapped was larch casebearer across the majority of our study period, as fungi typically defoliate the lower crowns and ground surveys confirmed the widespread presence of larch casebearer (Nelson *et al.*, 2008; Flowers *et al.*, 2009). In the last few years of the time series, fungal competitors became more abundant, despite the continued presence of larch casebearer (Flowers *et al.*, 2010, 2013).

All damage severities recorded by surveyors (i.e., trace, light, moderate, and severe) were retained for analyses and combined into a layer for each year with polygons indicating presence/absence of defoliation by larch casebearer. Spatial polygons for each year across both regions were rasterized into 4 × 4 km grid cells as follows. The defoliation polygons were first overlaid on a grid of 4 × 4 km cells using a geographic information system. Each cell was further subdivided into 100 smaller square cells and each sub-cell was assigned a 1 if the centroid of that cell was intersected by a defoliation polygon and recorded as a 0 otherwise. The percent of each grid cell covered by a polygon was calculated as the total number of sub cells covered by defoliation divided by 100 and recorded as an integer from 0 to 100.

The rasterized defoliation data were then subset to 1997-2016 to analyze recent trends in defoliation, as no defoliation by larch casebearer had been mapped before 2000 in Minnesota or since 1984 in Oregon/Washington (Figure 1.2). Data were further subset to include only grid cells that were positive for larch casebearer defoliation for at least 1 of the 20 years. This process resulted in 1,416 cells per year, totaling 28,320 cells across the 20-year time series. These cells were spatially referenced and used in analyses of spatial synchrony (see below). All GIS analyses were completed using R statistical software version 3.4.1 (R Core Team, 2017), and several packages enabled the analysis and processing of spatial data: GISTools (Brunsdon & Chen, 2014), maps (Becker *et al.*, 2016), raster (Hijmans, 2016), and rgdal (Bivand *et al.*, 2016).

Spatial synchrony

Spatial synchrony of defoliation events was first quantified for Minnesota, then Oregon/Washington, and then across both regions combined. We quantified spatial synchrony using nonparametric spatial covariance functions implemented via the Sncf command in the ncf package in R (Bjørnstad & Falck, 2001; Bjørnstad, 2016). The Sncf function fits a smoothing spline to the pairwise cross-correlations of randomly selected cells as a function of lag distance (Bjørnstad & Falck, 2001; Økland *et al.*, 2005; Bjørnstad, 2016). The spline and associated confidence intervals were calculated using 200 bootstrapped samples for each analysis.

Results

Synchrony of defoliation

Defoliation by larch casebearer was synchronous both within and between Minnesota (eastern larch forests) and Oregon/Washington (western larch forests), as denoted by positive correlations between zero and one in Figure 1.3. For Minnesota, populations were synchronous up to 108 km (95% CI: 82 – 311 km; Figure 1.3a). Defoliation in Oregon/Washington was significantly more synchronous than in Minnesota, with positive synchrony up to 331 km (95% CI: 320 – 339 km) or extending ~223 km farther than patterns observed in Minnesota (Figure 1.3b).

When quantifying synchrony across both regions by combining the data, synchrony decayed to 0 at 331 km (95% CI: 322 – 342 km; Figure 1.3c). There was evidence that populations in both regions fluctuated synchronously, as the correlation increased to 0.08 (95% CI: 0.05 – 0.11) at 2,150 km. Thus, when defoliation increases in eastern larch forests of Minnesota in a given year, defoliation in western larch forests of Oregon/Washington is expected to increase concomitantly.

Discussion

Defoliation of eastern larch and western larch fluctuated synchronously both within and between Minnesota and Oregon/Washington across 1997-2016. Within each region, dispersal, by either larch casebearer moths or natural enemies, may drive patterns of synchrony (Liebhold *et al.*, 2004). Given that dispersal between allopatric eastern larch and western larch forests by moths or natural enemies is extremely unlikely, climate is the most parsimonious explanation of synchrony observed at distances greater than 2000 km (Figure 1.3c). Simplified systems involving organisms without a co-evolutionary history may be more unstable than complex systems involving interactions between native organisms (Murdoch, 1975). Thus, an invasive insect such as larch casebearer without its full cohort of natural enemies may be prone to outbreaks following environmental or demographic stochasticity. Climate may act in several ways to moderate insect survival (Bale *et al.*, 2002; Van Asch & Visser, 2007) and synchrony in climatic variables between regions may produce similar patterns of defoliation. Investigation of the role of climate in the population dynamics of larch casebearer, including how changes therein may moderate host-parasitoid and parasitoid-hyperparasitoid interactions, may provide insight on the resurgence of larch casebearer both within and between regions.

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Figures

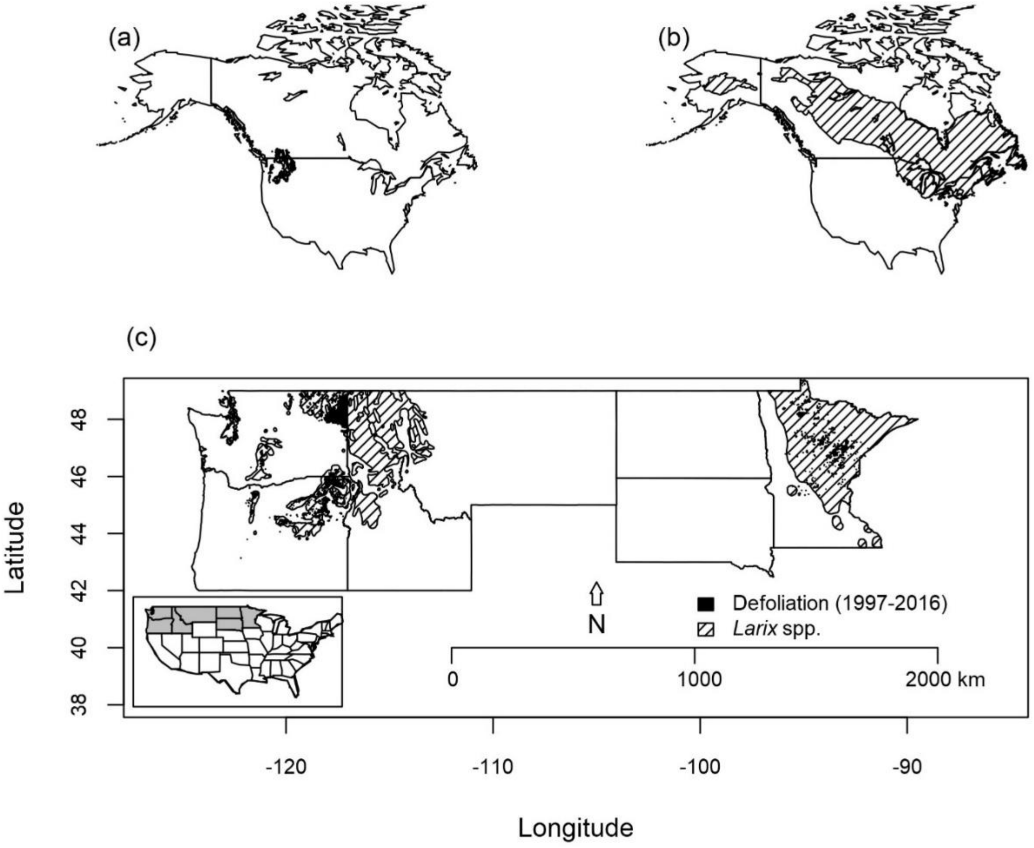

Figure 1.1 Ranges of (a) western larch *Larix occidentalis* Nutt. and (b) eastern larch *Larix laricina* (Du Roi) K. Koch. (c) Map of study region in the United States including cumulative mapped defoliation, 1997-2016. Hashed marks indicate tree distributions ranges of western larch in Oregon/Washington (west of longitude -105°) and the range eastern larch in Minnesota (east of longitude -105°).

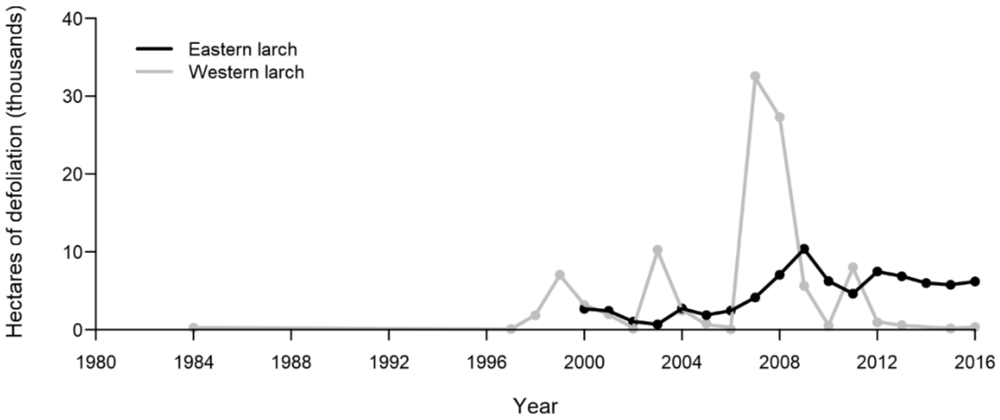

Figure 1.2 Time series of mapped defoliation in eastern larch forests of Minnesota, USA and western larch forests of Oregon and Washington, USA.

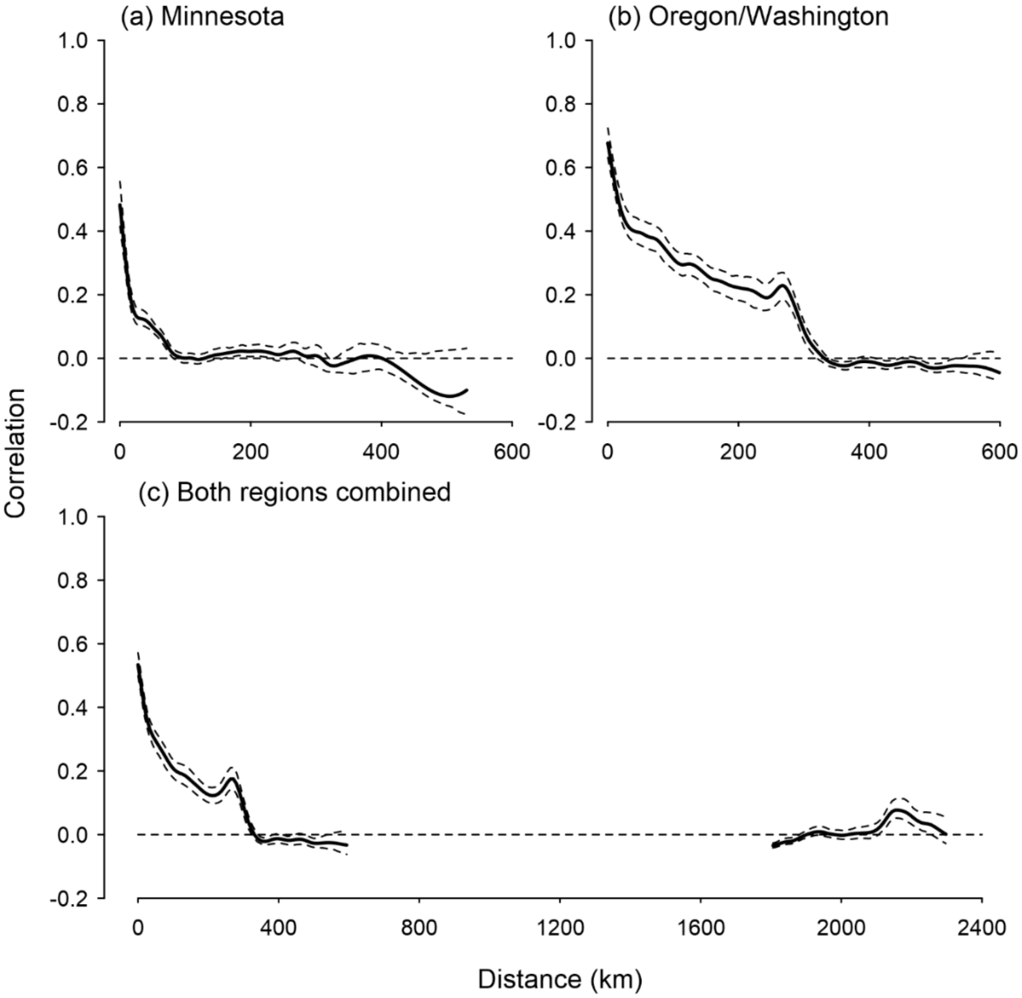

Figure 1.3 Synchrony of mapped defoliation (a) within Minnesota USA, (b) within Oregon and Washington, USA and (c) across Minnesota and Oregon/Washington combined. For panel c, synchrony at intermediate distances is not presented, as no *Larix* spp. occur at intermediate distances. Smoothing functions with dashed lines (95% confidence intervals) were estimated using non-parametric spatial covariance functions. Horizontal dashed line indicates 0, where no statistically significant synchrony was detected.

Chapter 2. The role of simulated spring water stress in interactions between eastern larch and larch casebearer

Coauthors: Aubree M. Kees, Mitchell P. Maddox III, Rebecca A. Montgomery, and Brian H. Aukema

Preface

Larch casebearer, *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae), is an invasive defolatior in North America where it infests eastern larch, *Larix laricina* (Du Roi) K. Koch (Pinaceae), and western larch, *Larix occidentalis* Nutt. Larch casebearer was once under successful importation biological control, but large scale outbreaks have been detected each year since 2000 in Minnesota, USA. In Minnesota, eastern larch is mostly found in peatland bogs or fens with complex hydrology. Given the potential for global climate change to alter precipitation and the seasonal flooding dynamics of eastern larch stands, we investigated the role of simulated waterlogging and drought on eastern larch-larch casebearer interactions over two years. We quantified the growth, survival, and monoterpene profiles of juvenile eastern larch in response to varying watering regimens and challenge from larch casebearer. We also measured how watering regimen affected the survival of 4th instars to adulthood. The growth and survival of eastern larch was negatively impacted by challenge from larch casebearer, waterlogging, and drought stress, though the strength of responses varied between years. The monoterpene profile of eastern larch did not change in response to water stress or challenge from larch casebearer but the concentrations of several monoterpenes decreased with time. No patterns emerged in the response of larch casebearer to watering regimen or monoterpenes. Thus, the interaction of watering stress and defoliation do not appear to moderate eastern larch – larch casebearer interactions, but rather act independently to impact growth and survival of juvenile trees.

Introduction

Water stress, such as waterlogging or drought, can moderate the phytochemistry of plants and their ability to tolerate or resist herbivory (Mattson & Haack, 1987; Jamieson *et al.*, 2012). Changes in plant defense in response to herbivory may come at a cost to plant growth, as plants allocate nutrients to the production of secondary compounds (e.g., "growth-differentiation balance hypothesis") (Herms & Mattson, 1992). Likewise, changes in the defensive capacity of plants due to water stress can impact the survival and growth of herbivorous insects (Mattson & Haack, 1987; Huberty & Denno, 2004) and the responses of such herbivores can be highly context dependent (Mattson, 1980; Mattson & Haack, 1987).

The defense systems and growth of the Pinaceae are well-studied; members of this taxon exhibit several constitutive and inducible defenses against herbivores, including monoterpenes, that are important to the interactions of the Pinaceae with several feeding guilds (Chen *et al.*, 2002; Raffa *et al.*, 2005; Mumm & Hilker, 2006; Seybold *et al.*, 2006). That is, monoterpenes can serve as attractants and defense compounds against bark beetles (Seybold *et al.*, 2006) as well as serve as attractants for natural enemies (Pettersson, 2001; Mumm & Hilker, 2006). Water stress is an important moderator for plant-insect interations, as members of the Pinaceae stressed by drought have shown increased susceptibility to attack from both endophytic (Gaylord *et al.*, 2013) and epiphytic (Jactel *et al.*, 2012) herbivores. The production of monoterpenes in response to soil waterlogging is less well studied, though there is evidence that waterlogging may not drive variation in foliar monoterpenes (Kainulainen *et al.*, 1992).

Eastern larch, *Larix laricina* (Du Roi) K. Koch (Pinaceae), is a deciduous conifer distributed throughout the boreal forest of North America (Burns & Honkala, 1990). In Minnesota, USA, eastern larch occurs in bogs, conifer swamps, and/or fens. Each wetland exhibits distinct hydrology that influences the nutritional status of needles (Tilton, 1978) and growth of resident larches (Girardin *et al.*, 2001). Seasonally flooded sites that eastern larch inhabits are nutrient-poor, and eastern larch is often unable to establish on upland sites that are highly suitable for growth due to interspecific competition (Duncan, 1954). While drought and flooding moderate survival and growth of eastern larch (Duncan, 1954), the role of water stress in defense chemistry is not well understood. Foliar monoterpenes of eastern larch include α-pinene, β-pinene, β-phellandrene, camphene, Δ-3-carene, limonene, myrcene, limonene, sabinene, and terpinolene, many of which are influenced by time of the growing season, soil nutrients, and/or shoot type (Powell & Raffa, 1999a). Given the plasticity of monoterpene profiles of eastern larch and the changes in growth and nutrient production by larch in response to site-specific hydrology, water stress may facilitate changes in defense chemistry and, thus, interactions of eastern larch with its herbivores.

Larch casebearer, *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae), is a needle-miner native to Europe that was detected near Northampton, Massachusetts in the late 1800s attacking planted European larch, *Larix decidua* Mill. (Hagen, 1886). The insect subsequently spread throughout most of the range of eastern larch and, following its establishment in Idaho in 1957 (Tunnock & Ryan, 1985), spread throughout most of the range of western larch. Moths of larch casebearer oviposit eggs individually onto larch needles (Herrick, 1911). Larvae hatch through the base of eggs into the needle where they mine for the first and second instars (Herrick, 1911; Thorpe, 1933). In late autumn during the second instar, larvae clip off the mined needle, line it with silk, and carry it as a case (Herrick, 1911). From the casebearing stage onwards, larvae feed by attaching their case to a needle and then mining in one or both directions without completely leaving their case. Larvae overwinter as third instars inside their cases attached with a silken plug to twigs on the host tree (Ryan, 1975). In spring, larvae molt into the fourth instar and then resume feeding (Herrick, 1911).

Spring-feeding larvae, which are required to activate after host trees flush new needles, are the most damaging part of the insect's life cycle (Thorpe, 1933), and defoliation may decrease growth of host trees (Tunnock *et al.*, 1969), predispose trees to attack from fungi or secondary agents (Tunnock *et al.*, 1969; Benoit & Blais, 1988), and/or kill trees when repeated annually (Craighead, 1950; Dowden, 1957). Pupation occurs in the case, which is again attached to host foliage, and adults emerge in late spring through summer (Herrick, 1911). An importation biological control program was established in the 1930s in eastern larch forests and 1960s in western larch forests and was eventually considered successful in both regions. Control has been attributed to two introduced parasitoids: *Agathis pumila* Ratzeburg (Hymenoptera: Braconidae) and *Chrysocharis laricinellae* Ratzeburg (Hymenoptera: Eulophidae) (Graham, 1948; Webb & Quednau, 1971; Otvos & Quednau, 1981; Ryan, 1990, 1997).

Larch casebearer has been undergoing a large-scale outbreak in Minnesota over the past 15 years. From the mid-1950s, when aerial sketch map surveys were first conducted in Minnesota, to 1999, no defoliation was detected, despite the presence of the introduced wasps (Cody *et al.*, 1967). Since 2000, thousands of acres of defoliation have been detected each year. Changes in plant defense driven by water stress may enable outbreaks of forest insects (Hart *et al.*, 2014). Given that mean annual precipitation in Minnesota has increased significantly across the previous century (Novotny & Stefan, 2007), we investigated the potential for both waterlogging and drought in spring to moderate interactions between eastern larch and larch casebearer. We elucidated the role of both forms of water stress in the growth and defense of juvenile eastern larch trees by quantifying the responses of trees to various watering regimens and challenge from larch casebearer. We also investigated the survival to adulthood of larvae in response to watering regimen and plant defense chemistry.

Materials and Methods

Eastern larch

Two studies were conducted: one in spring 2015 and the other in spring 2016. Three-year-old trees of eastern larch, referred to as juvenile trees interchangeably hereafter, were obtained on April 30, 2015 from the Minnesota State Forest Nursery in Badoura, Minnesota. The juvenile trees were bare-root lifted and shipped to the University of Minnesota, Saint Paul and stored at 4 °C overnight. Trees were potted the day after arrival in 10.2 cm × 24.1 cm pots (TP49, Stuewe & Sons, Tangent, Oregon) using Sunshine Mix #8 (Sun Gro® Horticulture, Agawam, MA) that contains Canadian sphagnum peat moss, perlite, vermiculite, starter nutrient charge (with gypsum), and dolomitic limestone. Seven grams of fertilizer (Vigoro® 16:4:8 NPK) with time-released nitrogen were added to the top of each pot and holes on the bottom of pots were left open, permitting drainage. Trees were stored in the greenhouse and watered with 300 ml of tap water twice daily for ten days. Following the ten-day period, trees were moved outside into a wooden, roofless cold frame on the north side of a greenhouse on the University of Minnesota, Saint Paul campus (44.9886 °N, 93.1802 °W), where both studies were conducted. We randomly selected 128 trees for the 2015 study and 88 trees for the 2016 study. All trees used were 3 or 4 years old in 2015 and 2016, respectively. Trees selected for use in 2016 were watered 2-3 times per week in 2015 until dormancy, at which point trees were stored above ground in the cold frame until use in 2016.

2015 study

Ten days after potting, 96 trees were assigned to one of six treatments involving variations of watering level and challenge by larch casebearer. Treatments were arranged in a factorial design with three watering levels (twice per day, once per day, and once per week) either with or without larvae. Trees treated with larvae had 15 larvae placed randomly onto foliage and each combination of treatments had 14-18 trees. Watering regimens were selected to mimic waterlogged soil, such as in a spring-flooded site, to extreme drought. All trees were watered the first day they were assigned to treatments and the watering regimens continued until June 5 2015, at which point all trees were watered 2-3 times per week until dormancy. Each bout of watering consisted of adding 300 ml of water to the topsoil. An additional 32 trees were watered once per day and had either 25% of the foliage removed (16 trees) or 75% of the foliage removed (16 trees). These mechanical defoliation treatments occurred on the first day of the study (i.e., 10 d after potting) and were achieved by clipping off the entire fascicle of needles for one out of four or three out of four fascicles with scissors, respectively. Trees were placed in baskets in groups of four such that in each basket, all trees were either challenged or not challenged by larch casebearer to prevent contamination of larvae-free trees. Baskets were randomly assigned to locations within the wooden frame and treatments aside from larval presence/absence were assigned randomly to baskets. Pots were left open, permitting ambient rainfall. Daily precipitation data recorded at a weather station (ID: USC00218450) within 0.5 km of our study site are provided in Figure 2.1a. Weather station data were obtained from the National Oceanic and Atmospheric Administration (NOAA, 2017). To quantify impacts of treatments on growth of eastern larch, the diameter of each tree was measured at 5 cm above the root collar prior to any experimental treatment and again in November after trees were dormant. Measurement location was marked with a paint pen to reduce measurement error. Tree survival was monitored weekly until November, at which point a “1” (survived until dormancy) or “0” (died during the growing season) was recorded for each tree.

Overwintering larch casebearers were collected by clipping infested twigs from eastern larch trees on April 15, 2015 from a site near Jacobson, MN (46.9982 °N, 93.1073 °W) for use in treatments involving challenge by larvae. Twigs with insects were stored at 6 °C (14L:10D) until use. Five days before the start of experiment, twigs with overwintering 3rd instars attached were placed inside plastic containers (width: 14 cm, depth: 10 cm, length: 26 cm) with mesh covers and into a growth chamber at 22 °C (14L:10D) to encourage activation. Active larvae were collected with a forceps from containers for use and were not fed prior to assays. Two days after the commencement of watering treatments, fifteen larvae were added to foliage on each of the trees selected for herbivory. Care was taken to distribute larvae homogenously throughout the canopy.

Larval counts were conducted the following day and, in rare circumstances where less than 15 larvae were detected, additional larvae were added to achieve a total of 15 per plant. Larvae were again counted 9 days after initial placement, at which point trees had a range of 7 to 15 larvae per tree (mean = 11.46, SE = 0.29). Larvae were left on trees until June 5, upon which all detected larvae were removed. The watering regimen continued until July 2, 2015, after which trees were watered 2-3 times per week. Needles were collected from each tree 24 h, 1 wk, and 3 wk after inoculation with larvae for quantification of monoterpenes (see *Needle analyses* below). The timeframe was selected based on previous studies of monoterpene production in juvenile eastern larch trees in response to fertilization (Powell & Raffa, 1999a). During each collection, six to ten needles were collected arbitrarily collected per tree, including needles with and without larval feeding damage as we were interested in a tree-wide response. Needles were placed into a zip-lock bag and onto dry ice immediately after collection and stored at -20 °C until analysis.

2016 study

The approach to watering in the 2015 study resulted in both waterlogging and mild drought treatments. In 2016, we altered watering treatments to examine drought more closely and modified our treatment density of larch casebearer larvae from presence/absence to a gradient of densities to gain insight on potential thresholds in the response of eastern larch to herbivory by larch casebearer. We also focused on quantifying the ability of larch casebearer to survive to adulthood in response to water stress and plant defense chemistry. On April 22, 2016, the 88 trees selected in May 2015 were assigned to treatments as follows: 24 trees were watered once per day, 40 trees were watered once per week, and 24 trees were watered once every two weeks. The watering regimen continued until May 26, at which point all trees were watered 2-3 times per week until dormancy. During each watering bout, trees were again provided 300 ml of tap water. Control trees appeared healthy throughout, and upon uprooting trees in 2017 after the experiment, there was sufficient space for continued root growth suggesting pots were not a source of tree stress across either year. Each pot was fit with a sleeve made from low density polyethylene tubing (diameter: 35.6 cm, thickness: 0.15 mm, ULINE®) to prevent rainfall from entering the pots. Sleeves were fit around the outside of the pot extending above the tip of the pot. The top of the sleeve was folded over the pot, around the stem of the tree, and secured with two small binder clips to prevent ambient rainfall from entering the pot aside from a small slit around the stem of the tree. See Figure 2.1b for precipitation at the study site in 2016.

Three to six trees per watering regimen were assigned to one of five levels of larval densities: 0, 5, 15, 30, and 50 larvae. The 7 d watering regimen with no larvae had an additional 16 trees. Larvae used in the 2016 study were collected on March 24, 2016 from the same site as in 2015 and stored as in 2015 until use. Larvae were placed onto trees in increments of five between April 22 and April 26, 2016, such that all trees selected for larval treatments would have five larvae before commencing placement of the next increment of five onto the trees. This process was repeated until all trees had their assigned number of larvae, and prevented any trees from receiving 50 larvae before another receive its first five. Two to four trees were randomly assigned to baskets except that trees within a basket had identical larval densities. Baskets location in the cold frame was assigned randomly. Following the same protocol as the 2015 study, the diameter and survival of each tree was measured at the start of the study and again after the onset of dormancy in late autumn.

We did not quantify temporal changes in needle chemistry in 2016. Thus, needle samples were collected on a single occasion (May 26, 2016, ~ 4 weeks after following challenge with larch casebearer), reflective of the timing of the final needle collection in 2015 (~ 3 weeks after challenge with larch casebearer). Needle collection protocol was further altered in 2016 to reduce variation and isolate the effects herbivory. Phytochemistry of eastern larch is moderated by shoot type (Powell & Raffa, 1999a), so samples were only taken from spur shoots on the main stem. Six to ten needles were arbitrarily selected from each of three fascicles on the main stem. Needles were placed into a zip-lock bag, onto dry ice immediately after collection, and stored at -20 °C until analysis. Both fascicles and needles with and without herbivory were selected, as we were interested in examining a tree-wide response, rather than localized induction. The watering regimen was continued until June 22, 2016, at which point all trees were watered two to three times per week.

Out of the trees originally selected to be challenged with larch casebearer, 36 were randomly selected, nine per level of larval density, to be fit with a cage on the top 25 cm of the tree terminal. Five larvae were placed into each cage. The five larvae included in the cage were part of the total number of larvae on the tree (e.g., a tree with 30 larvae would have 25 larvae outside of the cage and 5 inside the cage). Cages were constructed of low density polyethylene tubing (diameter: 12.7 cm, , thickness: 0.15 mm, ULINE®) with one side cut out and replaced with fine mesh for ventilation. For trees with caged terminals, the first five larvae placed onto the tree were always placed inside the cage. Moth emergence was monitored weekly and final counts of moth emergence were conducted on June 21, 2016, after which the terminal cages were removed. During cage removal, no living larvae were observed and all adults and pupae were placed into vials, stored at room temperature, and monitored for further emergence. Total number of moths produced per tree was recorded. Larvae not caged onto the terminal were not tracked or removed during the study, as defoliation was used as a proxy for larval density (see below). Survival to adulthood by larvae was only investigated in 2016.

Needle analyses

Needles analyses were conducted from autumn 2016 through spring 2017. Processing and analysis of needle samples followed a similar protocol to Rosenberger et al. (2017). All samples were stored at -20 °C until processing. Prior to extraction, needles were finely chopped with a small knife and the knife blade was washed with methanol between samples. Needle constituents were extracted twice with 0.75 mL (1.5 mL total) of hexane for 24 hours per extraction in a 2 mL vial at room temperature. Hexane was removed from the sample after each extraction with a 3 mL syringe. The resulting 1.5 ml extractant was passed through a 0.45 μm polyvinylidene fluoride syringe filter (Analytical Sales and Services Inc, Pompton Plains, NJ) in preparation for analysis via gas chromatography mass spectrometry (GCMS). For each sample, we pipetted 195 µl of the extractant and 5 µl of heptyl acetate (internal standard; final concentration of 0.025 mM) into 200 µl glass inserts, which were placed into 2 ml GCMS vials. The extracted needle sample was dried in a fume hood for 1 week at room temperature, weighed, and used to normalize the concentrations of organic extracts. For the 2015 study, all needles collected were used during extractions, whereas in 2016, a random sample from collected needles was used. GCMS analysis was carried out using a Shimadzu QP2010S equipped with a Restek Rxi-5ms column (30 m x 0.25 mm) using Helium as the carrier gas at a column flow rate of 0.60 mL/min. Initial oven temperature was 55 °C and the injection temperature was 220 °C. This temperature was held for 5 min, ramped to 70 °C at 1 °C per minute, and then ramped to 160 °C at 15 °C per minute where it was held for 2 minutes. After those two minutes, the oven was ramped to 250 °C at 30 °C per min and held for 4 minutes. Analytic standards of α-pinene, β-pinene, 3-carene, camphene, limonene, myrcene, sabinene, and terpinolene were used to generate calibration curves and response factors. These titration curves and response factors were used to determine final concentrations (mg of monoterpene per g of needle tissue) and ratios for each compound in the needle extracts.

Verification of experimental conditions

Additional trees were destructively sampled either before the 2015 study (44 trees) or after the 2016 study (twelve trees, treated equivalently to the other 2016 study trees) to develop allometric relationships between tree diameter and below ground biomass, above ground biomass and height. Diameter was measured at 5 cm above the root collar using a digital caliper (EZ CAL Digital Caliper). All soil, debris, and foliage were removed and discarded and the remaining plant material was dried at room temperature for several weeks. Each tree was then clipped at the root collar and remaining material above vs. below the root collar (referred to as above vs below ground biomass, respectively) was weighed to the nearest milligram. Tree height was measured from the root collar and recorded to the nearest half-centimeter. Simple linear regression was used to quantify the explanatory power of tree diameter in below ground biomass, above ground biomass, and tree height. Terms for above and below ground biomass were square root-transformed. Tree diameter was significantly positively correlated with each metric (see Results), and changes in diameter were used as proxy for the response of tree health to treatments.

During the 2016 study (see below), the efficacy of watering regimens was determined by taking soil moisture readings every 4 days between May 7-26 using an Etekcity® moisture meter. The meter recorded moisture levels on a continuous scale from 1-10, with 10 being the wettest measurement. Values were recorded to the nearest half-integer. Soil moisture readings were averaged per tree and an ANOVA and TukeyHSD were used to determine if water regimens resulted in different moisture levels of soils.

On May 26 and 27 2016, percent defoliation was estimated independently using visual estimates by three different people and values were averaged. This was done to confirm larval establishment and determine if variation in larval density was associated with needle consumption at the tree level. When analyzing variation in percent defoliation, a term for larval density was considered as the sole predictor.

Statistical analysis: 2015 study

A linear mixed-effects model was used to examine the tree diameter in November and tree survival as a function of watering regimen, presence/absence of larvae, their interaction, and tree diameter at the start of the study. A term for basket was fit as a random intercept. For comparisons of tree growth between type of challenge (no defoliation of any kind, presence of larch casebearer, 25% mechanical defoliation, and 75% mechanical defoliation), a linear mixed-effects model was again used with a term for basket fit as a random intercept. A term for tree diameter at the start of the study was also fit as a predictor, but watering regimen was not, as this analysis was limited to trees watered once per day to isolate the effects of defoliation. The concentration of each monoterpene (mg per g of foliage) was modeled as a function of watering regimen, type of challenge, days since May 11 (the start of the study), and percent increase in tree diameter across the study using a mixed-effects framework with terms for basket and a unique tree identifier fit as a random intercept. Concentrations of each monoterpene were log(y+0.01) transformed prior to analysis.

Statistical analysis: 2016 study

A mixed-effects framework was again used to examine the response of tree growth and tree survival to watering regimen, challenge from larch casebearer larvae, and their interaction as well as tree diameter at the start of the study with basket fit as a random (varying) intercept. In 2016, trees were challenged with a range of larvae from 0-50 and, thus, challenge from larch casebearer (i.e., larval density) was fit as a continuous predictor.

Moth emergence (percent emergence of moths out of five per cage) was modeled as a function of watering regimen, larval density, and concentration of each monoterpene using multiple logistic regression. A mixed effects framework was not used for models of moth emergence as only nine trees co-occurred in a basket with another caged tree (i.e., trees were randomly distributed throughout the study area). The concentration of each monoterpene (mg per g of foliage) was modeled as a function of watering regimen, larval density, and percent increase in tree diameter across the study using a mixed-effects framework with a term for basket fit as random intercept. All analyses were completed using R (R Core Team, 2017) and $\alpha = 0.05$ was used to define statistical significance. Mixed-effects models were implemented using the lme4 package (Bates *et al.*, 2013) and degrees of freedom were estimated using Satterthwaite's approximation via the lmerTest package (Kuznetsova *et al.*, 2014). For variable selection in multiple regression models, we used an iterative backwards selection procedure of candidate predictors until only significant predictors remained. Where significant differences occurred between levels of a category and variables had more than two levels, means were compared using Tukey's HSD test via the glht command in the multcomp package (Hothorn *et al.*, 2008). Model assumptions of heteroscedasticity were checked via graphical inspection of residuals and assumptions of equal variances were assessed using Levene's test implemented via the car package (Fox & Weisberg, 2011).

Results

Verification of experimental conditions

For the trees that were destructively sampled in 2015 and 2016 to develop allometric equations, tree diameter was positively correlated with both below ($F_{1,54}$ = 1093.48, $P < 0.0001$, Figure 2.2a) and above ground biomass ($F_{1,54}$ = 3011.31, $P <$ 0.0001, Figure 2.2b). Additionally, tree diameter was positively correlated with tree height such that a 1 mm increase in diameter was associated with a 10.5 cm increase in tree height ($F_{1,54}$ = 625.04, $P < 0.0001$, Figure 2.2c).

Watering regimens moderated soil moisture content. Thus, the ranges of watering regimen and larval densities were successful in providing varying amounts of stress to trees. On a scale of 1 to 10, soil receiving water daily was significantly higher in moisture readings by 3.41 (± 0.34 SE) points (TukeyHSD, $Z = 10.07$, $P < 0.0001$) and 4.97 (± 0.34

SE) points (TukeyHSD, $Z = 14.68$, $P < 0.0001$) than soil watered once per week and twice per month, respectively (Figure 2.3). Soil watered once per week was 1.56 (± 0.34 SE) points higher in moisture than soil watered twice per month (TukeyHSD, $Z = 4.61$, $P < 0.0001$). Higher densities of larch casebearers were associated with increased defoliation such that an increase of one in the number of larvae placed on a tree was associated with a 0.89% (± 0.10 SE) increase in visually estimated defoliation ($t_{34} = 8.64$, $P < 0.0001$, Figure 2.4).

2015 study

Average diameter of trees at the start of the 2015 study was 2.94 mm (± 0.04 SE) at 5 cm above the root collar. The initial stem diameter of trees accounted for significant variation in tree diameter measured in autumn, demonstrating the presence of variable growth rates across size classes ($t_{79} = 4.79$, $P < 0.0001$). After adjusting for initial diameter and the presence of larch casebearer (i.e., assessing a marginal fit), high frequency watering of at least once per day negatively impacted tree growth. For example, trees watered once per week increased 0.98 mm (± 0.31 SE) (TukeyHSD, $Z = 3.21$, $P = 0.0038$) and 1.42 mm (± 0.33 SE) (TukeyHSD, $Z = 4.35$, $P < 0.0001$) in diameter more than trees watered once per day or twice per day, respectively. There were no differences in growth between trees watered once per day and twice per day.

Challenge by larch casebearer significantly impacted tree growth, as the presence of larvae was associated with a 1.08 mm (± 0.34 SE) decrease in diameter (TukeyHSD, $Z = -3.20$, $P = 0.0034$). Watering regimen did not appear to moderate the effects of larch casebearer on tree growth, as there was no statistically significant interaction between watering regimen and the presence of larch casebearer. Neither watering regimen nor the presence of larch casebearer impacted tree survival, as no models of tree mortality yielded significant trends. However, only three trees (~2.3%) died throughout the course of the study and each was challenged with larch casebearer and watered once or more daily (i.e., two variables associated with decreased growth). Model coefficients and summary statistics are provided in Table 2.1.

Comparisons of mechanical defoliation to trees challenged with larch casebearers (which only included trees watered once per day) suggested that herbivory by larch casebearer was equivalent to removing 75% of the foliage (TukeyHSD, $Z = 0.81$, $P = 0.85$) in terms of tree growth. Presence of larch casebearer and removal of 75% of the foliage were associated with a 1.52 mm (± 0.42 SE) (TukeyHSD, $Z = -3.66$, $P = 0.0015$) and 1.17 mm (± 0.40 SE) (TukeyHSD, $Z = -2.92$, $P = 0.0184$) decrease in growth compared to controls, respectively. Similar findings emerged from comparisons of 25% mechanical defoliation compared to both the presence of larch casebearer and 75% mechanical defoliation (results not shown). The impacts of removing 25% of the foliage on tree diameter was not significantly different from controls, which were free any defoliation (TukeyHSD, $Z = 0.08$, $P = 0.99$). Model coefficients and summary statistics are provided in Table 2.1.

Trees of eastern larch did not appear to invest in monoterpene production in response to watering regimen or any type of challenge. Similarly, there was no evidence for a tradeoff between growth and investment in defense, as no significant relationships were detected between any monoterpene and percent increase in tree diameter. However, α-pinene, β-pinene, camphene, 3-carene, sabinene, and terpinolene decreased nonlinearly across the study period (Table 2.2, Figure 2.5). Patterns in monoterpene production may have been driven by a few select individuals with significantly high concentrations of a given monoterpene at the onset of the study, as several concentrations for individual plants appeared to be static through time (Figure 2.5). Neither limonene nor myrcene were associated with experimental treatments or changed significantly in concentration through time.

2016 study

In 2016, the eighty-eight trees were four years old and, at the start of the study in April, had an average diameter of 6.85 mm (± 0.13) at 5 cm above the root collar. Tree growth was again a function of watering regimen and initial diameter (Table 2.3). Initial tree diameter was again significant in explaining variation in final tree diameter ($t_{82} = 11.81$, $P < 0.0001$). The watering regimen differed from the 2015 study, as the largest interval between watering bouts was 14 d in 2016 compared to 7 d in 2015, the smallest interval between watering bouts was 1 d in 2016 compared to twice daily in 2015, and ambient rainfall was excluded in 2016. Thus, formal statistical comparisons between years were not conducted.

Qualitatively, tree growth in 2015 was decreased by waterlogging whereas in 2016 growth was hindered by drought. After accounting for initial tree diameter and larval density (i.e., assessing a marginal fit), trees watered every 14 d grew 0.58 mm (± 0.23 SE) less than trees watered every 7 d (TukeyHSD, $Z = -2.49$, $P = 0.0341$) and 0.71 mm (± 0.24 SE) less than those watered once per day (TukeyHSD, $Z = -2.93$, $P = 0.0094$). Contrary to the 2015 study, trees watered at 7 d intervals did not grow significantly less than those watered daily (TukeyHSD, $Z = 0.54$, $P = 0.85$). Tree growth was generally suppressed in response to increasing densities of larch casebearer, although the relationship was parabolic such that a density of 50 larvae per tree was associated with a slight increase in growth (Table 2.3). The effect of larch casebearer on tree growth was not moderated by watering regimen. That is, no statistically significant interaction between type of challenge and watering regimen was detected (results not shown).

Tree mortality was moderated by watering regimen and density of larch casebearer. The effect of watering regimen on tree mortality mirrored the effects of watering on tree growth such that trees watered at 14 d intervals were significantly more likely to die compared to trees watered daily (TukeyHSD, $Z = 2.61$, $P = 0.0230$). Trees watered at 7 d intervals were less likely to die than those watered at 14 d intervals, though this difference was not statistically significant (TukeyHSD, $Z = 2.14$, $P = 0.08$). There was also no statistically significant difference in expected mortality between trees watered every 7 d and those watered daily (TukeyHSD, $Z = 1.33$, $P = 0.37$). Defoliation was also stressful for trees, as increasing densities of larch caseberer were associated with a lower probability of tree survival ($Z = -2.00$, $P = 0.0456$).

Moth emergence by larch casebearer did not appear to be moderated by plant defense chemistry, as moth emergence was not associated with concentrations of monoterpenes. Watering level was also not associated with moth emergence. Indirect competition did not appear important either, as moth emergence did not vary with changes in larval densities. Across all treatments, average number of moths detected per cage was 1.6, equivalent to 30% survival. Lastly, the defense chemistry did not respond strongly to larval density or watering regimen with the exception of myrcene, which decreased significantly in response to increasing densities of larch casebearer ($t_{32} = -2.51$, *P* = 0.0173). Otherwise, all other monoterpenes were not statistically associated with percent increase in tree diameter, watering regimen or density of larch casebearer.

Discussion

Given the resurgence of larch casebearer and increases in precipitation in Minnesota, we aimed to elucidate the role of water stress in eastern larch-larch casebearer interactions. We found that drought, waterlogging, and feeding by larch casebearer act independently to decrease growth and survival of juvenile eastern larch. Furthermore, our study suggested that neither watering regimen nor challenge from larch casebearer influence the production of monoterpenes by eastern larch. Volatilization of monoterpenes was not measured and could have led to a failure to detect increases in concentrations (Litvak & Monson, 1998). Regardless, analysis of moth emergence suggested that monoterpenes do not enhance or hinder survival of larvae or pupae. It is possible that eastern larch does not invest significant resources in monoterpene production, as other defense compounds may be more important for combatting herbivores. For example, high concentrations of monoterpenes present in eastern larch did not influence performance of the gypsy moth *Lymantria dispar dispar* Linnaeus (Lepidoptera: Erebidae), but diterpene acids produced by eastern larch were strong feeding deterrents (Powell & Raffa, 1999b). Concentrations of other defense chemicals and/or nutrients important to larch casebearer or other feeding guilds on eastern larch may have changed in needles or other plant tissues (Werner, 1995).

Several monoterpenes decreased with time over the first month of the study in 2015 (Figure 2.2) and the concentrations of several monoterpenes likely continued to change as the growing season progressed (Powell & Raffa, 1999a). Decreasing monoterpenes in spring may have implications for spring synchrony between larch casebearer and eastern larch, as larvae that emerge earlier will need to contend with higher concentrations of monoterpenes. However, monoterpenes did not appear detrimental to survival for 4th instar larvae. Several spring-feeding lepidopterans such as gypsy moth and winter moth are susceptible to asynchrony of spring egg hatch and bud burst on some broad-leaf hosts, likely in part due to changes in defense chemicals such as phenolics (Meyer & Montgomery, 1987) and tannins (Feeny, 1970). Similarly, spring-feeding caterpillars such as spruce budworm *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae) and *C. pinus pinus* Freeman that feed on conifers are susceptible to spring asynchrony due to leaf senescence, physical changes in host tissues (e.g., lignification), and changes in foliar constituents (e.g., nutrients, sugars, phenolics, water) (Lawrence *et al.*, 1997; Pureswaran *et al.*, 2015; Régnière & Nealis, 2018). These species all overwinter as eggs or early (first or second) instars. Neonate larvae of the Lepidoptera are likely more susceptible to plant defenses than later instars (Zalucki *et al.*, 2000). Larch casebearer overwinters as a third instar and molts into the fourth instar before resumption of spring feeding and early instar larvae mine inside needles in late summer through autumn (Herrick, 1911; Thorpe, 1933). Thus, susceptible larvae may succumb to the defense system of larches in the first or second instar, resulting in a more robust cohort, on average, of spring feeding larch casebearers. As a result, the spring-feeding casebearers may be less influenced by changes in plant defense.

Both simulated waterlogging (2015 study) and drought (2016 study) in spring significantly decreased the growth of eastern larch. Throughout its expansive range, eastern larch experiences extreme variation in hydrology, occurring on both well-drained upland sites as well as bogs and fens prone to flooding (Duncan, 1954). The proportion of the plant subjected to flooding appears important for eastern larch, as complete submergence in water for three weeks caused greater than 90% mortality but flooding of the root system did not (Duncan, 1954). Our study suggested that decreased growth may result from waterlogged soil, although tree mortality was observed in 2016 in response to drought stress. It appeared that watering trees once per day to once per week was optimal for growth. However, in 2015, trees watered daily grew less than those watered weekly, whereas as no such trend was observed in 2016. Trees in 2016 were a year older, had larger diameters on average, and likely had greater above-ground biomass (Figure 2.1b). Thus, larger trees in 2016 may have facilitated faster depletion of soil water compared to 2015, and potentially decreased stress due to waterlogging. Moreover, trees in 2015 were not fit with a sleeve and so ambient rainfall could exacerbate stress from waterlogging and/or decreased stress from drought.

Challenge from larch casebearer significantly impacted tree growth in both 2015 and 2016. In 2015, the growth of larch trees was significantly impacted by the presence of larch casebearer (fifteen larvae per tree). Growth of trees in 2016 decreased with increasing density of larvae until the 50 larval increment, at which point trees appeared to increase diameter. Detection of a parabolic relationship between growth and larval density may be due to compensatory growth at intermediate levels of defoliation (25-50%), as observed in a congener of eastern larch being mechanically defoliated (Vanderklein & Reich, 1999). However, such a relationship may be a statistical artifact, as removing two potential leverage points from the analysis resulted in the term for larval density dropping from the model. Decreases in tree diameter may have occurred over short time scales that were not detectable using growth measured at the start and end of the growing season. Our data were not consistent with the growth-differentiation balance hypothesis (Herms & Mattson, 1992), as no tradeoff was detected between radial growth and production of monoterpenes. Again, trees were only measured before the study and after dormancy, and it is possible that a short term tradeoff was overlooked due to timescale of our diameter measurements.

Changing patterns of precipitation are expected under global climate change (Trenberth, 2011). Significant deviations from historical precipitation patterns may lead to changes in water stress (i.e., drought or waterlogging) for plants (Hanson & Weltzin, 2000; Kreuzwieser & Gessler, 2010) and facilitate changes in plant-insect interactions (Mattson & Haack, 1987). Our study did not provide evidence that recent, high precipitation in Minnesota has influenced populations of larch casebearer or the production of monoterpenes. There are several other ways in which altered precipitation regimens could impact larch casebearer – eastern larch interactions that were not quantified. Changes in other foliar constituents (e.g., other defense compounds, nutrients) may be observed (Tyrrell & Boerner, 1987) and altered volatile profiles of host trees may moderate attraction of native and introduced natural enemies (e.g., Mumm and Hilker 2006). Abundances of larch needle pathogens are also moderated by precipitation patterns (Hagle, 2004a, 2004b), and may present intense interspecific competition for foliage. Nonetheless, our study demonstrated that decreased provisions of water can cause significant growth loss and mortality for larch trees and that challenge from larch casebearer may impact growth additively. Thus, changes in precipitation that moderate water provisions to eastern larch may have implications for the establishment and growth of juvenile eastern larch, particularly when those trees are challenged with larch casebearer.

Acknowledgements

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Tables

Table 2.1 Summary statistics for mixed effects models of tree diameter for juvenile eastern larch trees at the end of the growing season for 2015. Predictors considered in model 1 were variables for tree diameter at the start of the study, density of larch casebearer larvae (0 or 15 per tree), and watering regimen (providing 300 ml of water twice per day, once per day or once per week). For model 2, all trees were watered once daily and predictors considered were tree diameter at the start of the study and presence/absence of larch casebearer larvae. Both models included a term for basket fit as a random intercept, and model 2 assumed a logit link and binomial error structure.

Response	Predictor	Estimate	SE	|*t*|	df[a]	*P*
1. Tree diameter	Intercept[b]	1.77	0.85	2.08	84	0.0408
	Initial diameter	0.29	0.27	4.79	79	<0.0001
	Watering regimen					
	Once per week	1.42	0.33	4.35	83	<0.0001
	Once per day	0.44	0.31	1.39	84	0.17
	Larvae present	-1.08	0.34	3.20	27	0.0034
2. Tree diameter	Intercept[c]	3.40	0.95	3.57	60	0.0007
	Initial diameter	0.98	0.31	3.14	60	0.0026
	Challenge					
	Larvae present	-1.52	0.42	3.66	41	0.0007
	25% defolation	-0.03	0.41	0.08	55	0.93
	75% defolation	-1.17	0.40	2.92	54	0.0051

[a] Degrees of freedom were estimated using Satterthwaite's approximation and rounded down to the nearest integer.

[b] Model reference level was a term for trees without larvae that were watered twice per day.

[c] Model reference level was a term for trees without larvae or mechanical defoliation.

Table 2.2 Summary statistics for mixed effects models of monoterpene concentrations (mg of monoterpene per g of needle tissue) in 2015 on days since onset of treatments (May 11, 2015). Other predictors considered in each model were variables for the increase in tree diameter, presence/absence of larch casebearer, and watering regimen (providing 300 ml of water twice daily, once daily or once weekly). Concentrations were log(y+0.01)-transformed prior to analysis and terms for tree number and basket were fit as random intercepts. Model fits and raw data are presented in Figure 2.5.

Monoterpene	Predictor	Estimate	SE	|*t*|	df[a]	*P*
α-pinene	Intercept	1.008	0.082	12.289	73	<0.0001
	Days	-0.025	0.005	-4.900	182	<0.0001
β-pinene	Intercept	0.369	0.081	4.577	94	<0.0001
	Days	-0.022	0.005	-4.119	183	<0.0001
camphene	Intercept	-0.902	0.069	-13.079	59	<0.0001
	Days	-0.020	0.004	-5.338	179	<0.0001
3-carene	Intercept	0.781	0.095	8.257	58	<0.0001
	Days	-0.077	0.005	-16.229	180	<0.0001
limonene	Intercept	-2.619	0.085	-30.76	171	<0.0001
	Days	0.006	0.005	1.23	135	0.22
myrcene	Intercept	-1.897	0.116	-16.39	35	<0.0001
	Days	-0.002	0.012	-0.15	43	0.88
sabinene	Intercept	-0.249	0.125	-1.992	117	0.0487
	Days	-0.108	0.009	-12.033	247	<0.0001
terpinolene	Intercept	-2.364	0.078	-30.325	50	<0.0001
	Days	-0.056	0.004	-12.424	149	<0.0001

[a] Degrees of freedom were estimated using Satterthwaite's approximation and rounded down to the nearest integer.

Table 2.3 Summary statistics for mixed effects models of the increase in tree diameter for juvenile eastern larch trees and tree survival for 2016. Predictors considered in models 1 and 2 were variables for tree diameter at the start of the study, density of larch casebearer larvae (0-50 per tree), and watering regimen (providing 300 ml of water once per day, once per week or twice per month). For model 2, we used a logit link with a binomial error structure.

Response	Predictor	Estimate	SE	Test statistic[a]	*P*
1. Tree diameter	Intercept[b]	2.27	0.64	$t_{82} = 3.57$	0.0006
	Initial diameter	0.97	0.08	$t_{82} = 11.81$	<0.0001
	Watering regimen				
	Once per week	-0.13	0.24	$t_{82} = -0.54$	0.59
	Twice per month	-0.71	0.24	$t_{82} = -2.93$	0.0043
	Larval density	-0.04	0.02	$t_{82} =-2.30$	0.0237
	Larval density^2	0.001	0.0003	$t_{82} =2.21$	0.0298
2. Tree survival	Intercept[b]	4.11	1.17	$Z = 3.50$	0.0005
	Watering regimen				
	Once per week	-1.53	1.15	$Z = -1.33$	0.18
	Twice per month	-2.93	1.12	$Z = -2.61$	0.0090
	Larval density	-0.03	0.02	$Z = -2.00$	0.0456

[a] Degrees of freedom for *t*-values were estimated using Satterthwaite's approximation and rounded down to the nearest integer.

[b] Model reference level was a term for trees watered once per day.

Figures

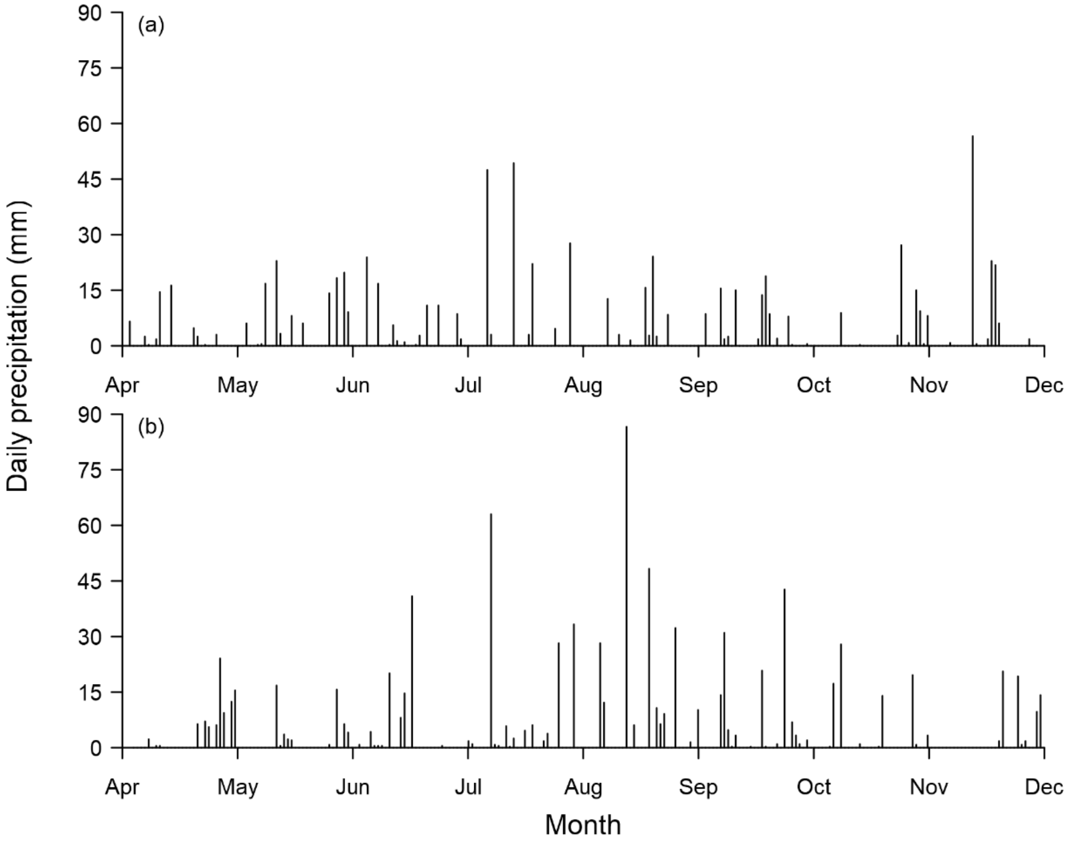

Figure 2.1 Daily precipitation data during our study from April 1 – December 1 in (a) 2015 and (b) 2016. Data were obtained from a weather station (ID: USC00218450) located within 0.5 km of our study site (44.9886 °N, 93.1802 °W).

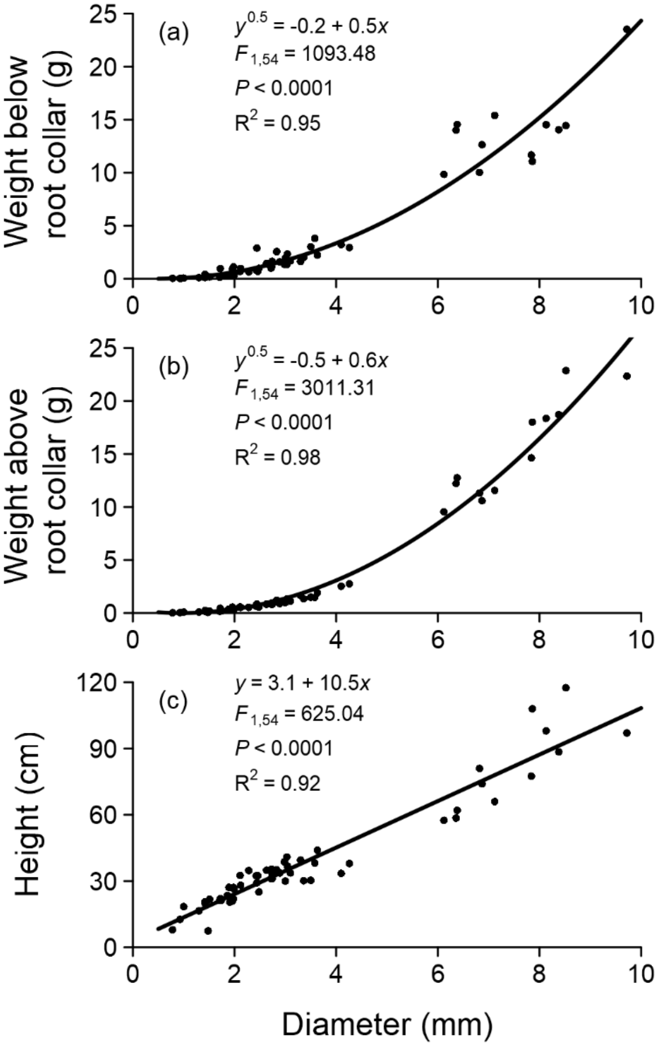

Figure 2.2 Utility of tree diameter as a proxy for overall tree growth for *Larix laricina*. Relationship of tree diameter measured 5 cm above the root collar with a) tree biomass below ground (i.e., below the root collar) b) tree biomass above ground (i.e., above the root collar) and c) tree height from the root collar.

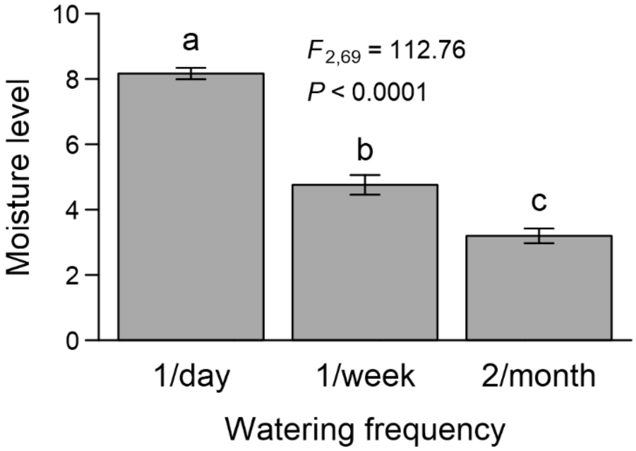

Figure 2.3 Effect of frequency of watering on soil moisture content measured with a moisture meter in 2016. Ten is the wettest value possible, indicating completely saturated soil.

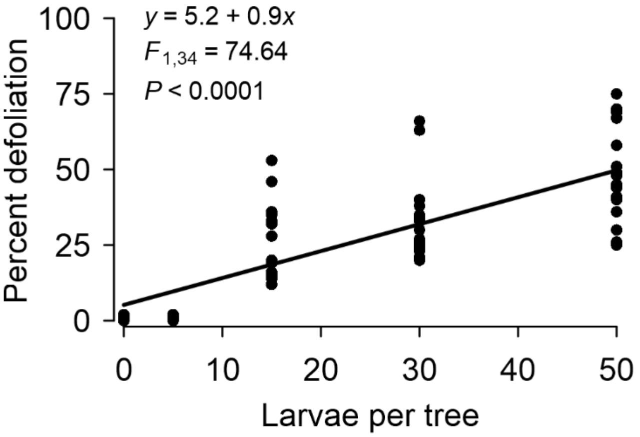

Figure 2.4 Number of larvae of larch casebearer added per tree vs. percent defoliation.

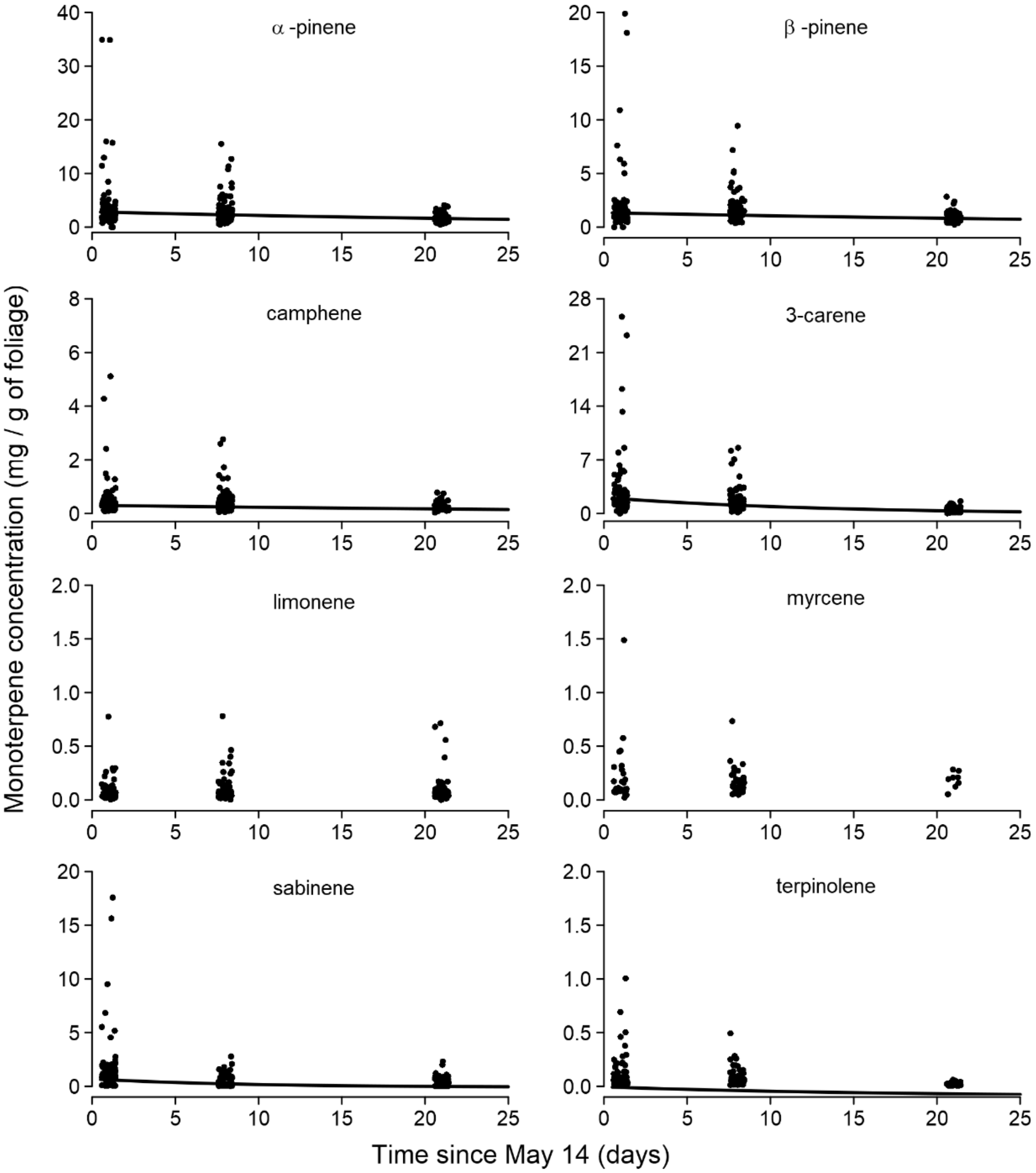

Figure 2.5 Relationship of several monoterpenes across time (May 14 – June 5, 2015) for eastern larch. See Table 2.2 for summary statistics for each linear model.

Chapter 3. Cold tolerance of the invasive larch casebearer and implications to invasion success

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Preface

Larch casebearer became established in North America in the late 1800s. Cold temperatures have been implicated in restricting the insect's range, yet the cold hardiness of larch casebearer has never been quantified. Motivated by a resurgence of larch casebearer in Minnesota since 2000, we investigated 1) mortality from acute and prolonged exposure to sub-zero temperatures and 2) freezing (i.e., supercooling) of larvae in September through April in 2015-16 and 2016-2017. We developed models linking exposure temperatures to proportional mortality and evaluated them using larvae exposed to field conditions. Finally, historical minimum temperature data were used to estimate changes in mortality across time and latitudes. We found that the cold tolerance of larch casebearer changes significantly with season, as both lower lethal temperatures and supercooling points are lowest in mid-winter and highest in spring and autumn. A model predicting survival using supercooling points provided conservative estimates of overwintering survival, as it overestimated survival by 4% and 8% in 2016 and 2017, respectively. Analysis of historical data suggested that overwintering survival has increased significantly over the previous half century.

Introduction

Larch casebearer *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae) is native to Europe, where it defoliates European larch *Larix decidua* Mill. It was first detected in Northampton, Massachusetts in the late 1800s defoliating planted European larch (Hagen, 1886), but eventually spread throughout most of the North American ranges of eastern larch *Larix laricina* (Du Roi) K. Koch and western larch *Larix occidentalis* Nutt. (Denton, 1979; Tunnock & Ryan, 1985). Moths emerge throughout summer and oviposit individual eggs onto larch needles. Larvae mine the needles for the first and second instar, eventually constructing a case by inhabiting the mined needle (Herrick, 1911). After molting into the third instar, larvae attach their case to a twig on the host tree and enter an obligate diapause to overwinter (Ryan, 1975). In spring, larvae molt into the fourth instar, resume feeding, and pupate inside their case attached to host foliage. Larvae enlarge their cases with silk or construct new cases out of needles (Bucheli *et al.*, 2002). Larval feeding in the spring causes the most damage to host trees. Defoliation can decrease growth of host trees (Tunnock *et al.*, 1969), predispose trees to attack from fungi or other secondary agents (Tunnock *et al.*, 1969; Benoit & Blais, 1988), and/or kill trees in multiyear infestations (Craighead, 1950; Dowden, 1957).

Following the invasion of larch casebearer in North America, an importation biological control program was established and deemed a success in both eastern and western larch forests (Graham, 1948; Webb & Quednau, 1971; Otvos & Quednau, 1981; Ryan, 1990, 1997). The success was attributed to *Agathis pumila* Ratzeburg (Hymenoptera: Braconidae) and *Chrysocharis laricinellae* Ratzeburg (Hymenoptera: Eulophidae); both parasitoids appeared to be distributed throughout the entire introduced range of larch caseberaer (Quednau, 1970). In eastern larch forests, *A. pumlia* appearred to be most important control agent (Quednau, 1970). Likewise, field studies in western larch forests identified *A. pumila* as the most important mortality factor for larch casebearer (Ryan, 1986, 1997). Following introductions of natural enemies, defoliation in western larch forests was reduced to sporadic occurrences until 1997 while aerial surveys in eastern larch forests did not detect defoliation by larch casebearer until 2000.

Since 2000, however, up to 30,000 ha of defoliation in Oregon/Washington and approximately 10,000 ha of defoliation in Minnesota have been detected each year (Figure 3.1), with no definitive explanation for the resurgence in insect populations. Cold, wet springs have been implicated as significant mortality factors for larch casebearer, although population regulation in such springs may be due to competition for foliage by fungi rather than temperature (Tunnock & Ryan, 1985). Historically, before the establishment of the parasitoid *A. pumila*, mortality during the needle mining stage and winter disappearance were regarded as the most important events affecting demography (Ryan, 1986, 1990, 1997). These results suggest that mortality from cold temperatures and/or winter predation may play a role in regulating the abundance of insects on the landscape. Cold temperatures, for example, have been implicated in limiting the northern and elevational limits of the invaded range, despite the apparent ability of larch casebearers to survive January temperatures around -40 °C (Shepherd & Ross, 1973). Larch casebearer has not yet been reported on alpine larch *Larix lyallii* Parl., a high elevation larch in western North America, where temperatures at some locations can reach as low as -57 °C (Arno & Habeck, 1972; Tunnock & Ryan, 1985).

Changes in winter temperatures and associated overwintering survival have been implicated in altered patterns of outbreaks and range limits of several insect populations (Crozier, 2003, 2004; Battisti *et al.*, 2005; Jepsen *et al.*, 2008; Hoch *et al.*, 2009; Lesk *et al.*, 2017). Forecasting such changes in survival in response to climate change is important for managing natural resources (Volney & Fleming, 2000; Walther *et al.*, 2002;

Estay *et al.*, 2009). Here, we investigate the lower lethal temperature and supercooling points for active/feeding and overwintering larch casebearer larvae from autumn through spring across two years. The first aim of the study was to quantify changes in the cold tolerance of larch casebearer associated with season and status (active vs. diapausing). We hypothesized that insects would be most cold hardy in mid-winter and the least cold hardy in spring when frosts are expected to cause significant mortality. We also conducted a lower lethal time study to determine if exposure length interacts with temperature to moderate survival. Correlation of temperatures with cold tolerance metrics may provide reliable estimates of distributional limits for select taxa (Andersen *et al.*, 2015). Thus, the final aim was to develop a model linking minimum temperatures to annual survival using laboratory derived cold tolerance metrics. We used this model to reconstruct overwintering survival based on historical climate data across a latitudinal range in Minnesota, USA. We hope that these investigations will provide insight into historical changes in overwintering survival in Minnesota, elucidate the role of overwintering mortality in the resurgence of larch casebearer, and enable forecasting of range limits under global climate change scenarios.

Materials and Methods

Insects

Several collections of larval larch casebearers were conducted from eastern larch at a single site near Jacobson, MN, USA (46.99 °N, 93.11 °W). Unless otherwise specified, all insects assayed originated from the Jacobson site. Larvae were also collected from two additional sites near Floodwood, MN, USA (46.97 °N, 92.99 °W) and McGregor, MN, USA (46.64 °N, 93.32 °W), 10 km and 42 km from Jacobson, MN, respectively. These additional collections were conducted to determine whether cold tolerance metrics of larch casebearer varied among locations. Site locations are depicted in Figure 3.1. Collections and cold tolerance assays were conducted from September to April in 2015-16 and 2016-17.

For assays taking place from September 2015 through October 2016, several collections of overwintering larvae were conducted. “Overwintering” refers to inactive larvae attached to branches via a silken plug and includes insects in diapause and insects that had either recently attached or were close to detaching in autumn and spring, respectively. For overwintering insects, we were not able to confirm an insect was in a true diapause, and thus the term “overwintering” is used in place of diapause throughout. Active, feeding larvae were also collected when available in autumn and spring and refers to insects that had not yet attached to the twig in autumn or that had detached in spring. During the autumn and spring, both overwintering and active larvae co-occurred on larch twigs and were easily differentiated, as the former were attached to foliage and moved when contacted whereas the latter were attached to twigs with a silken plug that completely sealed one end of the case.

For each trial, a minimum of 12 trees were randomly selected from a stand and live twigs with larvae within 2 m of the ground were collected. When active larvae were collected, twigs were placed in 1 L glass jars with 300-500 ml of tap water to prevent desiccation of foliage. Glass jars and twigs were stored outside in a wooden, roofless cold frame on the north side of a greenhouse on the Saint Paul campus of the University of Minnesota (44.99 °N, 93.18 °W). All overwintering insects were left attached to twigs, placed in a well-ventilated plastic container (volume: 17L, 46 × 31.1 × 19.4 cm) inside of the cold frame, and were not removed from twigs until immediately before use in cold tolerance assays. Insects were assayed within a week of the collection date. For assays taking place in or after November 2016, overwintering larvae were collected in early November from 60 trees (two twigs per tree) and stored outside as described above. Thus, for November 2016 onwards, insects were assayed from 1 week to several months after collection.

Lower lethal temperatures

Insects were assayed for lower lethal temperatures every 2-3 months in 2015-2016 and every 2-3 weeks in 2016-2017. For each trial, insects were carefully removed from twigs or foliage using forceps, placed into the bottom of a 1.5 ml microcentrifuge tube, and randomly assigned to an exposure temperature below 0 °C (range: 0 to -60 °C). Each microcentrifuge tube was sealed with a customized plastic dowel fit with an O-ring through which a 0.127-mm-diameter (36 AWG) type-T copper-constantan thermocouple, accurate to ± 0.17 °C, was threaded (Stephens *et al.*, 2015). When active larvae were assayed, the microcentrifuge tubes were fitted with a small piece of cotton to ensure insects remained near the thermocouples. The thermocouple was adjusted to contact the insect in the base of the tube. Sixteen insects were assayed at a time. The entire apparatus was cooled in one of two ways. For the first setup, each apparatus was placed individually into the center of a 20-cm polystyrene cube and into a-80 °C freezer. Polystyrene cubes were calibrated to cool at a rate of -1 °C per minute (Carrillo *et al.*, 2004; Stephens *et al.*, 2015). Under the second setup, each apparatus was placed into a an 18×150-mm (OD×L) Kimax glass test tube inside a cooling bath of silicon 180 oil

(Thermo Fischer Scientific A40, Waltham, MA) and cooled at approximately -1°C per minute (Cira et al. 2016).

Thermocouples were connected to USB-data loggers and temperatures were recorded once per second with TracerDAQ Pro software (Measurement Computing, Bourne, MA). Insects remained at the exposure temperature for <1 sec before being returned to room temperature. During each assay, control insects (*n* = 6-30) were handled equivalently but kept at room temperature. For assays on overwintering insects from October - December 1 in each year, insects were extracted from cases and survival was determined as active movement in response to tactile stimulation via a small brush. Insects assayed between December 1 – April were placed into growth chambers at 20 °C (18L:6D) inside 1.5 ml microcentrifuge tubes with 4-6 holes poked in the lids and survival was determined by observation of active wandering. The -80 °C freezer was used for all insects except for assays conducted in April 2015, as the chiller bath could only cool to -40 °C.

Supercooling points

Supercooling points were collected, on average, during every other lower lethal temperature assay. For supercooling assays, insects were treated equivalently to lower lethal temperature assays but were cooled until an exotherm was observed, indicative of the release of the latent heat of fusion (Carrillo *et al.*, 2004).

Active vs. Overwintering larvae

For assays that occurred in September 2016 and April-May 2016, active larvae were assayed in addition to overwintering larvae. For the assays in April-May 2016, both fed and starved larvae that were active were assayed. For fed larvae, naturally-activated larvae (i.e., larvae that had broken diapause and were actively wandering) were collected from the plastic storage container in the cold frame, placed onto potted eastern larch trees, and assayed after larvae were permitted to feed for a minimum of 24 hours. Only larvae that had attached and were feeding were recollected for assays. For comparison, naturally-activated, unfed larvae from the plastic storage container were collected and assayed the same day as feeding larvae were collected. Starved and fed larvae were assayed simultaneously. In April 2017, Supercooling points of overwintering and active/starved larvae were also investigated.

Supercooling points across sites

To quantify how supercooling points of larch casebearer varies among sites, insects were collected in early November 2016 from near Jacobson, Floodwood, and McGregor, MN, USA. Insects were stored outside of the greenhouse as described above and their supercooling points were measured in January 2017.

Deacclimation

We conducted a study to determine how quickly larch casebearer might de-acclimate from the cold-tolerant overwintering stage. In January 2017, 80 insects were placed individually into microcentrifuge tubes with 4-6 holes poked in each lid. Forty tubes were transferred into a greenhouse (10.5 – 30.3 °C, 12 – 43% RH) and the remaining 40 were placed outside into the plastic storage container (-14.2 – 3.7 °C, 54 – 100% RH). Temperatures and relative humidity in each location were recorded using HOBO Data-loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) and insects were held at natural photoperiods. Supercooling points were measured at 1, 3, 6, 13, and 21 days after placement into the respective locations to determine how quickly insects de-acclimate. A maximum of 16 insects (eight stored in the greenhouse, eight stored outside) was measured per sample day.

Lower lethal time

On December 30, 2016, 72 insects were placed into 1.5 ml microcentrifuge tubes and into freezers held at 2 °C, -2 °C, -23 °C, and -28 °C. Temperatures were again measured using HOBO Data-loggers. Cohorts of 4 to 10 insects from each exposure temperature were removed at 20, 24, and 34 days, placed at 20 °C (18L:6D), and monitored for survival every 2-3 days. The proportion of larvae activating (i.e., surviving) was recorded.

Field evaluation

We investigated the utility of cold tolerance metrics for predicting survival in the field. In 2016, 58 insects were collected from Jacobson in late March and stored outside for between 1 -3 weeks in Saint Paul before being transferred into 1.5 ml microcentrifuge tubes and into a growth chamber (20 °C, 18L:6D). Across April 2017, 29 and 45 insects from the November 2016 collection were transferred into either 1.5 ml microcentrifuge tubes or Petri dishes (9 × 50-mm polystyrene dishes, Falcon Labware, Oxnard, CA, USA), respectively. Tubes and Petri dishes were placed into a growth chamber (20 °C, 18L:6D). In both years, insects were monitored for survival every 2-3 days for several months.

The role of minimum temperatures in survival at the overwintering locations of Jacobson and Saint Paul were investigated as follows. We defined an “insect year” such that October 2015 – April 2016 would be insect year 2016, as mortality in October-December will affect insect pressure in spring the following year. Daily minimum temperatures from weather stations near Floodwood, MN and on the Saint Paul campus of the University of Minnesota were obtained for insect years 2016 and 2017, respectively, from the National Oceanic and Atmospheric Administration (NOAA, 2017). Weather stations were within 20 km of Jacobson and 1 km of Saint Paul. For predictions of survival using lower lethal temperature models, we obtained the absolute minimum temperature during the overwintering stage and the month in which it occurred. The minimum temperature was then entered into the lower lethal temperature model for the appropriate month to generate an estimate of survival. That is, if the coldest day in the insect year occurred in December, then the lower lethal temperature model for December would be used to estimate overwintering survival.

For predictions using supercooling points, we first bootstrapped 1000 samples to obtain estimates of the variance, s_i^2, for each model coefficient ($\hat{\beta}_0, \hat{\beta}_1, \hat{\beta}_2$) from our model employing ordinal day to predict supercooling points (see *Statistical analysis*). Bootstrap estimates of the variance were obtained by resampling residuals via the lm.boot() command in the simpleboot package in R (Peng, 2008). We then obtained the ordinal day on which the coldest temperature occurred in each insect year. The ordinal day (*D*, days since September 1) was then entered into our model and 1000 bootstrapped estimates of the supercooling point were generated using:

$$SCP = \beta_0 + \beta_1 \times D + \beta_2 \times D^2$$

where *SCP* = estimated supercooling point, $\beta_0 \sim N(\hat{\beta}_0, s_0^2)$, $\beta_1 \sim N(\hat{\beta}_1, s_1^2)$, and $\beta_2 \sim N(\hat{\beta}_2, s_2^2)$. Each $\hat{\beta}_i$was extracted from the multiple linear regression of supercooling points on ordinal day. This process was repeated 1000 times and for each iteration the proportion of estimates (i.e., supercooling points) lower than the observed minimum temperature was recorded as proportional survival. Thus, according to our model, if an insect's supercooling point was below the minimum temperature, then that insect survived the winter. Of the 1000 estimates of survival, the highest and lowest 2.5% were removed to obtain a confidence interval.

Trends in predicted overwintering survival

We obtained historical temperature data from 1964-2016 at a weather station in Grand Rapids MN, USA (Figure 3.1). Daily minimum temperatures were obtained from NOAA. For each insect year, the minimum temperature during the insect year and the ordinal day (= days since September 1) on which it occurred were obtained. Mortality per year was then estimated as described for the supercooling point model (see *Field evaluation*), which provided more conservative estimates of cold-driven mortality (see *Results*).

Statistical analysis

To model seasonal changes in cold tolerance, we combined data from both years of the study, split the data by month, and fit logistic regression models relating proportional survival of larvae to exposure temperature. To homogenize the number of insects sampled across time, the February model included insects sampled between February 1-March 3 and the March model included insects assayed between March 4-March 31. A term for year was considered as a predictor in each model but was not included in any final model (all $p > 0.05$). The temperature estimated to kill 50% of the population (LT_{50}) was then estimated for each month using the dose.p() function from the MASS package in R (Venables & Ripley, 2002). To determine how supercooling points changed through time, the effect of ordinal day and year on supercooling points was modeled using multiple linear regression.

For comparisons of lower lethal temperatures by status in autumn, the effects of temperature, status (fed vs. starved), and their interaction on proportional survival was assessed using logistic regression. For spring studies, the effects of temperature, status (fed vs. starved vs. overwintering), and their interaction on proportional survival was again assessed using logistic regression. We implemented TukeyHSDs via the glht() command in the multcomp package in R (Hothorn *et al.*, 2008) to compare lower lethal temperatures between active/fed, active/starved, and overwintering larvae. Differences in supercooling points between overwintering and active/feeding larvae in spring were compared using simple linear regression.

Variation in supercooling points by site was assessed using ANOVA. For analysis on deacclimation, supercooling points were modeled as a function of the interaction between length of storage (days, log-transformed) and storage location using ANCOVA. To determine how exposure time to cold temperatures influenced survival, we investigated the role of days of exposure, temperature, and their interaction in the proportion of larvae activating using logistic regression.

For analyses of historical trends in cold-driven mortality, the role of year in predicted proportional survival at Grand Rapids was modeled using simple linear regression. Proportions were logit transformed. Statistical significance for all models was determined using $\alpha = 0.05$. Analytical assumptions for all general linear models were evaluated via graphical inspection of residuals. All analyses were completed using R (R Core Team, 2017).

Results

Lower lethal temperature

Monthly observations from autumn (i.e., October) to spring (i.e., April) indicated that the lower lethal temperatures decreased going into winter and then increased with the onset of spring. That is, insects were most cold hardy in mid-winter and less cold hardy in autumn and spring. Parameter estimates for each month-specific logistic regression, along with monthly LT_{50} predictions, are provided in Table 3.1. For example, 50% mortality was estimated at temperatures of -28.9 (± 1.77 SE) °C in October, dropped to -40.8° (± 0.77 SE) °C in January, and increased to -27.8 (± 1.00 SE) °C in April (Table 3.1).

Supercooling points

We also found that the freezing point of larvae changes significantly across the overwintering period (Figure 3.2). The relationship was quadratic such that supercooling points were lowest in January and highest in autumn and spring. For example, the mean of supercooling points was -44.3 (± 0.3 SE) °C in January, compared to -36.5 (± 0.6 SE) °C and -30.3 (± 0.7 SE) °C in November and April.

Active vs. overwintering larvae

A change from active feeding to overwintering behavior was associated with increased cold tolerance, as overwintering larvae were more cold tolerant than active larvae despite that insects co-occurred on trees in autumn and spring. In autumn, the LT_{50} for overwintering insects was estimated at -28.6 °C compared to -17.4 °C for larvae still feeding (Figure 3.3a, Table 3.2). In April/May, overwintering larvae were again more cold hardy. Overwintering insects had significantly lower LT_{50} values than active insects that were feeding (TukeyHSD, $Z = 2.50$, $P = 0.0334$) or starved (TukeyHSD, $Z = 2.37$, P

= 0.0471). For example, overwintering insects had a predicted LT_{50} of -28.5 °C compared to -21.0 °C for active and fed larvae (Figure 3.3b, Table 3.2). Insects that were starved and active were not significantly different from insects that were fed, as starved larvae had an LT_{50} of -20.5 °C (TukeyHSD, $Z = -0.146$, $P = 0.99$). Insects that were active and starved in April had a supercooling point of -20.9, or 9.5°C degrees warmer than insects still in the overwintering stage (Figure 3.3c, Table 3.2).

Supercooling points across sites

Supercooling points did not vary significantly among sites ($F_{2,57} = 1.05$, $P = 0.36$). In January, insects collected from the Floodwood, Jacobson, and McGregor sites had mean (± SE) supercooling points of -42.8 (± 0.9 SE) °C, -43.9 (± 0.7 SE) °C, and -44.0 (± 0.4 SE) °C, respectively.

Deacclimation

Overwintering larch casebearers moved into warmer temperatures began to deacclimate one day after transfer (Figure 3.4, Table 3.2). For example, after 24 hours at greenhouse temperatures (10.5 – 30.3 °C), insects in the greenhouse had mean supercooling points of -41.0 °C (± 0.6 SE) compared to -44.9 °C (± 0.7 SE) for insects stored outside. At 21 days, insects in the greenhouse had a predicted mean supercooling point of -30.1 (1.5 ± SE) °C compared to -44.1 (0.5 ± SE) °C for insects stored outside.

Lower lethal time

Neither temperature, exposure time, or their interaction moderated survival. Across all treatments, insect survival was 90.3 ± 3.5 SE %. According to our lower lethal temperature models, the expected survival based on acute exposure to -22 °C and -27 °C in January is 98.9 % (95% CI: 95.3 – 99.8) and 96.8 % (95% CI: 90.4 – 99.0), respectively. Insects exposed to -22 °C and -27 °C for 20-32 days had an observed survival of 93.3 % and 76.9 %, respectively.

Field evaluation

Lower lethal temperature models (see Methods) predicted observed field mortality of insects better than models that incorporated supercooling points. In insect years 2016 and 2017, 94.8 ± 2.9 SE % and 91.9 ± 3.2 SE % of insects survived the winter when stored in the cold frame, respectively. Using lower lethal temperature models, predicted survival based on minimum temperature exposure was 88.1% (95 % CI: 77.1 – 94.2%) in 2016 and 84.7% (95 % CI: 54.7 – 96.2%) in 2017 (Table 3.3). Supercooling point models were more conservative, estimating survival of 98.5% (95 % CI: 97.7 – 99.6 %) in 2016 and 99.9% (95 % CI: 99.6 – 100.0 %) in 2017 (Table 3.3). Thus, these confidence intervals predicted from the lower lethal temperature model fall within one percentage point in 2016 but successfully included observed survival in 2017. These predictions were approximately 5 to 8% higher than the survival we observed, and the observed mortality did not fall within model confidence intervals.

Trends in predicted overwintering survival

Across all years, estimated proportional survival was 94.7 ± 1 SE % at the Grand Rapids weather station. The proportion of larvae estimated to survive winter increased from 1964 to present (Figure 3.5, Table 3.4). For example, on average, 84 % of larvae were estimated to survive minimum temperatures in 1965 compared to an average of 97 % in 2010.

Discussion

Our lower lethal temperature model estimated slightly lower survival than was observed in both years, whereas the supercooling point model overestimated survival by up to 8% (Table 3.3). Insects stored outside and transferred into growth chambers may succumb to other mortality factors (e.g., desiccation) in addition to cold exposure (Danks, 1978). Thus, the model incorporating supercooling or freezing points of insects is likely most useful in estimating mortality due to cold exposure and its use in forecasting will provide conservative estimates of overwintering mortality. Moreover, insect supercooling points did not vary among three sites investigated, as supercooling points were most similar between McGregor and Jacobson (vs. Floodwood), despite Jacobson being ~30 km farther away from McGregor than Floodwood. These findings suggest that our supercooling model may be useful in generating coarse estimates of mortality of larch caseberaer in response to minimum temperatures across northern Minnesota. To that end, predicted overwintering survival increased across our study period of 1964-2016 and suggests that less extreme winters (i.e., those with higher minimum temperatures) may have contributed to the resurgence of larch casebearer around Grand Rapids, Minnesota (Figure 3.5).

The lower lethal temperatures and supercooling points of larch casebearer are lowest during the months of December and January and higher in autumn and spring. These changes appear to be concomitant with transitions from the overwintering to active stage and vice versa, regardless of feeding status (Figure 3.3). Thus, the acquisition and loss of cold tolerance appear to be related to the onset or termination of overwintering behavior (i.e., diapause), respectively, and not the ingestion of plant material. Model predictions using historical data did not account for larvae that may have been active during October and April and may be conservative estimates of population mortality. Linking phenology of insects to cold tolerance may improve model predictions by enabling estimation of percent mortality of both overwintering and actively feeding larvae.

Other mortality factors associated with cold exposure may be important for overwintering survival. For example, the duration of cold exposures (i.e., lower lethal time) may interact with the intensity of cold exposure to moderate survival (Salt, 1961; Nedvěd *et al.*, 1998). Our lower lethal time study suggested that exposure to -27 °C did not kill significantly more insects than exposure to 2 °C. However, the lowest survival was observed at -27 °C and further investigation into lower exposure temperatures may provide insight on the role of exposure time × temperature interactions on survival of larch casebearer. The frequency of cold exposures may also be important (Marshall & Sinclair, 2015), such that locations with greater variance in temperature may result in different patterns of mortality compared to sites with similar mean temperatures but reduced variance. When held at high temperatures above 10 °C, for example, larvae of larch casebearer began to deacclimate within a week (Figure 3.4), although such greenhouse temperatures are not ecologically relevant for January temperatures in Minnesota. Our model did not account for the potential for short exposure to warmer temperatures during the overwintering stage to temporarily elevate lower lethal temperatures, as observed for mountain pine beetle *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae) (Régnière & Bentz, 2007). However, deacclimation by mountain pine beetle in response to warming appears much more rapid than for larch casebearer (Régnière & Bentz, 2007). Nonetheless, due to the potential importance of variance in winter temperatures (e.g, heat waves followed by cold snaps) and duration of exposures for survival of larch casebearer, our model provides conservative estimates for overwintering mortality.

Patterns of cold tolerance displayed by larch casebearer are comparable to defoliators native to northern Minnesota. In their most cold hardy conditions in January, 50% of larch casebearer larvae are expected to survive temperatures down to approximately -40 °C. The supercooling points of overwintering, pharate larvae of forest tent caterpillar *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae) are -40 °C (Uelmen *et al.*, 2016) whereas larvae of spruce budworm *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) supercool to -42 °C in February (Han & Bauce, 1993, 1995). These insects are sympatric with larch casebearer in large areas of its introduced range on eastern larch. Similar seasonal changes in supercooling points of larch casebearer were observed with spruce budworm, as supercooling points were closer to -34 °C in autumn and spring (Han & Bauce, 1995). Thus, the cold tolerance of larch casebearer has likely contributed to its invasive success in this region.

Several factors, including spring phenological synchrony (Visser & Holleman, 2001; Van Asch & Visser, 2007), efficacy of natural enemies (Dwyer *et al.*, 2004), forest structure (Roland, 1993), and interactions therein (Hunter & Elkinton, 2000), may contribute to outbreaks of forest lepidopterans in addition to potential changes in overwintering survival (Neuvonen *et al.*, 1999). Indeed, forecasting the impacts of climate change on insects will require understanding how intra- and interspecific interactions are moderated by changes in annual temperatures (Fleming & Volney, 1995;

Volney & Fleming, 2000; Bentz *et al.*, 2010; Van der Putten *et al.*, 2010). Linking landscape patterns in population dynamics to overwintering survival remains challenging. For example, for two North American *Dendroctonus* spp., predictions of population change in response to changes in overwintering survival are variable (Trân *et al.*, 2007; Sambaraju *et al.*, 2012; Weed *et al.*, 2015). Nonetheless, increased overwintering survival can facilitate shifts in the distribution and abundance of insects and, in turn, influence population growth rates. The model developed here suggests that overwintering survival has contributed to the increase in abundance of larch casebearer around Grand Rapids, Minnesota and will aid in forecasting potential changes in the elevation and latitudinal range limits of this insect.

Acknowledgements

We thank Theresa Cira for training with thermocouples, Aubree Kees for assistance with cold tolerance assays and monitoring insect survival, and members of the Salt Institute at the University of Minnesota for helpful discussions of insect overwintering biology. Funding was provided by USDA Forest Service award 15-DG-1142004-237, the College of Food, Agricultural, and Natural Resource Sciences at the University of Minnesota, Minnesota Agricultural Experiment Station project MIN-17-082, and a University of Minnesota Doctoral Dissertation Fellowship to SF.

Tables

Table 3.1 Month-specific logistic regression models linking survival of larch casebearer to acute exposure at a range of temperatures between 0 and -60 °C. Data were collected from November 2015 – April 2017. Data within a month were combined across years, as annual variation was statistically insignificant.

Month	*n*	Intercept ± SE	Temperature ± SE	LT_{50}
October	48	6.881 ± 1.932**	0.238 ± 0.070**	-28.90
November	160	13.349 ± 2.457***	0.359 ± 0.064***	-37.19
December	30	8.368 ± 2.779*	0.230 ± 0.074*	-36.35
January	201	10.255 ± 1.620***	0.252 ± 0.038***	-40.77
February	61	10.892 ± 3.610*	0.277 ± 0.088*	-39.34
March	96	5.914 ± 1.440***	0.189 ± 0.042***	-31.31
April	201	5.609 ± 0.814***	0.202 ± 0.028***	-27.79

All models followed the general equation *P*(Insect survives) =1 / (1 + exp[-(intercept + β_1* temperature)]). February included insects assayed between February 1 – March 3 and March include insects assayed from March 4 – March 31.

*$|Z| \geq 1.96$, $P \leq 0.05$

**$|Z| \geq 3.30$, $P \leq 0.001$

***$|Z| \geq 3.90$, $P \leq 0.0001$

Table 3.2 Models of lower lethal temperatures and supercooling points comparing overwintering and active casebearer larvae. Data and models are presented in Figure 3.3 or Figure 3.4. The level for fed represents insects that were actively wandering and had been fed foliage for at least 24 hours. The level for starved is equivalent to "fed" except that insects were not provided foliage.

Figure	Month	*n* insects	Response	Predictor	Estimate	SE	|Test statistic|	*P*
3.3a	Sep 2016	63	Survival	Intercept[a]	5.010	1.384	$Z = 3.62$	0.0003
				Temperature	0.288	0.075	$Z = 3.83$	0.0001
				Overwintering (vs. fed)	3.238	1.091	$Z = 2.97$	0.0030
3.3b	Apr/May 2016	216	Survival	Intercept[a]	3.583	0.547	$Z = 6.56$	<0.0001
				Temperature	0.171	0.026	$Z = 6.52$	<0.0001
				Starved (vs. fed)	-0.081	0.559	$Z = 0.15$	0.88
				Overwintering (vs. fed)	1.300	0.521	$Z = 2.50$	0.0126
3.3c	Apr 2017	26	SCP[b]	Intercept[a]	-20.876	1.068	$t = 19.55$	<0.0001
				Overwintering (vs. fed)	-9.474	1.510	$t = 6.27$	<0.0001
3.4	Jan 2017	68	SCP[c]	Intercept[d]	-44.302	0.884	$t = 50.10$	<0.0001
				log(Days)	0.007	0.076	$t = 0.09$	0.93
				Greenhouse (vs. outside)	5.179	1.226	$t = 4.22$	0.0001
				Greenhouse (vs. outside) × log(Days)	0.469	0.106	$t = 4.41$	<0.0001

[a] For example, to estimate the probability of survival of an overwintering larvae in September, P(Survival) = 1 / (1 + exp(-(5.01 + 0.29 × T + 3.24))), where T is the lowest temperature to which a larva was exposed.

[b] Model statistics: $F_{1,24} = 39.38$, $P < 0.0001$, Adjusted $R^2 = 0.61$

[c] Model statistics: $F_{3,64} = 59.76$, $P < 0.0001$, Adjusted $R^2 = 0.73$

[d] The model reference level is "outside", indicating insects that were stored outside.

Table 3.3 Validation for the models linking mortality to monthly minimum temperatures in select months from 2015-2017. Insects were collected from near Jacobson, MN, USA and either immediately assayed for survival (Jacobson, 2016), or stored outside in Saint Paul, MN, USA. After storage, insects were placed at 20 °C (18L:6D) to determine survivorship.

Year	Month	*n*	Sourced	Observed survival	Predicted survival SCP[a] (95% CI)	Predicted survival LLT[b] (95% CI)
2016	Apr	58	Jacobson	94.8	98.4 (97.6 – 99.5)	88.1 (77.1 – 94.2)
2017	Apr/May	74	Saint Paul	91.9	99.9 (99.6 – 100.0)	84.7 (54.7 – 96.2)

[a] LLT = estimates produced using models of proportional survival as a function of exposure temperature

[b] SCP = estimates produced using a model of supercooling points as a function of day

Figures

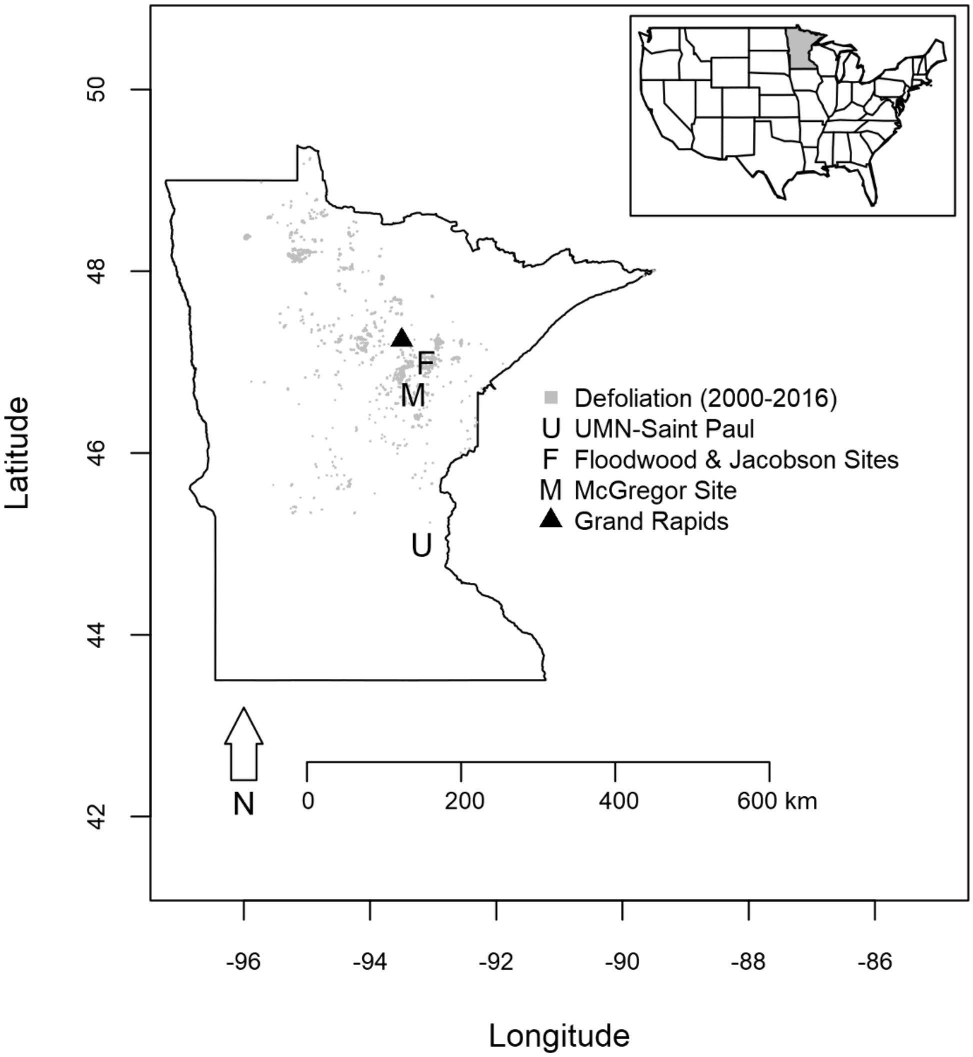

Figure 3.1 Locations of cumulative defoliation by larch casebearer detected via aerial surveys (2000-2016) and collection sites in Minnesota, USA. Markers for the Jacobson site and Floodwood site are overlapping due to proximity of sites (10 km).

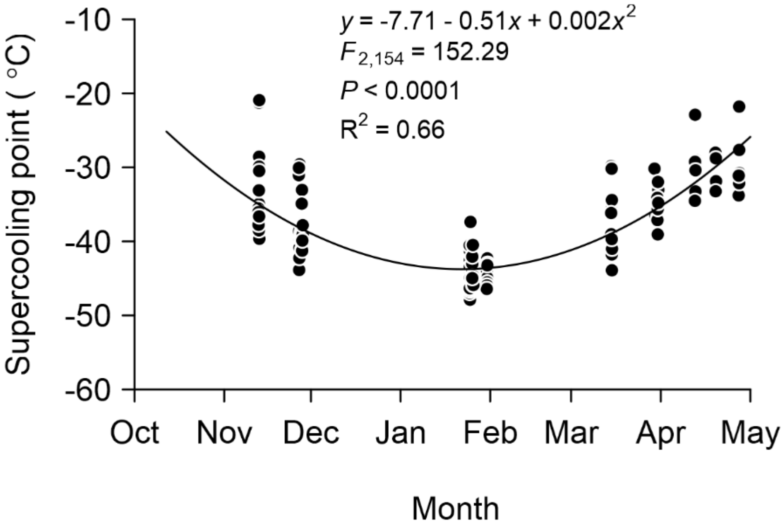

Figure 3.2 Changes in supercooling points of overwintering larch casebearer larvae. Ordinal day, *x*, in equation is the number of days since September 1. Data are jittered in the *x*-direction.

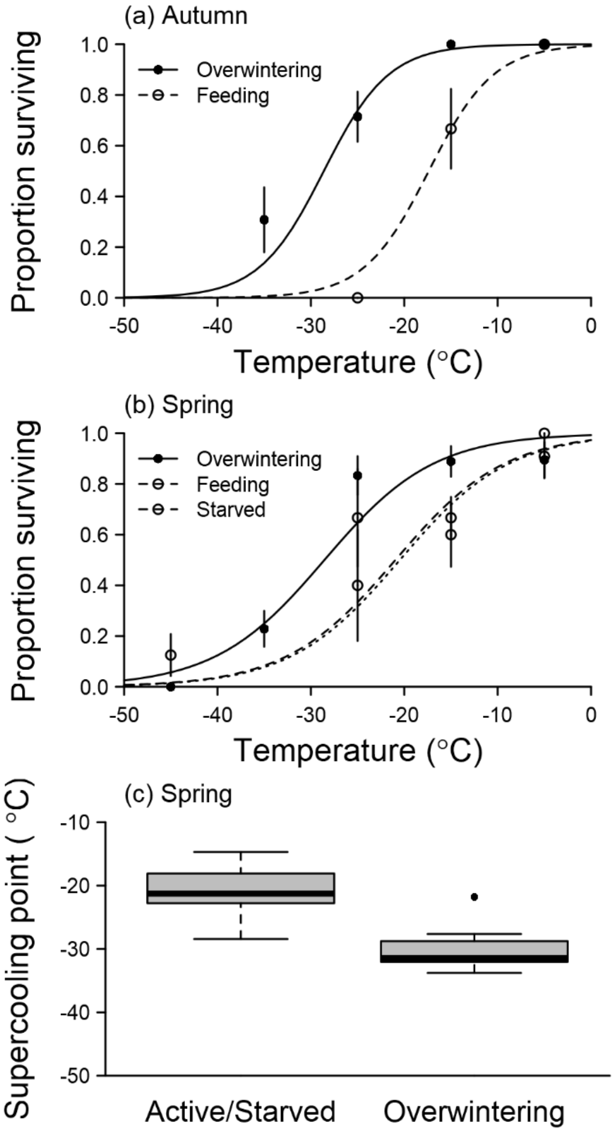

Figure 3.3 (a) Change in lower lethal temperatures between overwintering and feeding larvae in September 2016, (b) Change in lower lethal temperatures between overwintering, feeding, and active/starved larvae in April/May 2016, and (c) Change in supercooling points between overwintering and starved larvae in April 2017. Model coefficients are provided in Table 3.2. Circles and error bars in panels a and b denote means ± SEs and lines are predicted using models in Table 3.2.

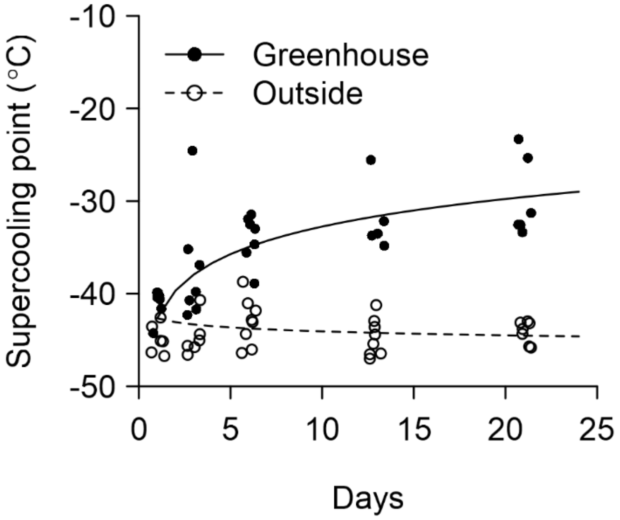

Figure 3.4 Deacclimation or change in supercooling points through time of larch casebearer larvae placed in the greenhouse at ambient light (10.5 – 30.3 °C, 12 – 43% RH) and outside at ambient light (-14.2 – 3.7 °C, 54 – 100% RH). All larvae were placed into microcentrifuge tubes and treatment locations on January 14, 2017 and subsamples of larvae were assayed for supercooling points incrementally for up to 21 days. Model l coefficients are provided in Table 3.2.

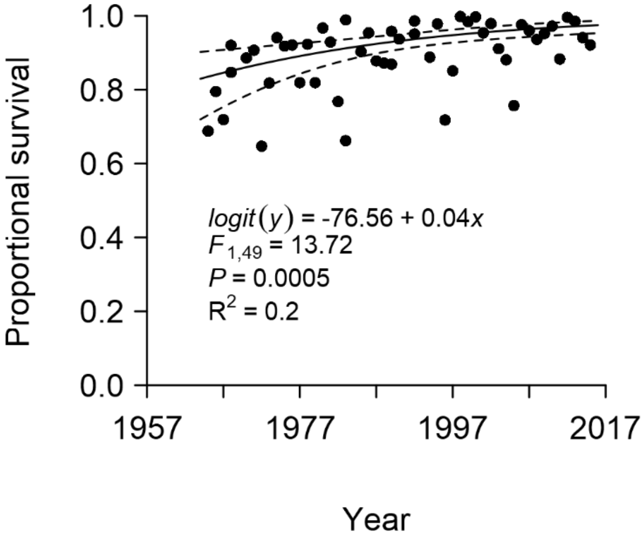

Figure 3.5 Trends in predicted overwintering survival at Grand Rapids (1964-2016). Dashed lines are confidence intervals.

Chapter 4. Spring phenological synchrony and invasion success by a non-native defoliator on a deciduous conifer

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Preface

Eastern larch, *Larix laricina* (Du Roi) K. Koch (Pinaceae), is a deciduous conifer distributed throughout the boreal forest of North America. Larch casebearer, *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae), is an invasive needle miner that defoliates eastern larch across the southern regions of the tree’s range. After years of successful importation biological control, larch casebearer is undergoing a large-scale resurgence in several locations throughout its invaded range. We conducted a series of growth chamber, greenhouse, and cold frame studies to elucidate the role of spring phenology – specifically the temporal patterns of feeding larvae and flush of host foliage – in the invasion success of larch casebearer. We constructed degree-day models of spring activity based on findings from the suite of experiments and investigated sensitivity of larch casebearer to early and delayed emergence relative to bud break. Both species have similar lower developmental thresholds of ~5 °C, though eastern larch required a mean of 29 (± 0.55 SE) degree-days (DD) after December 1 to break bud compared to a mean of 275.8 (± 11) DD after February 20 for larvae to become active. Due to disparate responses of eastern larch and larch casebearer to temperature and photoperiod, larch casebearer consistently activated several days after bud break. Starved larvae survived an average of 19 days across 6-31 °C; their longevity decreased with increasing temperatures. Survival was independent of number of days between activation and bud break (range 0-58 days). The robustness of larch casebearer to changes in the timing of larval feeding relative to bud break in spring has likely contributed to this insect's successful establishment and spread on eastern larch.

Introduction

Insects and plants integrate temperature and photoperiod to time annual rhythms of activity (Rathcke & Lacey, 1985; Cleland *et al.*, 2007; Van Asch & Visser, 2007). Temperature can moderate plant phenology by fulfilling both forcing and chilling requirements (Laube *et al.*, 2014) and insects typically rely on temperatures between developmental thresholds to complete development (Tauber & Tauber, 1973, 1976). Phenological synchrony – such as the timing of insect herbivore activity in relation to host plant resources – can facilitate establishment and spread of both native and non-native insects, increase insect abundances, and moderate patterns of host plant use (Battisti *et al.*, 2005; Ward & Masters, 2007; Gray, 2010; Jepsen *et al.*, 2011; Pureswaran *et al.*, 2015). Phenological mismatch can influence insect fitness and host susceptibility (Feeny, 1976), as insects with seasonally shifted emergence contend with altered phytochemistry and physical properties of plant tissues (Fuentealba *et al.*, 2017, 2018; Régnière & Nealis, 2018) or starvation when feeding on deciduous hosts (Van Asch & Visser, 2007). Global climate change may drive shifts in phenological matching if the herbivore and plant respond differentially to shifts in patterns of degree-day accumulation (Walther *et al.*, 2002; Kharouba *et al.*, 2015; Posledovich *et al.*, 2018). Understanding relative changes in the timing of phenological events between plants and their insect herbivores is paramount for forecasting plant-insect interactions under global climate change (Visser & Both, 2005).

Larch casebearer, *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae), is a needle-miner native to Europe that became established in North America in the late 1800s. The insect was discovered near Northampton, Massachusetts feeding on planted European larch *Larix decidua* Mill. (Hagen, 1886) and eventually spread throughout most of the range of eastern larch, *Larix laricina* (Du Roi) K. Koch (Pinaceae). In the late 1950s, larch casebearer was discovered defoliating western larch, *Larix occidentalis* Nutt. (Pinaceae), in northern Idaho (Denton, 1957) and subsequently spread throughout most of western larch's range (Tunnock & Ryan, 1985; Ryan *et al.*, 1987). An importation biological control program was established across the invaded range of larch casebearer and has been implicated to control casebearer populations (Graham, 1948; Webb & Quednau, 1971; Otvos & Quednau, 1981; Ryan, 1990, 1997), although they have resurged since 2000 for reasons unknown (Chapter 1).

Larch casebearers overwinter as third instars in cases attached to twigs with silken plugs (Herrick, 1911; Tunnock *et al.*, 1969). The insect appears to be strictly univoltine with an obligate diapause moderated by photoperiod (Ryan, 1975). The depth of diapause decreases nonlinearly through winter: Ryan (1974) found that time required for activation of larvae (i.e., active wandering after winter diapause is broken) exposed to 18L:6D and 20 °C was ~30 days in November, ~8 days in February, and ~2 days in April. Chilling does not appear to moderate timing of diapause termination by larch casebearer (Ryan, 1974a, 1975) or affect developmental rates (Supplementary Information 1). The effects of a range of photoperiods and temperature on activation of overwintering larvae have yet to be investigated.

Phenological synchrony between larval feeding and bud break has been sufficient for larch casebearer to establish across large geographic regions in the allopatric ranges of eastern and western larch. However, variation in phenological patterns across the genus *Larix* and consequences of spring phenological synchrony for larch casebearer are not well understood. Bud break by European larch changes significantly across sites and elevation, though does not appear to be driven by photoperiod (Basler & Körner, 2012). The most important determinant of bud break by European larch is temperature, whereas photoperiod is the most important driver of leaf senescence in autumn (Migliavacca *et al.*, 2008). Eastern larch flushes new needles in early spring from both short shoots and long shoots (Clausen & Kozlowski, 1967; Remphrey & Powell, 1984, 1985; Powell, 1987; Powell & Raffa, 1999a). The timing and degree-days required for bud break by eastern larch decreases with accumulation of chilling hours and at warmer forcing temperatures (Nanninga *et al.*, 2017). The degree-days required for bud break in relation to chilling days have not been quantified for eastern larch. Photoperiod does not appear to moderate bud break by *Larix decidua* (Basler & Körner, 2012; Way & Montgomery, 2015) and preliminary analyses suggested it does not moderate bud break by eastern larch (Supplementary Information 2).

The activity of larch casebearer in relation to the timing of bud break and dormancy by eastern larch remains unknown, and may be an important determinant of eastern larch-larch casebearer interactions. Phenological synchrony by eastern larch and larch casebearer are determined by the magnitude of responses to cues that trigger spring activation. Given the resurgence of larch casebearer in parts of its invaded range after years of successful biological control (Chapter 1) and the potential of climate warming to destabilize phenological synchrony with its host (Bale *et al.*, 2002), we quantified the potential for changes in spring phenology to facilitate outbreaks of larch casebearer. Several laboratory, greenhouse, and cold frame studies were used to understand the spring phenology of both eastern larch and larch casebearer.

Our first aim was to quantify the bud break response by eastern larch to temperature (heat and chilling accumulation) and to quantify spring feeding response by larch casebearer to temperature (heat accumulation) and photoperiod. We then developed and evaluated simple degree-day models to predict bud break and larval feeding. Finally, we studied larval survival in relation to early and delayed spring emergence by larch casebearer relative to bud break. Our overarching goals were to understand the 1) importance of timing between bud break by eastern larch and spring feeding by casebearers and 2) potential for climate change, which may have driven changes in spring phenology, to facilitate the recent resurgence of larch casebearer in several areas of the invaded range (Chapter 1).

Materials and Methods

Eastern larch

Three-year-old eastern larch trees were bare-root lifted from the Minnesota State Forest Nursery in Badoura, Minnesota, shipped to the St. Paul campus of the University of Minnesota on April 30, 2015, and stored overnight at 4 °C. All trees were potted on May 1, 2015 in 10.2 cm × 24.1 cm pots (TP49, Stuewe & Sons, Tangent, Oregon) using Sunshine Mix #8 (Sun Gro® Horticulture, Agawam, MA, USA), which contains Canadian sphagnum peat moss, perlite, vermiculite, starter nutrient charge (with gypsum), and dolomitic limestone. Seven grams of fertilizer (Vigoro® 16:4:8 NPK) were added to the top of each pot at planting. Plants were watered every 2-3 days and stored outside in a wooden, roofless cold frame adjacent to a greenhouse (where greenhouse studies were later conducted). Unless specified otherwise, these potted trees were used for all assays.

Bud break was recorded as the first day when green needle tissue was visible in an open bud anywhere on a tree or twig. HOBO Temperature/Relative Humidity Data Loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) were used to record and confirm accuracy of temperatures in the field, outdoor cold frame, greenhouse, and growth chambers. For outside temperatures, all loggers were housed inside radiation shields (field) or in the plastic containers (outdoor cold frame).

Intact trees versus cuttings

Either potted trees or twigs (length: ~10 cm) clipped from potted trees were used for all experiments. To determine if cuttings were a good proxy for tree phenology, twenty-four trees were brought into the greenhouse on December 2015, January 2016, and February 2016, for a total of seventy-two trees. On the day of transfer, a single cutting was removed from the mid-canopy of each tree and placed in a 100 ml plastic cup with ~30 ml of tap water. Source trees were watered 2-3 times per week with ~500 ml of tap water and cups with cuttings adjacent to their source trees were refilled as necessary. A branch on the donor tree at approximately equal height to where each cutting originated was marked for tracking and trees and cuttings were monitored every 2-3 days to record day of bud break.

To determine if four-year-old trees served as a useful approximation of mature eastern larch trees, two ~25 cm cuttings, one each from the north and south side of a tree, were collected from twenty-four eastern larches (1.0 – 20.2 cm diam at ground level) in January 2016 at a field site near Jacobson, MN, USA (44.9886 °N, 93.1802 °W). Field collected branches were placed into ~1 L glass jars in the greenhouse, filled with ~300 ml of water and monitored every 2-3 days for bud break. Chilling days, defined as the number of days with a mean temperature between -8 °C and 10 °C, were calculated for the field site based on daily maximum and minimum temperatures recorded with a HOBO temperature logger (Onset Computer Corporation, Bourne, Massachusetts, USA) inside a radiation shield attached to a branch 2 m above ground. The interval of chilling temperatures was developed by iteratively changing high and low chilling thresholds to achieve the best fit between predicted bud break (Eqn. 4.2, *Statistical analysis*) and observed bud break (see *Eastern larch degree-day model*). If the mean temperature fell anywhere within the chilling interval, a chilling day would be recorded. Thus, mean temperatures of -5 °C and 5 °C resulted in equivalent units of chilling according to our criteria. Other models of bud break by western larch have used a chilling interval of -4.7 to 16 °C (Harrington *et al.*, 2010; Harrington & Gould, 2015). Daily degree-days (DDs) above our estimated base temperature (T_{base} = 5°C, see *Eastern larch developmental threshold*) required for bud break after December 1 were estimated from paired daily minimum-maximum temperatures using a simple degree-day calculation:

$$\frac{T_{max} + T_{min}}{2} - T_{base} \qquad [4.1]$$

Eastern larch developmental threshold

To determine the range of temperatures suitable for development of eastern larch, twigs clipped from potted trees were placed at six different temperatures: 6, 11, 16, 21, 26, and 31 °C. Photoperiod was 14L:10D. Ten to 11 twigs were placed into each chamber on March 24, 2016 and monitored every 2-3 days for bud break. Days until bread break were recorded and the lower developmental threshold that provided the best fit was estimated using the method developed by Ikemoto & Takai (2000) (Eqn. 4.2, *Statistical analysis*).

Eastern larch chilling requirements

Chilling days and degree-days can affect the probability that a tree will break bud (Cannell & Smith, 1983; Harrington *et al.*, 2010; Harrington & Gould, 2015). Subsets of potted trees were transferred from the cold frame into the greenhouse every ~3 weeks between December 2015 and March 2016. After transfer, trees were watered every 2-3 days with ~500 ml and monitored for bud break. The proportion of trees that broke bud and the DDs to bud break after December 1 were recorded. A chilling day was defined as any day after November 1 when mean temperature $[(T_{max} + T_{min})/2]$ was between -8 ° and 10 °C.

Eastern larch degree-day model

To develop and evaluate a degree-day model for predicting date of bud break, we used field collected branches (see *Intact trees versus cuttings*) in addition to a cohort of 108 potted eastern larch trees placed into the cold frame in November 2016. Trees in the cold frame were monitored for bud break every 2-3 days beginning in early March and DDs above 5 °C required for bud break after December 1 were calculated using Eqn. 4.1.

The fit line from our model relating DDs to bud break to chilling days (Eqn. 4.2) represented the “possibility line” for bud break (Cannell & Smith, 1983; Harrington *et al.*, 2010) and was used as our degree-day model. Expected DDs according to this model vs. observed DDs at bud break were compared qualitatively as follows. For each day, accumulated chilling days were input into Eqn. 4.2 until observed DDs were greater than the value predicted by Eqn. 4.2. When that condition was met, the predicted value was recorded as the expected DDs. We assumed a parallel model in which a tree could accumulate DDs and a chilling day on the same day, for example, when mean temperature was above 5 °C but below 10 °C (Hunter & Lechowicz, 1992).

Larch casebearers

For assays taking place from autumn 2015 until autumn 2016, several collections of overwintering, third instar casebearers were conducted at the Jacobson field site as follows. A minimum of 12 trees was randomly selected from a stand and live twigs with larvae within 2 m of the ground were collected. All insects were left attached to twigs, placed in a well-ventilated plastic container (volume: 17L, 46 × 31.1 × 19.4 cm) inside of the cold frame, and were not removed from twigs until immediately before use in phenology assays. Insects were assayed within a week of the collection date. For assays taking place in or after November 2016, larvae were collected in early November from 60 trees (2-4 twigs per tree) and stored outside as described above. Thus, for November 2016 onwards, insects were assayed from 1 week to several months after collection.

For use in assays, randomly selected larvae were carefully removed from twigs using forceps and randomly assigned to 9 × 50-mm polystyrene dishes (Falcon Labware, Oxnard, CA, USA) or 1 ml microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA, USA). Diapause termination was indexed by larval activation, as larvae begin to actively wander. Preliminary analyses demonstrated that larval handling does not affect phenological patterns (Supplementary Information 3). Moreover, phenology patterns from the Jacobson site may be extrapolated to other locations in northern Minnesota, as larvae sourced from different locations display similar phenological patterns (Supplementary Information 4).

Larch casebearer developmental threshold

On 14 April, 2016, ten larvae were placed into each of the same six growth chambers used in the eastern larch developmental threshold study. Larvae were monitored every 2-3 days after transfer and days to activation were recorded.

Larch casebearer and photoperiod

To elucidate the role of photoperiod in diapause development and termination, larvae were transferred to growth chambers set to a range of photoperiods. Transfers occurred at two-week intervals from 15 December, 2016 to 17 April, 2017. Growth chambers held at a nominal temperature of 20 °C and three photoperiods: 12L:12D, 14L:10D, and 18L:6D. An additional 24L:0D treatment was added on February 1 and continued throughout the remainder of the study. Fifteen larvae placed into three Petri dishes were transferred into each chamber at each interval. Exact hours of light per 24 hours were determined using HOBO Pendant Temperature/Light Data Loggers (Onset Computer Corporation, Bourne, Massachusetts, USA). The proportion of larvae activated and DDs accumulated since December 1 at activation were recorded.

Larch casebearer degree-day model

We placed 50 and 95 larvae in groups of 4-6 in Petri dishes and placed them in the outdoor cold frame on 8 December, 2015 and 10 November, 2016, respectively. To evaluate robustness of our degree-day model, we evaluated historical activation of naturally occurring larvae on eastern larch from 1997-2002 in Wooster, OH, USA. The set date on which DDs would begin to accumulate was determined by iteratively adjusting the start date and comparing observed vs. predicted activation in both St Paul and Wooster. This process resulted in a model to predict cumulative activation of larvae that used a starting date for DD accumulation of February 20. Predicted degree-days required for 50% activation (± 95% confidence limits) were estimated and then linked to DDs accumulated in Wooster to obtain the estimated date of emergence and associated confidence intervals. That is, the first day that observed DDs surpassed the number estimated for 50% cumulative activation was recorded as the day of activation. Weather station data were obtained from the National Oceanic and Atmospheric Administration (NOAA, 2017).

Survival of early emerging casebearers

A study was begun on 24 March, 2016 to simulate larval emergence prior to bud break. Several hundred larvae collected in November 2016 as described above were placed into Petri dishes (20-30 larvae per dish) and into a growth chamber set to 20 °C (83% RH, 18L:6D) to bring insects out of diapause. Insects were monitored daily. Activated insects were transferred into 1.5 ml microcentrifuge tubes with perforated lids and randomly assigned to one of the six growths chambers used for the lower developmental threshold studies. Each growth chamber was assigned a total of 15 larvae. Survival was monitored every 2-3 days until 100% mortality was observed in all chambers. Inactive larvae were gently contacted with a small brush and immobile ones were designated dead. Time and DDs to death were recorded for each specimen.

Consequences of delayed emergence

Effects of spring phenological synchrony on larch casebearer survival were investigated by incrementally transferring 47 four-year-old potted eastern larch trees into the greenhouse between January 11 and March 6, 2017. Trees were searched extensively to remove all overwintering arthropods. While trees were dormant in late November, lateral branches were removed to enable attachment of a cage to each tree terminal. Cages were constructed of low density polyethylene tubing (diameter: 12.7 cm, thickness: 0.15 mm, ULINE®) with one side cut out and replaced with fine mesh for ventilation. Larvae collected in November from the Jacobson site were assigned to trees as follows. Several hundred larvae were placed into the 18L:6D chamber used in the photoperiod study to promote activation. Between March 22 and April 3, sets of three activated larvae were caged onto shoots on the main stem of each tree and monitored every 2-3 days for moth emergence. The time between bud break and placement of each set was recorded as the "phenological synchrony" and ranged from 0-58 days after bud break. Moth emergence was tracked individually and the proportion of moths activating and days until moth emergence were recorded.

Statistical analysis

To determine the utility of cuttings as a proxy for tree phenology, the number of days to bud break by an individual tree was regressed on the number days to bud break by corresponding cuttings. The influences of tree diameter and cardinal direction from which the cutting was taken on days until bud break were assessed using a linear mixed-effects model with a term for tree fit as random intercept.

Across all analyses, actual temperatures and photoperiods rather than nominal temperatures were analyzed. For both species, developmental rates (1/days to bud break or activation) were log transformed and regressed on temperature. Optimum developmental temperatures were estimated using the delta method (Fox & Weisberg, 2011) and the lower developmental thresholds were estimated using reduced major axis (model II) regression (Ikemoto & Takai, 2000):

$$D \times T = k + \mathrm{t}D \qquad [4.2]$$

where D is the days required for activation, T is the temperature, and k and t are the intercept and slope parameters equivalent to the effective cumulative temperature (i.e., DDs to activation) and the lower developmental threshold (T_{base}), respectively (Ikemoto & Takai, 2000; Hartley *et al.*, 2010).This approach was implemented in R via the lmodel2 package (Legendre, 2018), which provides 95% confidence intervals for the slope (= the lower developmental threshold). The confidence intervals were then used to formally compare thresholds between species.

The effect of chilling on proportion of trees breaking bud was estimated using logistic regression. Similar to Cannell & Smith (1983), nonlinear regression was used to quantify the effect of chilling days on DDs to bud break as:

$$DD = b_0 \times \exp(-b_1 * CD) \qquad [4.3]$$

where *DD* was the degree-days accumulated at bud break, *CD* were the chilling days accumulated at activation, and b_0 and b_1 were coefficients estimated using the nls() function in R (R Core Team, 2017). Confidence intervals for predictions were estimated using the delta method (Fox & Weisberg, 2011).

The role of photoperiod, transfer day, and their interaction in the proportion of larvae activated was modeled using a generalized linear mixed effects model with a binomial error structure and logit link function. The DDs to activation was modeled as a function of the same predictors using a linear mixed effects model. In both models, an identifier term for Petri dish was fit as a random intercept.

In our study of survival of early emerging larvae, the time to death and DDs accumulated at death were both log-transformed and regressed on temperature using simple linear regression. For our study on delayed emergence, the effect of days of phenological synchrony (= date larvae were placed onto a tree – date of bud break) on the probability of moth emergence was assessed using a generalized linear mixed-effects models with a binomial error structure and logit link function. The effect of days of phenological synchrony on days until moth emergence were regressed on phenological synchrony (i.e., days between bud break and larval activation) using a linear mixed-effects model. In both models, a term for tree was fit as a random intercept. For all analyses, assumptions of heteroscedasticity and normality of errors were checked via graphical inspections of residual plots. All analyses were completed using R statistical software (R Core Team, 2017) and $\alpha = 0.05$ was set to determine statistical significance. Mixed effects models were fit using the lme4 package (Bates *et al.*, 2013) using Satterthwaite's approximation for the degrees of freedom implemented via the lmerTest package (Kuznetsova *et al.*, 2014).

Results

Intact trees vs. cuttings

Temperatures in the greenhouse during this study averaged 19 °C (range: 10 – 37 °C) and humidity averaged 39% RH (range: 10 – 89% RH, natural photoperiod). Results indicated that bud break times from cuttings were a good proxy for their source trees, regardless of tree size and cutting origin. For example, the slope of the regression of days required for bud break by intact, potted trees on days for their corresponding cuttings was not significantly different from unity (slope: 0.90, 95% CI: 0.77 – 1.03, $F_{1,65} = 197.81$, $P < 0.0001$, Figure 4.1). Bud break times of field-collected cuttings were independent of tree diameter and the side of the tree (north/south) from which the cutting was taken (results not shown). For example, cuttings from the south side broke bud 0.18 ± 0.74 days earlier than branches from the north side of the tree.

Eastern larch: developmental thresholds

Actual temperatures in the growth chambers were 6.4 ± 0.05 SE °C (90% RH), 10.1 ± 0.02 SE °C (83% RH), 15.5 ± 0.01 SE °C (60% RH), 20.7 ± 0.01 SE °C (55% RH), 27.2 ± 0.04 SE °C (91% RH), and 31.6 ± 0.03 SE °C (85% RH). Development rate increased with temperature until a maximum of 0.24 days^{-1} at 28.3 ± 2.4 SE °C, above which development was slowed (Figure 4.2a). Both a linear ($F_{1,53} = 37.33$, $P < 0.0001$) and quadratic ($F_{1,53} = 16.95$, $P < 0.0001$) term for temperature were included in the final model. Analysis of the data using the method developed by Ikemoto & Takai (2000) produced an estimated lower developmental threshold of 5.0 °C (CI: 3.2 – 6.8 °C).

Eastern larch chilling requirements

All larch trees brought into the greenhouse broke bud and grew new needles except for three out of 138 trees (< 3%); the three were transferred in the initial transfer month of December, when 25 out of 28 trees in that cohort broke bud. Thus, chilling days did not affect the probability of bud break ($Z = 0.005$, $P > 0.99$). However, DDs to bud break decreased with number of accumulated chilling days (Figure 4.3). The values estimated for b_0 and b_1 in Eqn. 4.2 were 991.45 ± 88.53 SE and 0.0272 ± 0.0021 SE (Table 4.1). To illustrate the solution of Equation 2, trees brought into the greenhouse after 50 chilling days outdoors would break bud at 255 DDs above °5 C, whereas trees that had experienced 120 chilling days would break bud after just 38 DDs.

Eastern larch degree-day model

Degree-days required for bud break by trees stored outside for the entirety of winter were between 17 and 53 DDs with a mean of 29 ± 0.6 SE. This translated to a mean day of bud break of April 4 (range: March 31 and April 10) in the outdoor cold frame in spring 2017. Our nonlinear model linking DDs to bud break to chilling days (Table 4.1) predicted that mean bud break should have occurred at 31 DDs (CI: 20 – 43 DDs), equivalent to April 7, the first day that observed DDs was above the "possibility line" presented in Figure 4.3. For field-collected branches, observed mean bud break occurred at 193 ± 6.5 DDs whereas bud break was estimated to occur at 200 DDs. Degree-days to bud break by by field-collected cuttings, which were transferred on January 16, was similar to potted trees transferred into the greenhouse on January 4 and February 2, which required 194 DDs and 141 DDs to break bud, respectively (Figure 4.3).

Larch casebearer developmental threshold

The developmental rate of larch casebearer was positively correlated with temperature up to a maximum 0.12 days^{-1} at 32.3 ± 5.3 SE °C (Figure 4.2b). Both linear ($F_{1,39} = 25.59$, $P < 0.0001$) and quadratic ($F_{1,39} = 7.93$, $P < 0.0076$) terms for temperature were included in the final model. Analysis of the data between 6 and 27 °C using the method developed by Ikemoto & Takai (2000) yielded an estimate of 4.0 °C (CI: 2.9 – 5.1 °C) for the lower developmental threshold, approximately 1 °C lower than the lower threshold estimated for eastern larch. However, the confidence interval for the lower developmental threshold of larch casebearer included 5 °C, so we adopted 5 °C as the base temperature for both eastern larch and larch casebearer for cohesiveness.

Larch casebearer and photoperiod

Actual hours of light per 24 hours and temperatures for the growth chambers were 12.1L (20.8 ± 0.02 SE °C, 77% RH), 14.5L (21.4 ± 0.01 SE °C, 71% RH), 18.7L (20.0 ± 0.09 SE °C, 63% RH), and 24 L (21.5 ± 0.07 SE °C, 70% RH). Photoperiod and ordinal day of transfer were both positively associated with the proportion of larvae that activated. Across the study, 36.8 ± 4.1 SE % of larvae exposed to 12L activated compared to 73.7 ± 3.8 at 14L, 90.3± 2.5 at 18L, and 93.3 ± 2.6 exposed to 24L. There was no statistically significant interaction between photoperiod and ordinal day. However, when transferred on December 15 and April 17, 26.7 ± 11.4 and 68.8 ± 11.6 % of insects exposed to 12L activated compared to 93.3 ± 6.4 and 100.0 ± 0.0 % of insects activating on those same dates, respectively, when exposed to 18L. On the last transfer day (April 17), mean percent activation was 88.5 ± 4.1 % (range: 68.8 – 100.0 %) across all photoperiods.

The DDs to activation was negatively correlated with both photoperiod and transfer day (Table 4.2). That is, insects at high photoperiods required fewer DDs to activate. Across the study, insects held at 12L, 14L, 18L and 24L activated at 319 ± 21.6, 372 ± 20.9, 284 ± 7.4, and 255 ± 12.8 DDs, respectively. For insects transferred at the start of the experiment on December 15, activation occurred at 425 ± 30.9 SE DDs compared to 221 ± 9.1 SE DDs for larvae transferred on April 17. There was no statistically significant interaction between photoperiod and transfer day.

Larch casebearer degree-day model

In 2015 and 2016, 84.0% and 89.5% of insects activated, respectively. Activation across 2016 and 2017 was observed between April 18 and June 12 , which translated translated to 100 and 728 DDs, respectively, with a mean of 276 ± 11 SE. Logistic models of cumulative activation suggested that 5% of larvae activated by 69.4 ± 23.3 SE, 50% by 273 ± 22.8, and 95% by 477 ± 23.4 DDs, respectively (Figure 4.4a). Regressing cumulative activation on month of transfer suggested that 5% of insects activated by April 19, 50% by May 10, and 95% by May 31 (Figure 4.4b).

Our model predictions matched historical activation in 5 out of the 6 years in a different state (Table 4.3). In the first year, 1997, the predicted date of activation preceded the observed by 11 days. In the subsequent five years, however, differences between predicted and observed values were within 1-2 days, and the 95% confidence intervals for predicted dates included the observed dates in all of the five years.

Survival of early emerging casebearers

Larvae placed at 6 °C survived for a mean of 76 days (Figure 4.5a). Survival decreased nonlinearly with temperature such that larvae held at 6°C, 19 °C and 31 °C died after a mean (± SE) of 76.1 (± 5.8), 11.9 (± 1.3) and 7.5 (± 0.8) days, respectively. In converting the time held at specific temperatures to DD, it was estimated that larvae survived an average of 153 ± 8.9 SE DD. Larvae at higher temperatures survived for more degree-days, which may reflect mortality due to desiccation rather than temperature for larvae held at lower temperatures (Figure 4.5b).

Consequences of delayed emergence

Average temperatures in the greenhouse across this study were 20.8 ± 0.3 °C (range: 11.5 – 30.6) and humidity was 39% RH. Delayed emergence in relation to bud break did not affect survival to adulthood (holding all else equal; $Z = -0.25$, $P = 0.81$). Across all trees, an average of 1.31 moths emerged out of three larvae placed onto trees and time to emergence averaged 49.5 ± 2 SE days ($t_{34} = 1.81$, $P = 0.08$), regardless of time since bud break.

Discussion

Phenological synchrony between the onset of spring feeding by forest Lepidoptera and the flush of host tree foliage can be an important determinant of insect survival and fitness (Feeny, 1976; Van-Asch & Visser, 2007). Here, we have identified three aspects of this native tree-invasive defoliator system that have contributed to larch casebearer's establishment and spread in eastern larch forests of North America. First, larch casebearer consistently activates after bud break by eastern larch, leading to robust phenological matching between insect and host. Eastern larch and larch casebearer have similar lower developmental thresholds; however, activation of larvae requires over 200 DDs more than bud break by trees that have accumulated chilling days typical of Minnesota. These phenological patterns suggest that even the earliest active larvae will become active only after local hosts break buds and needles are available for consumption. Second, delayed emergence by larch casebearer does not affect survivorship or development time of larvae to adulthood. Larch casebearer larvae are fourth instars in spring and feed inside needles in the early instar. Thus, larvae may be well adapted to feed on foliage of a variety of ages, types (long vs. short shoots), and defensive capacities. We did not detect an effect of phenological synchrony on development rate (i.e., time to moth emergence), but other sublethal effects, such as changes in fecundity, may vary with host quality and be important for insect population dynamics (Awmack & Leather, 2002). Third, larvae appear able to survive periods without food, consistent with findings that earlier emergence by larvae relative to bud break does not affect mortality of larch casebearer. Degree-days survived by starved larvae increased with temperature and our model estimated that larvae held at 6 °C survive for over 50 DDs on average (Figure 4.5b), more than 20 DDs longer than required for bud break by eastern larch in 2016.

Our findings suggest that activation and development of casebearer larvae across winter and into spring is moderated by photoperiod, similar to other temperate forest Lepidoptera that are sympatric with larch casebearer in much of its introduced range (Régnière, 1990). Given sufficient chilling and heat accumulation, eastern larch appears able to break bud at any time after December 1. Thus, warmer winters and springs may drive earlier bud break without concomitant shifts by larch casebearer, ensuring that larvae will consistently have foliage available for consumption. The effect of chilling on bud break by eastern larch may be moderate (Nanninga *et al.*, 2017) and there does not appear to be a minimum threshold required for bud break to occur. Nonetheless, the average days between larval activation and bud break by eastern larch may decrease with further climate change, which could delay bud break by decreasing chilling accumulation (Yu *et al.*, 2010; Luedeling *et al.*, 2011).

The degree-day models for eastern larch performed well when evaluated using planted trees stored outside. The model for eastern larch predicted mean bud break of potted, juvenile trees would occur at 31 DDs compared to an observed mean bud break of 29 DD, equivalent to a difference of three days in 2017 (April 7 vs. April 4). Observed DDs at bud break for cuttings sampled from the Jacobson site was 193, compared to 199 DDs predicted from our exponential model (Table 4.1). Thus, barring an interaction of cutting and tree size, our model appears reliable in forecasting future and reconstructing historical phenology of eastern larch across a range of tree age and size classes in Minnesota.

The degree-day model for larch casebearer also performed well when evaluated using an independent data set of insects naturally infesting eastern larch trees in Wooster, O.H., USA. Our findings suggested that larvae transferred during winter were more likely to activate and require fewer DDs to activate than those transferred earlier (Table 4.2). Taken together, this suggests that the depth of diapause is decreasing through time and that insects may become incrementally less responsive to photoperiod as winter progresses (Tauber & Tauber, 1970; Régnière, 1990). Thus, ambient photoperiod and factors influencing the depth of diapause may be important for spring activation. Despite the complex role of photoperiod in the activation of larvae, our degree-day model using a base temperature of 5 °C and start date of 20 February predicted spring activation of larch casebearer in the field within two days in six out of seven years. Thus, our model may be useful for predicting spring phenology of larch casebearer on eastern larch at latitudes similar to Wooster, OH and Saint Paul, MN, USA. Extrapolation of models to western larch forests should be done with caution, as host effects may moderate phenology (Goehring & Oberhauser, 2002).

Foliage flush of the tree prior to larval activation of the insect, driven and maintained by disparate responses to environmental cues in a changing climate, has likely contributed to the insect's establishment across a large geographic region. Ultimate reasons for the resurgence of larch casebearer are likely multifaceted, however. Warmer temperatures, for example, can increase developmental rates (Dale *et al.*, 2001; Bale *et* *al.*, 2002), shift timing of spring activity (Badeck *et al.*, 2004; Cleland *et al.*, 2007), decrease chilling accumulation in plants (Yu *et al.*, 2010; Luedeling *et al.*, 2011), and increase growing season length (Chmielewski & Rötzer, 2001; Walther *et al.*, 2002). Each of these affects may have altered other intraspecific interactions and interactions across trophic levels (Harrington *et al.*, 1999). For example, deviations from historical climatic patterns could have facilitated decreases in the efficacy of imported natural enemies (Hellmann *et al.*, 2008), potentially by inducing phenological asynchrony between foraging wasps and susceptible host life stages (Hance *et al.*, 2007). Elucidating factors behind the casebearer's continent-wide resurgence remain the subject of ongoing research.

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Tables

Table 4.1 Model parameters and summary statistics for a nonlinear regression model linking degree-days required for bud break (DD, T_{base} = 5 °C) to chilling days accumulated (CD) (Eqn. 4.2 in text).

Parameter	Coefficient	SE	|*t*|	df	*P*
b_0	991.4498	88.5309	11.20	87	<0.0001
b_1	0.0272	0.0021	12.85	87	<0.0001

Table 4.2 The role of photoperiod and transfer day in the proportion of larvae activated and degree-days required for activation by larch casebearer. Both models had fixed effects for predictors and a term for Petri dish fit as a random intercept.

Response	Predictor	Coefficient	SE	|Test statistic|	*P*
Proportion activated[a]	Intercept	-4.9912	0.6621	$Z = 7.54$	<0.0001
	Photoperiod	0.3365	0.0413	$Z = 8.14$	<0.0001
	Day	0.0093	0.0030	$Z = 3.15$	0.0017
DDs to activation	Intercept	561.0749	34.0720	$t_{253} = 16.47$	<0.0001
	Photoperiod	-7.0358	1.7504	$t_{349} = 4.02$	<0.0001
	Day	-1.5013	0.1921	$t_{349} = 7.82$	<0.0001

[a] Model fit with a binomial error structure and logit link function.

Table 4.3 Dates when first active larvae were observed and predicted dates of 50% larch casebearer activation (along with 95% confidence limits, CL) in Wooster, OH, USA between 1997-2002.

Year	Observed DD[a]	Observed Date	Predicted date	Error	Early CL	Late CL
1997	356.5	Apr 15	Apr 04	-11	Apr 01	Apr 09
1998	274.8	Apr 06	Apr 06	0	Mar 31	Apr 10
1999	267.8	Apr 08	Apr 08	0	Apr 06	Apr 13
2000	308.6	Mar 25	Mar 23	-2	Mar 15	Mar 26
2001	252.3	Apr 10	Apr 11	1	Apr 09	Apr 14
2002	261.5	Apr 08	Apr 09	1	Apr 02	Apr 11
Mean:	286.9	Apr 06	Apr 05	-1.8	Mar 31	Apr 08

[a] T_{base} = 5 °C, onward from 20 February in each year.

Figures

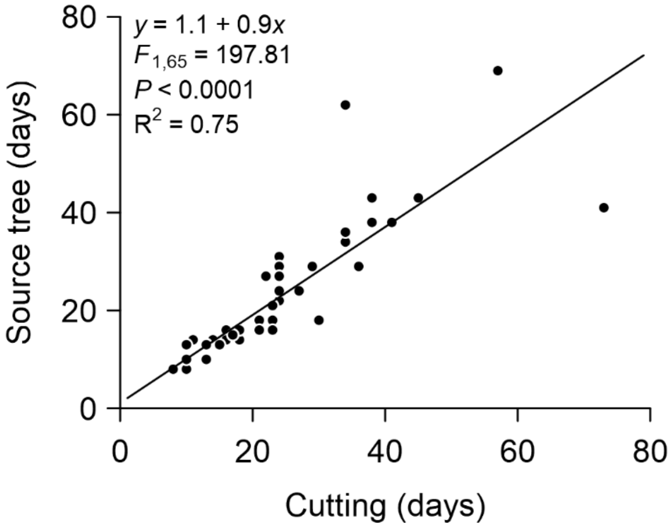

Figure 4.1 Days required for bud break by individual potted trees of eastern larch and corresponding cuttings housed in the greenhouse after transfer from outdoor cold frame monthly from December 2015 to February 2016 (*n* = 24 per month).

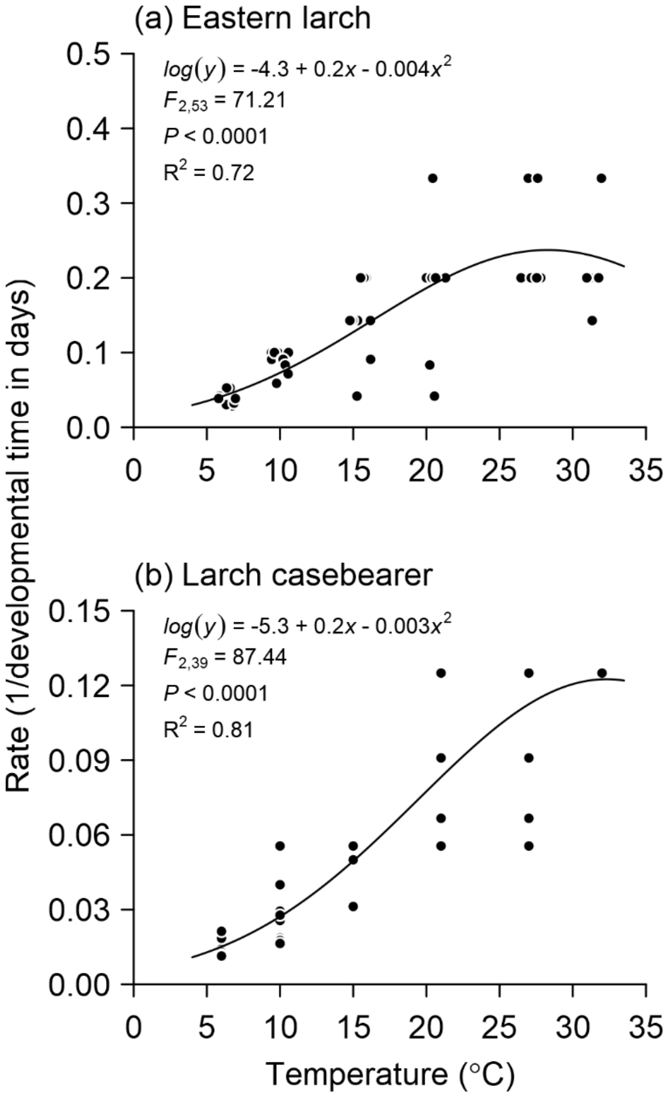

Figure 4.2 The effects of temperature on developmental rates for (a) bud break by eastern larch and (b) activation by larch casebearer. Points in panel (a) are jittered.

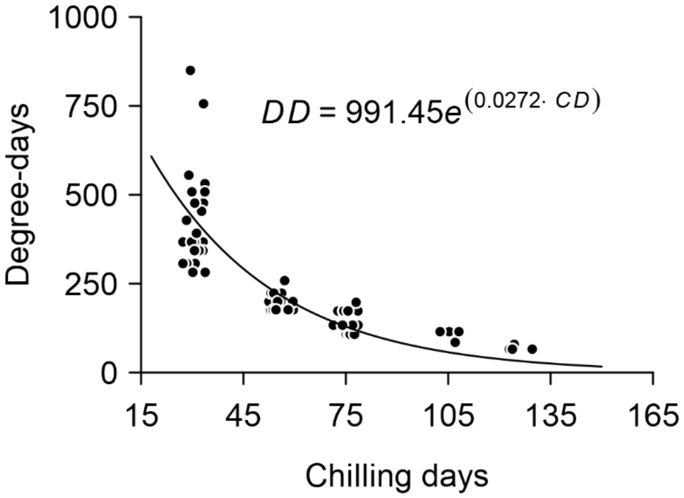

Figure 4.3 Chilling days experienced by eastern larch housed outdoors in the cold frame before sequential transfers at 2-3-wk intervals into a greenhouse and subsequent growing degree-days to bud break after 1 December. The fit line indicates the “possibility line”, as any point at or above the line indicates an individual expected to break bud. Summary statistics for the fit line are provided in Table 4.1. Points are jittered for graphical clarity.

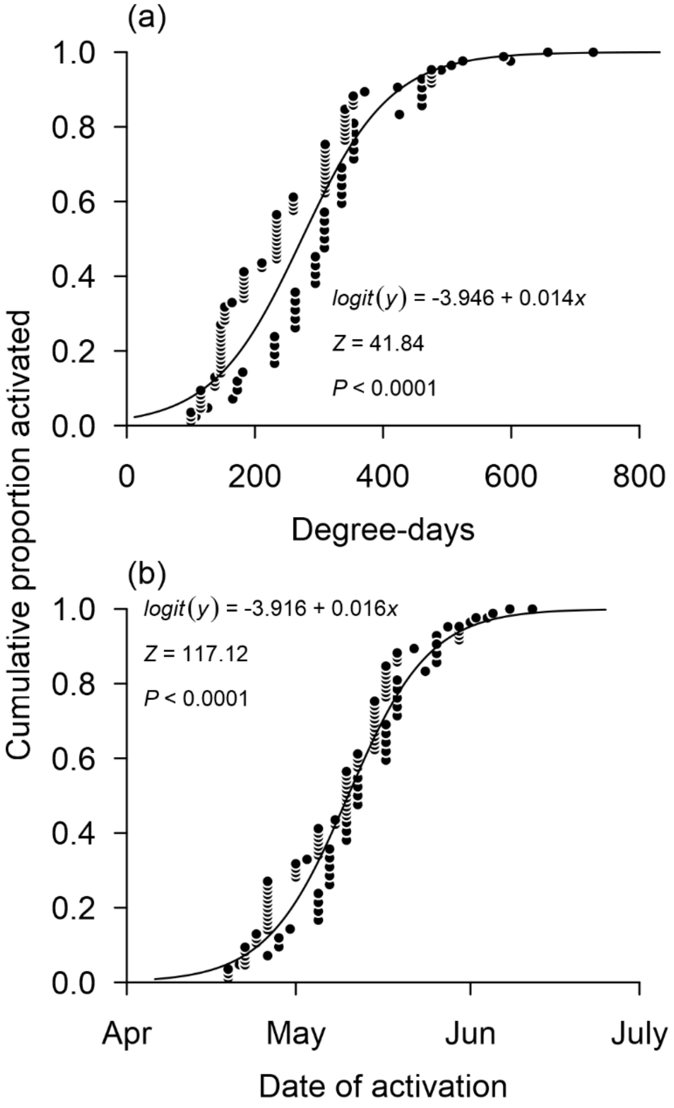

Figure 4.4 Cumulative proportion of larvae activated as a function of (a) degree-days accumulated after February 20 and (b) days after January 1 in 2016 and 2017 for larch casebearer.

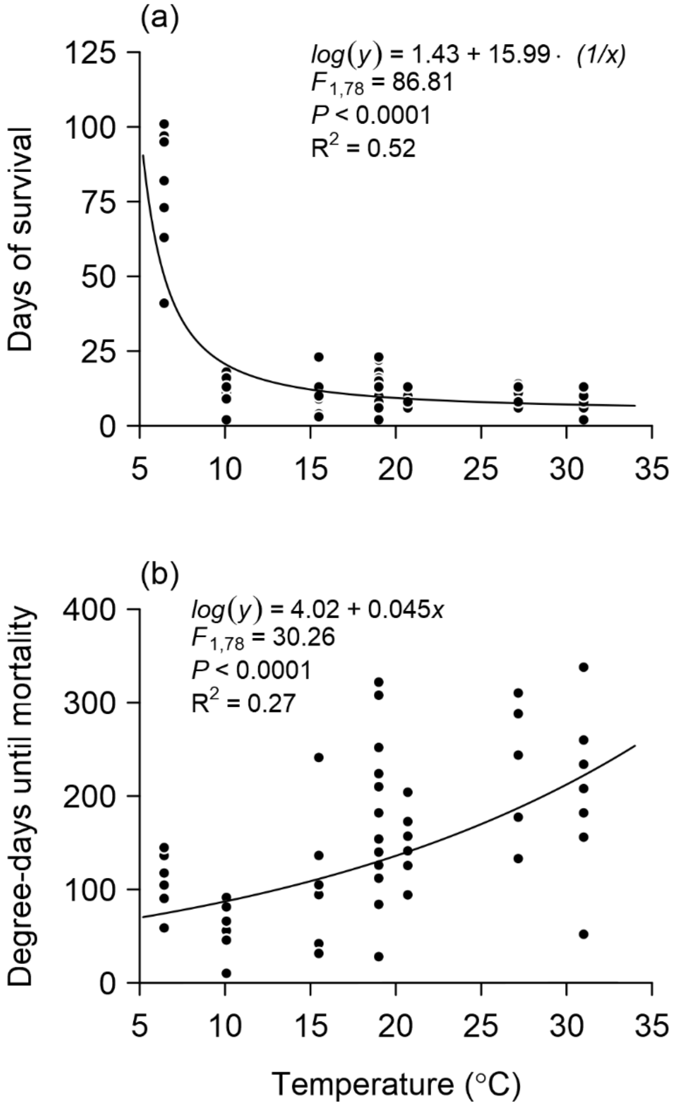

Figure 4.5 The role of temperature in (a) longevity after activation and (b) degree-days until death by casebearer larvae deprived of food.

Supplementary Information 1

Larch casebearer chilling requirements

The role of chilling in activation of casebearer larvae was investigated across winter 2015-2016. Larvae were collected within a week of assays as described in the main text (see *Larch casebearers*), placed in groups of 4-6 into Petri dishes, and transferred into the greenhouse. Transfers days were as described for the chilling study on eastern larch (see *Eastern larch chilling requirements*). Insects were monitored every 2-3 days for activation. Six petri dishes (~30 larvae) were moved per increment. For simplicity, a chilling day for larch casebearer was defined equivalently to a chilling day for eastern larch. The proportion of larvae activated, DDs required to activate (accumulated above 5 °C after transfer), and chilling days since November 1, 2015 were recorded.

Statistical analysis

The effect of chilling days on the proportion of larvae activated was modeled using a generalized linear mixed-effects model with a logit link function and binomial error structure. The effect of chilling days on DDs (log-transformed) to activation was modeled using a linear mixed-effects model. A term for Petri dish was fit as a random intercept in both models. Assumptions of heteroscedasticity and normality of errors were checked via graphical inspection of residuals.

Results and Discussion

The proportion of larvae activated increased with chilling days such that the percent of larvae activated was 0.00 % (± 0.0 SE) after 34 chilling days compared to 40.0% (± 8.9 SE) after 133 chilling days (slope: 0.036 ± 0.009, $Z = 4.00$, $P < 0.0001$).

However, the number DDs to activation were not moderated by the accumulation of chilling days. Because chilling had no effect on development rates, the effect of chilling day on proportion activated was assumed to be due to decreasing depths of diapause with ordinal day. That is, larvae transferred earlier in winter were in a deeper diapause, required more DDs to activate, and thus, were at greater risk of desiccation than insects transferred later in winter.

Supplementary Information 2

Eastern larch and photoperiod

The role of photoperiod in bud break was investigated in winter 2015-2016. Twigs clipped from potted trees were placed individually into 100 ml plastic cups with ~30 ml of tap water that was filled as necessary. Three twigs were placed into each of six growth chambers on December 8, January 4, and February 2, totaling 54 twigs. The experiment was designed as a two-way factorial with two nominal temperatures (16 °C and 22 °C) and three photoperiods (8L, 11L, and 14L). Approximate photoperiods were confirmed manually at the onset of the study. The proportion of trees that broke bud, DDs required for bud break (accumulated above 5 °C after December 1), and chilling days accumulated by trees in the cold frame prior to cutting were recorded.

Statistical analysis

The roles of photoperiod, temperature, and chilling days and all possible interactions in the proportion of twigs breaking bud was modeled using logistic regression. The DDs to bud break was log-transformed and regressed on the same set of predictors using multiple linear regression. Assumptions of heteroscedasticity and normality of errors were checked via graphical inspection of residuals.

Results and Discussion

Actual temperatures were 17.2 ± 0.05 SE °C (87% RH, 8L:16D), 16.0 ± 0.02 SE °C (32% RH, 11L:13D), 15.8 ± 0.09 SE °C (30% RH, 14L:10D), 22.2 ± 0.02 SE °C (23% RH, 8L:16D), 23.2 ± 0.23 SE °C (64% RH, 11L:13D), and 21.8 ± 0.04 SE °C (62% RH, 14L:10D). Across all treatments, 87 ± 5 SE % of cuttings broke bud and no variables explained variation in the probability of bud break. A variable for chilling days was negatively correlated with DDs to bud break ($F_{1,45} = 191.47$, $P < 0.0001$). Otherwise, no other variables explained variation in DDs to bud break.

Supplementary Information 3

Larval handling

A study was conducted in January 2016 to determine if insect handling (i.e., excision vs. plucking from twig) influenced phenology. Twigs with larvae were collected as described in the main text (see *Larch casebearers*). Twenty twigs were then randomly selected and one larva was removed from each twig by using a utility knife to cut around the area where the larva had attached its case to the twig (i.e., the larva and/or silk attachment were never contacted; referred to as “excised larvae”). A second larva from the same twig was removed with forceps by pulling the distal end of the case (referred to as “plucked larvae”). Cases are translucent and larva are visible through the case. Thus, care was taken to ensure that no larvae were punctured or compressed by the forceps, that the openings on the proximal ends of cases were still plugged with silk, and thus, that larvae remained undisturbed in cases. Larvae originating from the same twigs were placed into Petri dishes (2 larvae per dish, one excised and one plucked) and into a growth chamber (17.1 ± 0.3 SE °C, 83% RH, 18L:6D). Larvae were monitored every 2-3 days for activation. The proportion of larvae that activated and days until activation were recorded.

Statistical analysis

The effect of larval extraction (excised vs. plucked) on the proportion of larvae activated was investigated using a generalized linear mixed-effects model with a logit link function and binomial error structure. The effect of larval extraction on days required for activation was modeled using a linear mixed-effects models. For both models, a term for twig was fit as a random intercept. Assumptions of heteroscedasticity and normality of errors were checked via graphical inspection of residuals.

Results and Discussion

The proportion of larvae activating was not moderated by handling ($Z = -0.48$, $P = 0.63$) nor were the days required to activate ($t_{19} = -0.04$, $P = 0.97$). Eighty-five percent of excised larvae activated compared to 90% of plucked larvae. Activation time for excised was 24.2 days compared to 24.3 days for plucked larvae.

Supplementary Information 4

Larval activation by site and collection date

To assess robustness of phenological patterns to other locations, phenology was studied at two additional locations to the Jacobson site, one near Floodwood, MN, USA (46.9683 °N, 92.9912 °W) and the other near McGregor, MN, USA (46.6446 °N, 93.3166 °W). The additional sites were 10 km and 42 km distant from the site in Jacobson, respectively. In November 2016, twigs with larvae were collected from 12 trees at each site and stored in the cold frame as describe in the main text (see *Larch casebearers*). On January 16, 2017, twigs with larvae were collected from 12 trees at the Jacobson site and stored in the cold frame. On January 17, 2017, 145 larvae (i.e., 30 larvae from the two additional sites from the November collection and 30 and 50 larvae from the November and January collections from the Jacobson site, respectively) were placed into Petri dishes in groups of 4-6 and into the 14L and 18L chambers from the larch casebearer photoperiod study (see *Larch casebearer and photoperiod*). Larvae were monitored every 2-3 days and the proportion of larvae activated and the DDs to activation after transfer were recorded.

Statistical analysis

For analyses on larval activation by site, only larvae collected in November were considered, as these larvae had three months to acclimatize to the ambient environment of the cold frame. For that analysis, the effect of photoperiod, site, and their interaction on the proportion of larvae activated was modeled using a generalized linear mixed-effects model with a binomial error structure and logit link function. The effects of the same predictors on DDs required to activate after transfer were evaluated using a linear mixed-effects models. A term for Petri dish was fit as a random intercept in both models.

For analyses on collection date, only larvae from the Jacobson site were considered. The role of collection date, photoperiod and their interaction in the proportion of larvae activated was modeled using a generalized linear mixed effects model with a binomial error structure and logit link function. The effect of the same set of predictors on DDs to activation after transfer was assessed using a linear mixed-effects model with a term for Petri dish fit as a random intercept. Assumptions of heteroscedasticity and normality of errors were checked via graphical inspection of residuals.

Results and Discussion

Neither site nor collection date explained variation in the proportion of larvae activated or DDs to activation. In both analyses, photoperiod was positively associated with the proportion of larvae activated (results nots shown) but not with DDs to activation. Thus, our results using insects from the Jacobson site appear generalizable to proximate sites in modeling phenology of larch casebearer throughout northern Minnesota.

Chapter 5. Implications of heat accumulation across the growing season to population dynamics of an invasive defoliator

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Preface

Larch casebearer is an invasive needle miner in North America that defoliates eastern larch. Historically, larch casebearer has been controlled by a successful importation biological control program but has resurged in parts of its invaded range in the USA since 2000. We investigated the role of changing phenology from spring activation to fall dormancy in outbreaks by larch casebearer. We quantified degree-days required to develop through each of larch casebearer life stages at three sites in Minnesota, USA and developed models predicting cumulative abundance of each stage. We used these degree-day models to predict 1) synchrony of spring feeding by larvae with bud break and 2) cumulative abundance of overwintering larvae in cases. These predictions allowed us to determine if patterns of estimated spring phenology of feeding larvae and bud break or proportions of larvae developing to the overwintering stage have changed through time (1964-2016) using climate data from Grand Rapids, Minnesota. The consequences of warmer autumns and winters (i.e., pre-spring warming) for the incidence and timing of spring activation of larvae, which may influence growing season length and patterns of fall dormancy, were also investigated. Our results suggested that no significant changes have occurred in estimated timing of larval activation relative to bud break, but that estimated proportions of larvae reaching the overwintering casebearing stage have increased through time. Pre-spring warming resulted in delayed spring activation, suggesting that warmer temperatures may act antagonistically on casebearer development depending on time of year and intensity of warming. That is, warmer autumns and/or winters could delay spring activation whereas warmer springs and summers may accelerate spring activation and subsequent developmental rates. Nonetheless, our results provide evidence that longer growing seasons enable more larvae to complete development have helped facilitate the resurgence of larch casebearer in Minnesota.

Introduction

In temperate latitudes, growing season length is determined by timing of onset of spring activity and fall dormancy for both plants and herbivorous insects, which moderates plant-insect interactions (Parmesan, 2006). For example, earlier bud break by plants and spring emergence by insects induced by earlier warmer temperatures in spring can lengthen growing seasons (Schwartz *et al.*, 2006; Cleland *et al.*, 2007; Körner & Basler, 2010; Way, 2011). Likewise, the onset of dormancy in autumn, such as leaf drop by plants and diapause induction by insects, may be delayed by autumnal warming and increase growing season length (Gallinat *et al.*, 2015). For plants and insects that rely on photoperiod to time dormancy, the timing of the growing season may be fixed (Tauber & Tauber, 1976; Way, 2011; Gallinat *et al.*, 2015). Temperature can also moderate developmental rates (Bale *et al.*, 2002) and influence insect voltinism (Tobin *et al.*, 2008). Insects must accumulate sufficient growing degree-days and reach their overwintering stage to persist, and development must occur at a time when host foliage is available for consumption. Thus, the direction (earlier vs. later) and magnitude (days vs weeks) of changes in the timing of phenological events by both plants and insects are important determinants of insect fitness (Visser & Both, 2005).

Larch casebearer, *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae), is a needle-mining moth that is native to Europe. In the late 1800s, larch casebearer was discovered to be defoliating planted European larch *Larix decidua* Mill. trees near Northampton, Massachusetts (Hagen, 1886). Over the next century, the insect spread throughout most of the ranges of eastern larch, *Larix laricina* (Du Roi) K. Koch (Pinaceae), a deciduous conifer distributed throughout the boreal forest of North America (Burns & Honkala, 1990). Larch casebearer eventually spread throughout forests of western larch, *Larix occidentalis* Nutt. (Pinaceae), presumably following an introduction from eastern larch forests in the 1950s (Denton, 1957; Tunnock & Ryan, 1985; Ryan *et al.*, 1987).

Larch casebearers overwinter as third instars in cases that are attached with silken plugs near dormant buds on larch twigs (Tunnock *et al.*, 1969). In spring, surviving larvae become active, molt into fourth instars, and resume feeding without leaving their cases, which larvae enlarge as they grow. Pupation occurs inside cases beginning in mid to late spring and adult moths are active from late spring through late summer (Herrick, 1911). Females place eggs singly onto larch needles and neonate larvae then mine through the base of the egg shells into the underlying larch needles. During the second instar, larvae construct cases from hollowed out needles, molt to third instars, and then attach their cases to twigs in advance of winter (Herrick, 1911; Thorpe, 1933).

Eastern larch breaks bud in spring, the timing of which is moderated by the accumulation of chilling days and heating units (Nanninga *et al.*, 2017). Drivers of the timing of needle drop in autumn are less understood, although it is thought that temperature is less important than photoperiod, which moderates the timing of needle drop by *L. decidua* (Migliavacca *et al.*, 2008; Moser *et al.*, 2009). The timing of spring bud break and needle drop in autumn are important determinants of larch casebearer survival, as these events bracket the growing season for larch casebearer irrespective of the insect's response to temperature. For example, as needles undergo senescence and abscission on eastern larch each fall, cases housing third instar larvae may sometimes be observed fixed to detaching needles. Larvae attached to such needles are 1) at elevated risk of being dropped from the tree and 2) feeding on a quickly diminishing resource as trees enter dormancy. Thus, such larvae are unlikely to overwinter successfully and complete development the following spring.

After years of successful importation biological control in both eastern and western larch forests (Graham, 1948; Webb & Quednau, 1971; Otvos & Quednau, 1981; Ryan, 1990, 1997), larch casebearer has undergone a largescale resurgence at several locations across its invaded range in North America beginning around 2000 for reasons unknown (Chapter 1). Given the potential for climate change to facilitate changes in plant-insect interactions (Bale *et al.*, 2002; Walther *et al.*, 2002; Weed *et al.*, 2013), we conducted a series of experiments examining whether aspects of temperature related to this invasive insect's development and ecology with its host may be contributing to its recent resurgence in parts of North America. Our first objective was to quantify current and historical patterns of phenology and changes therein, with special consideration of larval activity in spring and autumn. To do this, we studied how the phenology of larch casebearer development progressed throughout the growing season in northern Minnesota, and how that progression related to the presence of eastern larch foliage. We applied degree-day models to weather station data from 1964-2016 at Grand Rapids, Minnesota, USA to predict the phenology of larval activation relative to bud break and cumulative abundance of overwintering larvae in autumn. Our second objective was to determine if warming outside of the growing season (i.e., in autumn and winter) could drive changes in spring phenology, which could in turn influence growing season length. To that end, we conducted two larval transfer experiments to understand how warm temperature exposure in either autumn or winter might moderate spring activation of larvae.

Materials and Methods

Field phenology

We characterized the presence (i.e., bud break, needle drop in autumn) and weight of larch foliage along with all life stages of larch casebearer between spring and autumn. Branch samples from nine trees were collected from three sites (three trees per site) near Floodwood, MN, USA (46.9683 °N, 92.9912 °W), Jacobson, MN (46.9982 °N, 93.1073 °W), and McGregor, MN (46.6446 °N, 93.3166 °W) (Figure 5.1). Sampling occurred weekly between April 19, 2016 and November 9, 2016 and the same trees were sampled each week. All sites were sampled on the same day. Sampling began after trees had broken bud but before significant needle growth or activation by any larvae had occurred. On each sampling day, the distal ~25 cm of one branch per tree was clipped, placed into a 1-gallon plastic bag inside a cooler with wet ice, transported to the lab, and frozen at -20 °C for several weeks prior to processing.

For processing, branches were first dried at room temperature (22.8 ± 0.1 SE °C, 37% RH) for a minimum of 4 days. Foliage was then removed from each branch and weighed. Buds and overwintering casebearers attached to the branch were counted, and linear branch length was measured to the nearest cm. The foliage was then homogenized and a 0.25 g subsample was weighed out and thoroughly examined for all life stages of larch casebearer. If less than 0.25 g of foliage was present, as occurred in early spring and late autumn, then all foliage was examined. Life stages in foliage or attached to twigs were characterized as either an egg, needle miner, feeding casebearer, overwintering casebearer, or pupa.

One prism trap (Alpha Scents Inc., Portland, OR) with a sticky card was placed at each of the three study sites. Traps were baited with larch casebearer sex pheromone (McMillian & Borden, 1974; Priesner *et al.*, 1982) and were deployed from May 19, 2016 until November 9, 2016. A second trap was added to each site on May 25. Traps were checked weekly and pheromone baits were replaced every 2-3 weeks. Abundance of all phenological stages was expressed as individuals per gram of foliage, except for overwintering larvae and adults, which were expressed on a per bud and per trap basis, respectively. HOBO Data-loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) were placed at each field site inside radiation shields attached to a branch 2 m above ground. Loggers recorded daily maximum and minimum temperatures. Daily degree-days (DDs) above a chosen base temperature (T_{base} = 5°C) required for bud break after January 1 for eastern larch and February 20 for larch casebearer were estimated from paired daily minimum-maximum temperatures using a simple degree-day calculation:

$$\frac{T_{max} + T_{min}}{2} - T_{base}$$

Phenological simulations

We asked if changes in historical accumulation of DDs, as one aspect of climate change, could moderate the proportion of casebearer populations reaching the overwintering stage. We compiled daily temperature data spanning 1964-2016 at a weather station in Grand Rapids, Minnesota, USA (Figure 5.1) from the National Oceanic and Atmospheric Administration (NOAA, 2017). For each year of climate data, we used degree-days models based on our field phenology data to estimate the day at which 50% of spring feeding larvae, the F_0 population in each year, would have been active (i.e., the day of median activation). We used models estimating the day at which 50% of eastern larch trees break bud (= day of median bud break; Chapter 4) to estimate phenological synchrony (= day of median activation by larvae – day of median bud break). For each year of climate data we also estimated the proportion of larvae in the F_1 population that would have reached the overwintering stage as a function of DDs accumulated after February 20. Thus, this model captured the role importance of development by both spring larvae and their offspring. We assumed that foliage was available for development until November 30 of each year. Thus, the proportions of larvae reaching the overwintering stage each year were estimated by substituting the number of DDs accumulated between February 20 and November 30 (base =5.0 °C) into our models linking cumulative abundance of overwintering larvae with accumulation of DDs.

Effects of warming in autumn

An experiment was commenced in autumn 2016 to evaluate changes in spring activation of larvae exposed to warm temperatures in autumn vs. larvae stored outside at cooler ambient temperatures. On September 30, 2016, 12 branches approximately 30 cm in length with actively feeding larvae were collected from the Jacobson site. Branches were placed into 1 L glass jars with tap water immediately after cutting and stored in a roofless, wooden cold frame outdoors on the Saint Paul campus of the University of Minnesota, MN, USA. On October 12, 2016, the glass jars with twigs and larvae were transferred into a heated greenhouse where larvae were permitted to feed and go dormant until the experiment was initiated on November 10. From October 12 – November 9, mean temperature in the greenhouse was 18.3 ± 0.2 SE °C (range:16.7 – 20.2, 53% RH). On November 6, several hundred dormant larvae were collected from 60 trees at the Jacobson site. Between 2-4 live twigs per tree with larvae within 2 m of the ground were collected. From October 12 – November 5, mean temperature was 6.2 ± 0.6 °C (range: 1.2 – 10.8, 76% RH) at the Jacobson site. All insects were left attached to twigs and placed in a well-ventilated plastic container (volume: 17L, 46 × 31.1 × 19.4 cm) inside of the cold frame, which experienced a mean temperature of 9.8 ± 2.2 °C (range: 7.6 – 11.9, 74% RH) from November 6 to November 9. On November 10, 40 dormant larvae from the heated greenhouse and 100 dormant larvae from the November 6 collection (i.e., insects exposed to cooler temperatures than those in the greenhouse) were placed separately in groups of four to six in Petri dishes and transferred into a plastic container in the cold frame (volume: 17L, 46 × 31.1 × 19.4 cm). Larvae were monitored for activation every 2-3 days beginning in March and proportion activating and DDs after February 20 required to activate were recorded. All insects were exposed to natural photoperiods across the study.

Effects of warming in winter

We also conducted a study in winter 2017 to evaluate changes in spring activation of larvae exposed to warm temperatures in winter vs. larvae stored outside at ambient temperatures. Larvae were collected as part of the November 6 collection described above for the study on the effects of autumnal warming. On January 13, 2017, we transferred 402 larvae from the plastic containers in the cold frame into Petri dishes in groups of 4-6. Of those larvae, 160 were stored outside in the plastic container in the cold frame (-0.6 ± 0.5 °C, range: -12.5 – 8.2, 76% RH, natural photoperiod), 122 were placed into the greenhouse (18.6 ± 0.3 °C, range: 14.2 – 24.6, 30% RH, natural photoperiod), and 120 into a growth chamber held at 12L:12D (20.8 ± 0.02 SE °C, 77% RH). Forty larvae were transferred on day zero (January 13) and then approximately sixty larvae (~twenty from each storage location) were transferred to a growth chamber held at 18L:6D (20.0 ± 0.09 SE °C, 63% RH) (i.e., conditions known to facilitate activation) every two weeks from January 13 to April 3, 2017. Larvae were monitored every 2-3 days for activation and the proportion of larvae activated and DDs required to activate after transfer were recorded. Thirty larvae (< 8%, all stored in either the 12L growth chamber or greenhouse) activated before transfer to the 18L:6D growth chamber and were excluded from analysis.

Statistical analysis

Data were combined from all three sites for analyses. Weight of foliage (g per linear cm of branch) was modeled as a function of DDs using a generalized additive model. Sequential field sampling revealed seven distinct life history stages: larvae feeding in spring, pupae, adults, eggs, needle mining larvae, autumnal larvae in cases, and overwintering larvae in cases. Generalized linear mixed-effects models were used to predict cumulative abundance of each stage as a function of accumulated DDs. Terms for site and tree were included in the model as random effects and a binomial error structure and logit link function were used. Due to a lack of fit for logistic models of abundance of pupae and eggs, two generalized additive models were also fit. Otherwise, emphasis is put on logistic models for other life stages due to ease of use and interpretation. Separate simple linear regressions were used to assess the effects of year on spring phenology (i.e., days between bud break and larval feeding) and proportion of larvae reaching the overwintering stage. Proportions for the latter were logit-transformed prior to analyses (Warton & Hui, 2011).

Results of the autumn warming study were analyzed in a mixed-effects framework. The roles of exposure location (outside vs. greenhouse) in the proportion of larvae activated and DDs required to activate after February 20 were evaluated using a generalized linear mixed-effects model and linear mixed-effects model, respectively. For the former, a binomial error structure and a logit link function were used. In both models, a term for Petri dish was fit as a random intercept.

Results of the winter warming study were also analyzed in a mixed-effects framework. The roles of exposure location, transfer day, and their interaction in the proportion of larvae activated and DDs required to activate after transfer were evaluated using a generalized linear mixed-effects model and linear mixed-effects model, respectively. For the generalized mixed-effects models, a binomial error structure and a logit link function were again used and both models included a term for Petri dish fit as a random intercept. All analyses were completed using R statistical software (R Core Team, 2017) and used $\alpha = 0.05$ to determine statistical significance. Generalized additive models were fit using the mgcv package (Wood, 2011) and mixed effects models were fit using the lme4 package (Bates *et al.*, 2013) using Satterthwaite's approximation for the degrees of freedom implemented via the lmerTest package (Kuznetsova *et al.*, 2014). Model assumptions of homoscedasticity and normality of errors were checked via graphical inspection of residuals.

Results

Field phenology

All trees had broken bud with visual evidence of minute green needles beginning to flush prior to sampling for larch casebearer, which began on April 19. At this point, 61 DD had accumulated. Needle weights were approximately 0g until May 5, despite the presence of very minute needles in recently broken buds. The relationship of larch foliage, measured as weight of needles per length of branch, to DDs was trapezoidal with sharp increases and decreases in spring and autumn, respectively (Figure 5.2a).

Actively feeding casebearer life stages were detected between 105 and 1900 DDs, corresponding to sampling days of May 5 and October 20. The cumulative abundance curves for all casebearer life stages were significantly and positively associated with DDs (Figure 5.2b-h). The date of first observation along with predictions from generalized additive models for 5%, 50%, and 95% cumulative abundance for each life stage are provided in Table 5.1. Model statistics for generalized additive models of needle weight and cumulative abundance of pupae and eggs are provided in Table 5.2. Increases in foliage following spring bud break and decreases in foliage in autumn were concomitant with the appearance of feeding and overwintering larvae, respectively (Figure 5.3).

Phenological simulations

Grand Rapids was chosen as a focus of phenological simulations because defoliation of eastern larch by larch casebearer has been detected in the region frequently via aerial surveys since 2000 (Figure 5.1). Phenological synchrony was estimated using our model of cumulative abundance of spring feeding larvae (Figure 5.2b) and degree-day models estimating median bud break by eastern larch trees (Chapter 4). Estimated phenological synchrony has not changed significantly through time (model statistics: $F_{1,50} = 1.628$, $P = 0.21$). That is, the amount of time between foliage flushing and spring activation of overwintering larvae of larch casebearer has not changed from 1964-2016; the estimated median activation of larvae has consistenly lagged 28 ± 1 SE days behind median bud break (range: 12 – 52; Figure 5.4a).

Even though the amount of time between spring needle flush and spring activation of larch casebearer has not changed through time, we found that the estimated proportion of larvae that successfully reach the overwintering stage each fall has increased through time over the past five decades (Figure 5.4b). Before 1975, less than 25% of actively feeding larvae were likely to successfully develop to overwintering stages, on average. Since 2000, however, this proportion has increased to more than 90%. These estimates were derived from the phenology model estimating the cumulative abundance of overwintering cases (Figure 5.2h).

Effects of warming in autumn

Larvae that had entered the overwintering phase and were stored outside at ambient temperatures for the entirety of autumn were significantly more likely to activate than insects reared to overwintering in the greenhouse under warmer conditions for one month in autumn (Figure 5.5a). That is, 89.5 ± 3.1 SE % of the insects stored outside activated compared to 55.0 ± 7.9 SE % of insects stored in the greenhouse. We did not determine if this difference was due to mortality rather than terminal diapause. Warm exposure in autumn delayed spring activation, as insects stored outside required 255.1 ± 13.4 SE DD for activation compared to 362.6 ± 35.2 SE for insects stored in the greenhouse (Figure 5.5b). For larvae stored outside in autumn, larval activation was first observed on April 19 and continued until June 12. Insects exposed to the warm autumn treatment activated from April 26 until June 21.

Effects of warming in winter

Likewise, warm exposure during winter decreased activation and negatively impacted developmental rate. Because insects were transferred to a growth chamber that facilitated activation (18L:6D), larval activation was observed from January 20 to April 26. Across the entire study, 89.4 ± 2.4 SE % of insects stored outside activated compared to 27.7 ± 4.2 SE % and 44.0 ± 5.0 SE % of larvae stored in the greenhouse and 12L growth chamber, respectively. However, the effect of winter warming depended on time of transfer; insects transferred from outside were increasingly more likely to activate through time (Figure 5.6a) whereas insects stored in the greenhouse (Figure 5.6b) or 12L growth chamber (Figure 5.6c) were less likely to activate through time. On average, insects stored outside required 304 ± 7 SE DDs to activate, compared to 298 ± 18 SE DDs and 270 ± 15 SE DDs required for insects transferred from the greenhouse and 12L growth chamber, respectively. The effect of winter warming again depended on time of transfer; insects transferred from outside (Figure 5.6d) and the greenhouse (Figure 5.6e) required fewer DDs to activate through time compared to insects from the 12L growth chamber (Figure 5.6f), which required more degree-days to activate through time (Table 5.3).

Discussion

The timing of the onset and end of the growing season, in addition to its length, can significantly affect insect population dynamics (Volney & Fleming, 2000; Bale *et al.*, 2002). Our study suggests that increasing mean temperatures of growing seasons contribute to the annual survival of this invasive defoliator. In a region where larch casebearer has been detected via aerial surveys since 2000 in Minnesota (Figure 5.1), no significant changes in estimated spring phenological synchrony have occurred since the 1950s (Figure 5.4a), but estimated proportions of the population successfully reaching the overwintering stage has increased (Figure 5.4b). Thus, warming during spring and the growing season that accelerates development of the F_0 and F_1 generations, rather than changing spring phenological synchrony, are likely important for casebearer population dynamics. As host trees enter dormancy each autumn, larvae still developing as needle miners face increased risk of overwintering mortality if they are dropped with needles from the tree as larches are deciduous conifers. At present, the length of time required for actively feeding, autumnal larvae in cases (Figure 5.2g) to attach to branches and enter diapause is unknown, but could refine predictions of the impact of the impact of growing season length on population dynamics of this insect.

Interactive effects of photoperiod with temperature across either the host or insect likely also influence the proportion of larvae that complete development to the overwintering stage. Eastern larch may be responsive to photoperiod for dormancy, similar to European larch (Migliavacca *et al.*, 2008; Moser *et al.*, 2009). However, another congener of eastern larch, alpine larch *Larix lyallii*, drops needles in response to temperature and the role of photoperiod appears limited (Worrall, 1993). In forests of western larch, larvae at different elevations with presumably distinct climatic regimes appear to enter the casebearing stage synchronously (Ryan, 1974b), suggesting that photoperiod is more important to the biology of larch casebearer than the host. Indeed, in the laboratory, larvae do not complete their life cycle without sufficient exposure to long (18L:6D) and short (12L:12D) photoperiods (Ryan, 1975).

Some insects display a critical stage, such that the exposure to certain photoperiods during that stage induces diapause (Tauber & Tauber, 1976; Tobin *et al.*, 2008). Our field study corroborates previous laboratory studies that indicate larch casebearer is strictly univoltine with an obligate diapause (Ryan, 1975). At present, it is unknown if larch casebearer exhibits a critical photoperiod-sensitive life stage. If it does, then climate-driven changes in summer developmental rates could expose the life stage(s) to alternate photoperiods to which the critical stages have not been historically exposed (Tobin *et al.*, 2008). Such exposure could, in turn, moderate incidences of diapause. Thus, interactions of temperature and photoperiod could influence the incidence and/or depth of diapause, and thus moderate the length of the growing season rather than changing voltinism.

Factors that moderate the depth of diapause may be important determinants of spring activation and associated plant-insect phenological synchrony (Van Asch & Visser, 2007; Kharouba *et al.*, 2015; Fuentealba *et al.*, 2017). We found that larvae exposed to warm temperatures in autumn and winter activated later and at lower percentages than insects stored outside, suggesting that heat-injury may have reduced survival and delayed development of the survivors (Colinet *et al.*, 2015). Shifts in the timing of spring activation by casebearers in response to pre-spring warming could ultimately influence the length of the growing season. For example, our data suggest that an anomalously warm autumn could delay activation by over 100 DDs, which would truncate the DDs available for pupal-adult development and egg-casebearer development of the subsequent generation. Further work is needed to determine the range of temperatures during diapause that slow development or influence its depth.

Increases in heat accumulation may drive latitudinal or elevational shifts in the ranges of some insects (Parmesan *et al.*, 1999; Battisti *et al.*, 2005; Wilson *et al.*, 2007; Chen *et al.*, 2011). While there may be several northern locations throughout the range of eastern larch where larch casebearer does not accumulate sufficient degree-days to reach the overwintering stage, our finding that higher estimated proportions of larvae reach the overwintering stage in warmer growing seasons may cause concern for further range and/or host shifts for this invasive insect in North America. For example, larch casebearer has never been detected on alpine larch and has not infested western larch across the northern limits of that species' distribution, presumably due to late spring and/or early autumnal frosts (Tunnock & Ryan, 1985) and/or insufficient growing DDs. Moreover, the northern extent of larch casebearer's range on eastern larch is not well described. Given that global climate has facilitated latitudinal and elevational shifts of other western forests insects (Logan & Powell, 2001; Logan *et al.*, 2010; Sambaraju *et al.*, 2012), previously uninhabited sites at high elevations or latitudes such as stands of alpine larch likely face increased risks from this invasive insect with warmer growing seasons.

In summary, the resurgence of larch casebearer appears to be facilitated, in part, by longer and warmer growing seasons. Warming during the growing season can influence insect population dynamics in several ways depending on how different trophic levels respond (Harrington *et al.*, 1999; Bale *et al.*, 2002; Jamieson *et al.*, 2012). Further work is needed to disentangle the responses of eastern larch to climate, including how warmer growing seasons might impact nutritional and defense status of host trees that influence developmental rates of larch casebearer (Scriber & Slansky, 1981). Changes in spring activation or fall dormancy can drive shifts in growing season length (Gallinat *et al.*, 2015) and, depending on phenological responses of eastern larch, may determine growing season length for larch casebearer. Given our findings that greater estimated proportions of larvae are reaching the overwintering stage, no changes in phenological synchrony were observed, and that larch casebearer is very robust to changes in spring phenological synchrony (Chapter 4), it appears that changes in timing of dormancy in autumn by larch casebearer relative to eastern larch may be an important driver of casebearer population dynamics. Such phenomena are likely contributing to the resurgence of this invasive defoliator on eastern larch in several parts of the invaded range in North America, raising concerns for further geographic and/or host range expansions.

Acknowledgements

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Tables

Table 5.1 Date of first observation and estimated cumulative emergence (5%, 50%, and 95%) of life stages of larch casebearer across three sites in Minnesota, USA., 2016. See Figure 5.2 for graphical representation of statistical functions.

Life stage	First observed	Predicted DDs		
		5%	50%	95%
Spring larvae	May 5	61	244	427
Pupae	May 12	278	482	1,728
Adults	May 25	507	712	917
Eggs	Jun 17	610	893	1,840
Miners	Jun 8	851	1,383	1,916
Autumn larvae in cases	Aug 18	1,617	1,751	1,885
Overwintering larvae in cases	Aug 18	1,820	1,888	1,956

Table 5.2 Model statistics for generalized additive models in Figure 5.2. The models were fit using a generalized additive model framework with needle weight (panel a) or cumulative proportional abundance of insect life stage (panels b,d) as a function of degree-days (base = 5°C) accumulated since Janaury 1 for eastern larch and February 20 for larch casebearers.

Panel	Response	Intercept ± SE	S(DDs)[a]	
			χ^2	R^2
a	Needle weight	0.026 ± 0.001***	400***	62.0
c	Pupae	0.498 ± 0.013***	19,180***	98.0
d	Eggs	0.429 ± 0.009***	39,931***	98.6

*$|Z| \geq 1.96$ and/or $P \leq 0.05$

**$|Z| \geq 3.30$ and/or $P \leq 0.001$

***$|Z| \geq 3.90$ and/or $P \leq 0.0001$

[a] Smoothing parameter fit in generalized additive model

Table 5.3 The effect of warm temperature exposure during winter on the proportion of larvae activated and degree-days required to activate by larch casebearer. Models were fit using a mixed-effects framework with a term for Petri dish fit as a random intercept.

Response	Predictor	Coefficient	SE	|Test statistic|	*P*
Proportion activated[a]	Intercept	1.71	0.44	$Z = 3.85$	0.0001
	Growth chamber (vs. outside)	1.22	0.92	$Z = 1.33$	0.18
	Greenhouse (vs. outside)	2.261	0.98	$Z = 2.32$	0.0205
	Day of transfer	0.028	0.01	$Z = 2.03$	0.0428
	Greenhouse × Day of transfer	-0.178	0.04	$Z = -4.54$	<0.0001
	Growth chamber × Day of transfer	-0.160	0.03	$Z = -4.98$	<0.0001
DDs required to activate (base = 5°C)	Intercept	354.76	15.58	$t_{36} = 22.77$	<0.0001
	Growth chamber (vs. outside)	-23.69	52.51	$t_{44} = -0.56$	0.58
	Greenhouse (vs. outside)	-124.02	35.98	$t_{40} = -3.45$	0.0013
	Day of transfer	-1.54	0.36	$t_{34} = -4.24$	0.0002
	Greenhouse × Day of transfer	-1.09	2.19	$t_{75} = -0.50$	0.62
	Growth chamber × Day of transfer	3.63	1.42	$t_{61} = 2.56$	0.0129

[a] Model fit with a binomial error structure and logit link function.

[b] Model fit using a linear mixed-effects model framework. Degrees of freedom for test statistics have been rounded down to nearest integer.

Figures

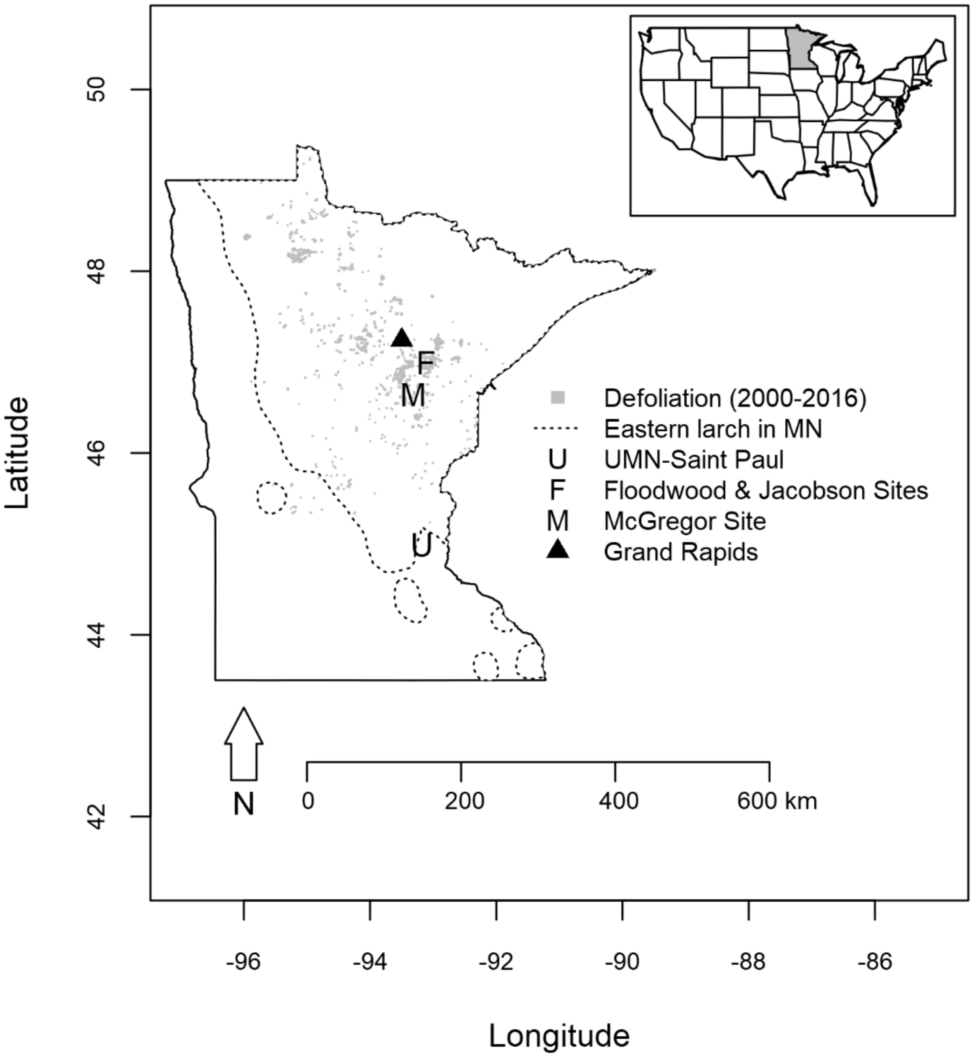

Figure 5.1 Map of study area in Minnesota, USA, showing range of eastern larch, locations of defoliation records since 2000, field sampling sites, and weather station in Grand Rapids, MN.

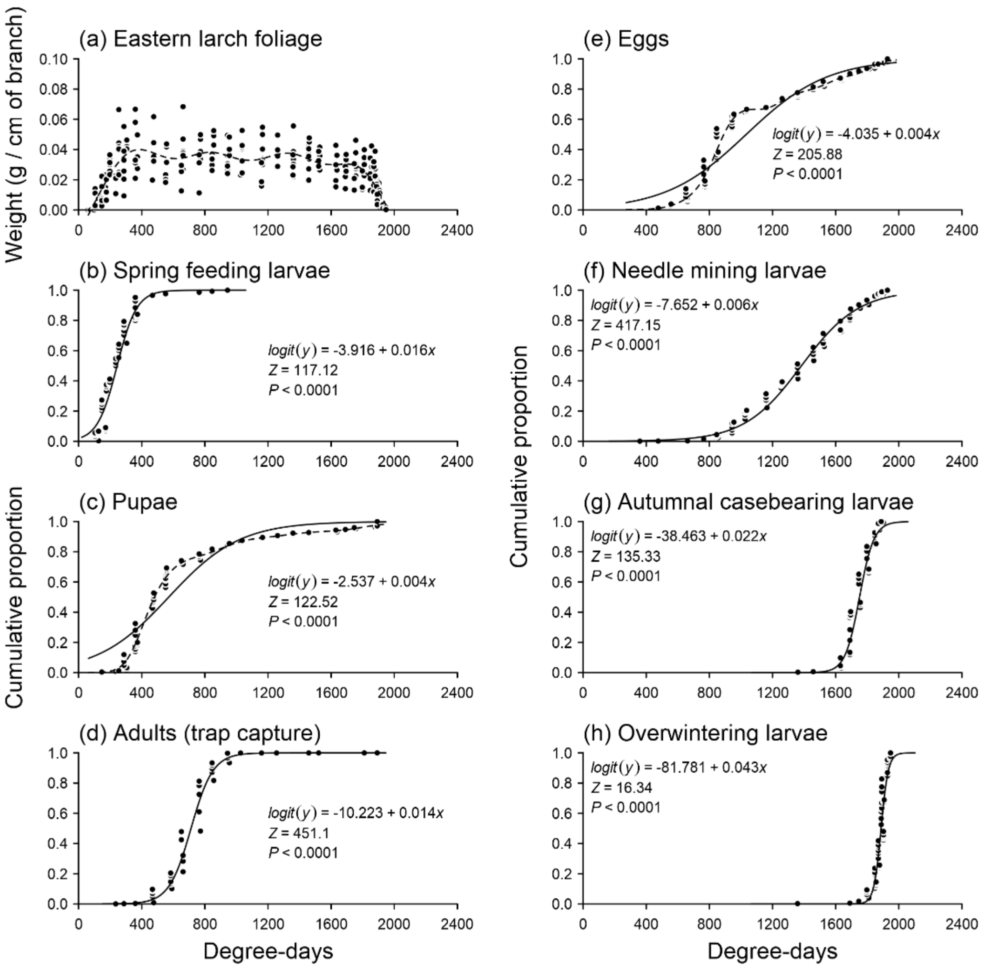

Figure 5.2 Cumulative abundance of larch casebearer life stages from April 19 to November 9, 2016 as a function of degree-days (base = 5°C) across three sites in Minnesota, USA (see Figure 5.1 for site locations). Solid lines are fits from generalized linear mixed-effects models with binomial errors and logit link functions. Dashed lines (panels a, c, and e) are fits from generalized additive models. Abundance of all phenological stages were expressed per gram of foliage, except overwintering larvae and adults, which were expressed per bud and per trap, respectively.

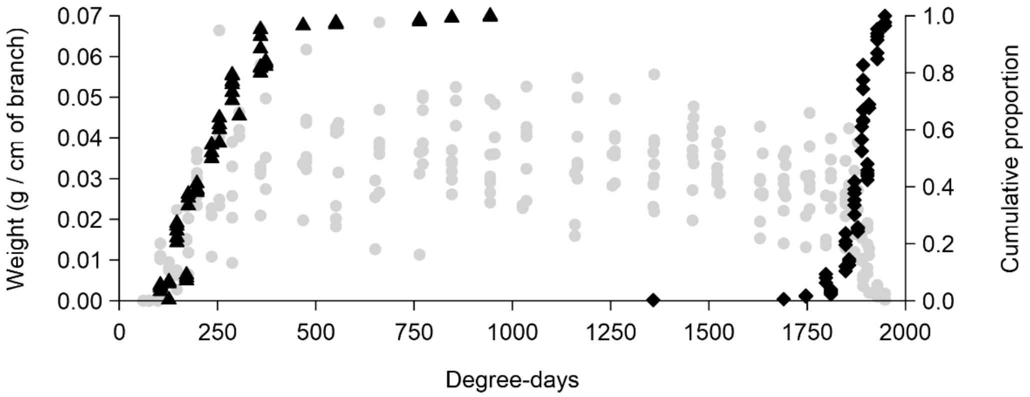

Figure 5.3 Grey circles indicate needle weights (left y-axis) as a function of degree-days at three study sites in northern Minnesota, 2016 based on weekly sampling throughout the growing season (19 April – 9 November 2016). Triangles indicate larvae feeding in spring whereas diamonds indicate overwintering larvae in cases appearing in autumn (right y-axis).

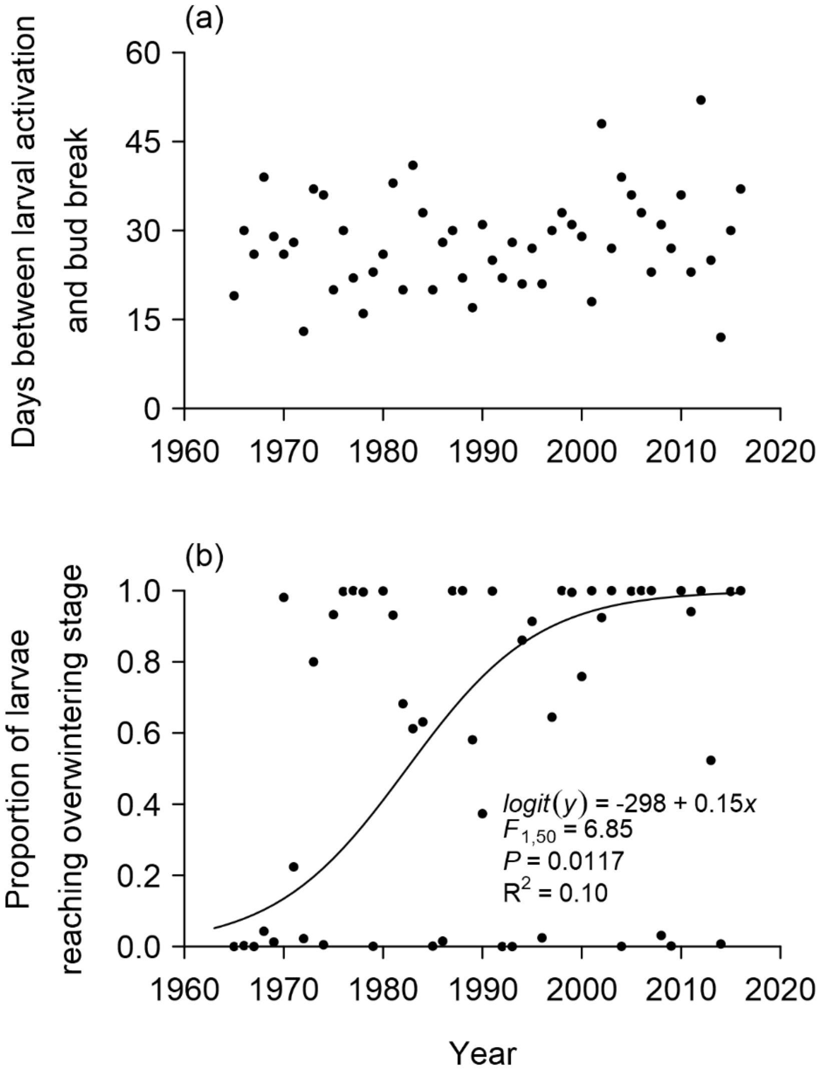

Figure 5.4 (a) Spring phenological synchrony (i.e., the length of time between spring needle flush by eastern larch and subsequent activation of overwintering larch casebearer larvae) and (b) proportion of larvae accumulating sufficient degree-days (base = 5°C) after February 20 to reach the overwintering stage as a function of year between 1964-2016 in Grand Rapids, Minnesota, USA.

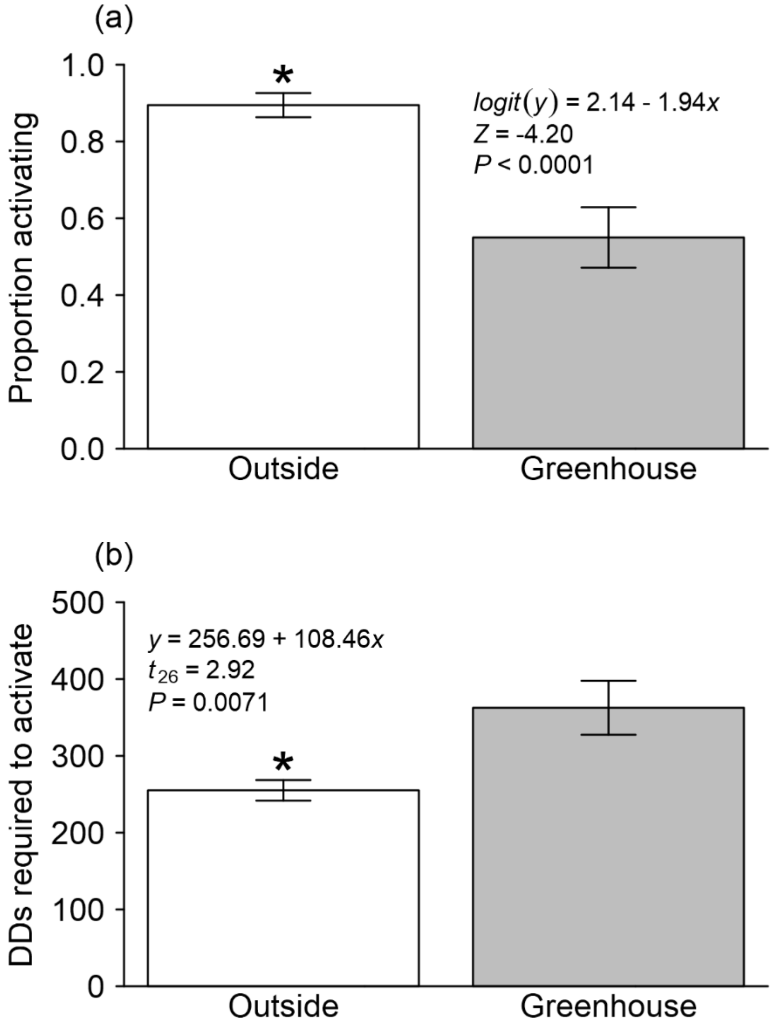

Figure 5.5 Differences in (a) proportion of larvae activating and (b) DDs required to activate (base = 5°C) after February 20 by exposure location (outside vs. greenhouse) during previous autumn. In equations, *x* is equal to one when larvae were stored in the greenhouse and zero if stored outside. Test statistics on graph pertain to slope coefficients for *x* in equations; * indicates significant difference between groups.

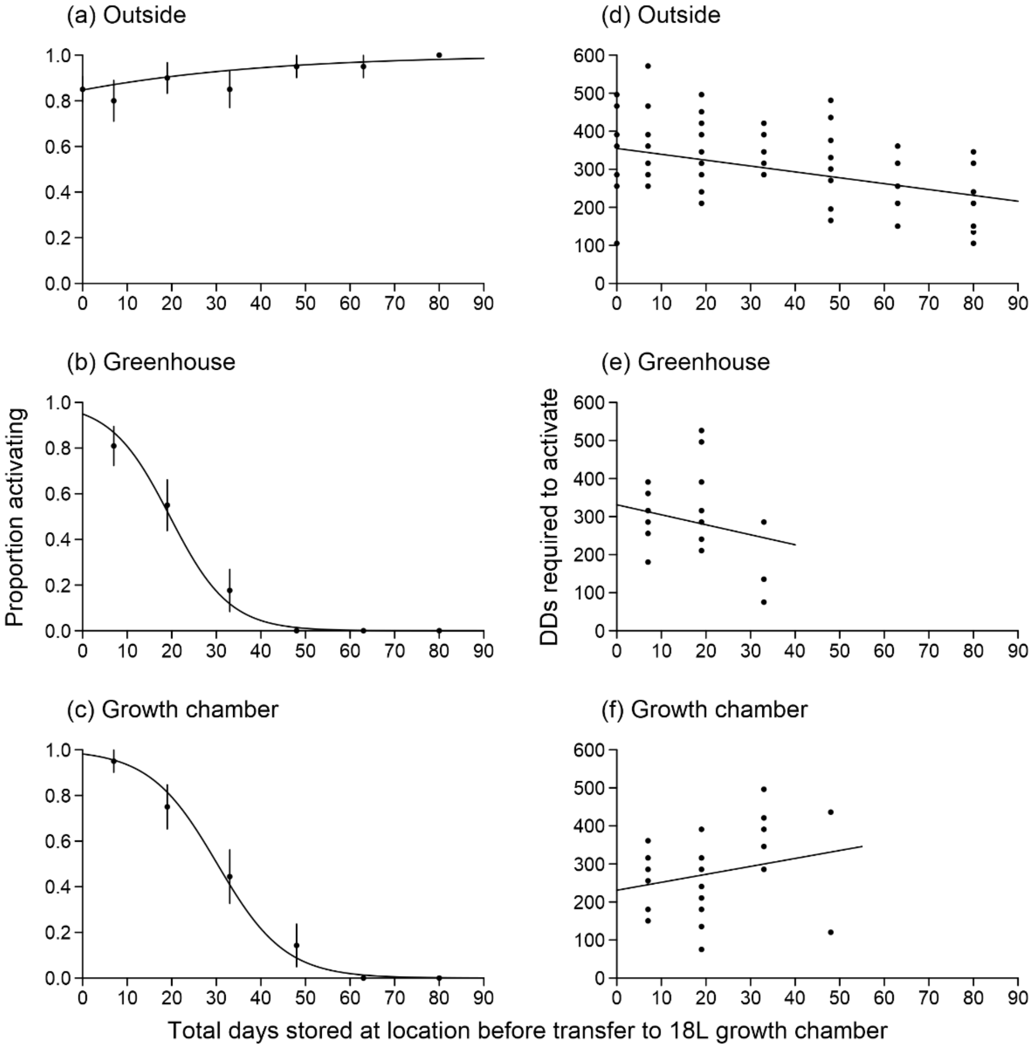

Figure 5.6 Proportion of larvae activating (panels a-c) and DDs required to activate (base = 5°C; panels d-f) after transfer as a function of exposure location (outside, greenhouse, or growth chamber) in winter and days stored at each location. Fit lines in panels a-c are from a generalized linear mixed-effects model with a logit link function and binomial error structure and fit lines in panels d-f are from a linear mixed-effects model. Model summary statistics are provided in Table 5.3.

Chapter 6 Predisposing factors for landscape-scale outbreaks by an invasive defoliator and a native, tree-killing bark beetle

Coauthors: Brian H. Aukema

Preface

Biotic disturbances such as insects are highly responsive to climatic change and can have widespread ecological and economic impacts on forests. Quantifying the responses of introduced and native insects to climate, including how dynamics of one agent may moderate those of another, is important for forecasting forest ecosystem function and structure. We investigated drivers of outbreaks by larch casebearer, an invasive defoliator, and eastern larch beetle, a native tree-killing bark beetle, in a lowland conifer ecosystem between 2002-2016 in Minnesota, USA. For each insect, we evaluated the explanatory power of temporal, spatial, and climatic predictors in landscape patterns by developing spatiotemporal autologistic models. Models were fit using rasterized aerial survey data (i.e., polygons identified by sketch-mappers surveying from fixed-wing aircrafts). Climatic variables included indicators of minimum temperatures, growing season length and intensity, and precipitation (drought/flooding). The role of defoliation by larch casebearer, which may predispose trees to attack by secondary agents, in tree-killing outbreaks of eastern larch beetle was also investigated. For both species, the most important predictors of an outbreak pertained to the proximity of other conspecific outbreaks in space and time. For larch casebearer, outbreaks were more likely with warmer Octobers, warmer Septembers, greater degree-day accumulation across the growing season, and greater total precipitation in May. The dynamics of eastern larch beetle were influenced by several climatic predictors pertaining to growing season length

and drought; warmer and dryer years were positively associated with outbreaks. We also found that beetle outbreaks were positively correlated with defoliation by larch casebearer. Our results demonstrate that climate can drive large scale outbreaks of introduced and non-native disturbance agents on a single host species, and that interactions at the tree level between such agents may scale up to manifest across large temporal and spatial scales.

Introduction

Climate change is likely to alter patterns of forest disturbance and cause widespread and long-lasting impacts on the ecology of forest ecosystems and the services they provide (Ayres & Lombardero, 2000; Dale *et al.*, 2001; Weed *et al.*, 2013). Forests experience a variety of natural disturbances such as fire, drought, insects, pathogens, hurricanes, windstorms, ice storms and landslides (Dale *et al.*, 2001). Disturbances by insects can impact nutrient cycling, succession, and species composition (McCullough *et al.*, 1998; Kurz *et al.*, 2008; Hicke *et al.*, 2012) and provide pulsed resources for other trophic levels such as soil microbes and mammalian and avian predators (Yang, 2004; Yang *et al.*, 2008). Such impacts can be difficult to predict when native flora and fauna have not coevolved with the introduced species (Kenis *et al.*, 2009). Understanding how to forecast and balance risks posed by different disturbance agents and the potential for interactions between agents to modulate the severity of impacts is critical for effective forest management under global climate change (McCullough *et al.*, 1998; Dale *et al.*, 2001; Millar *et al.*, 2007; Nealis *et al.*, 2009).

Climate is an important driver of the spatiotemporal patterns of both native and non-native insects. For example, outbreaks of mountain pine beetle, *Dendroctonus* *ponderosae* Hopkins (Coleoptera: Curculionaide) are driven by warmer temperatures (Aukema *et al.*, 2006, 2008; Sambaraju *et al.*, 2012) and drought (Chapman *et al.*, 2012) and outbreaks of spruce beetle, *Dendroctonus rufipennis* Hopkins (Coleoptera: Curculionaide), have been correlated with decreases in precipitation (Hart *et al.*, 2014, 2017). Temperature is associated with changes in the range boundaries of the non-native gypsy moth, *Lymantria dispar dispar* Linnaeus (Lepidoptera: Erebidae), in North America (Tobin *et al.*, 2014) and moderates spread rates of *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) in the southern hemisphere (Lantschner *et al.*, 2014). Biotic disturbances may also interact, as defoliation can facilitate the colonization of host trees by bark beetles (Wright *et al.*, 1984; Raffa *et al.*, 1998; Wallin & Raffa, 2001), potentially by influencing subcortical plant defense systems (Wallin & Raffa, 1999). Interactions at the tree level may scale up to manifest across large geographic scales (Goodsman *et al.*, 2015) and such interactions may complicate predictions of disturbance (Dale *et al.*, 2001). Unfortunately, there are few studies of interacting biotic disturbance agents at the landscape level, especially from different feeding guilds or across native/invasive species (Goodsman *et al.*, 2015).

In this paper, we study outbreak dynamics and landscape-scale interactions between a non-native defoliator, larch casebearer (*Coleophora laricella* Hübner; Lepidoptera: Coleophoridae), and a native, tree killing bark beetle, eastern larch beetle (*Dendroctonus simplex* LeConte; Coleoptera: Curculionidae), that is deviating strongly from historical patterns of behavior (McKee & Aukema, 2016). These insects are sympatric across forests of eastern larch or tamarack, *Larix laricina* (Du Roi) K. Koch (Pinaceae), an early successional species that is an important component of lowland conifer forests in the Great Lakes Region of North America (Duncan, 1954). The southern margin of tamarack in North America stretches through the state of Minnesota, USA.

The invasive defoliating moth, larch casebearer, is a needle-miner native to Europe but became established in North America in the late 1800s. Larch casebearer was discovered near Northampton, Massachusetts attacking planted European larch, *Larix decidua* Mill. (Pinaceae) (Hagen, 1886). The insect then spread throughout most of the ranges of tamarack and eventually became established on western larch, *Larix occidentalis* Nutt. (Pinaceae), in the late 1950s (Tunnock & Ryan, 1985). After years of successful importation biological control, larch casebearer has appeared to undergo a resurgence in parts of its invaded range in tamarack forests of Minnesota (Chapter 1) For example, aerial surveys conducted via fixed wing aircraft in Minnesota first detected defoliation by larch casebearer in 2000, even though the insect has been present in Minnesota for several decades (Cody *et al.*, 1967; Tunnock & Ryan, 1985). Since 2000, 672 to 10,382 ha of defoliation by larch casebearer have been mapped each year (Figure 6.1).

Sympatric with larch casebearer in the Great Lakes Region, eastern larch beetle is a native, tree-killing bark beetle that attacks tamarack (Wood, 1982). Eastern larch beetle is distributed across the range of tamarack, which extends from Alaska, across the boreal forest of Canada and the Great Lakes Region of the United States, to eastern Canada and Northeastern United States (Burns & Honkala, 1990; Seybold *et al.*, 2002). Outbreaks of eastern larch beetle have historically lasted 2-3 years, and have been associated with trees weakened by drought, flooding, defoliation and/or other stressors (Werner, 1986; Langor

& Raske, 1989). An outbreak was first detected via aerial surveys in 2002 in Minnesota, within a few years of the first detections of larch casebearer. Since then, eastern larch beetle has killed between 519 and 85,444 ha of mature tamarack annually in Minnesota (Figure 6.1), totaling more than 426,000 m^3 of lost volume (Crocker *et al.*, 2016). This outbreak is longer than any previous known outbreak in the previous century (McKee & Aukema, 2016).

Successive years of defoliation by larval feeding of larch casebearers may decrease growth (Tunnock *et al.*, 1969) and/or kill host trees (Craighead, 1950; Dowden, 1957). Defoliation has been hypothesized to predispose trees to attack from secondary agents such as eastern larch beetle in eastern larch forests and western larch borer (*Tetropium velutinum* LeConte; Coleoptera: Cerambycidae) and root rots in western larch forests, although such interactions have not been well studied (Tunnock *et al.*, 1969; Tunnock & Ryan, 1985; Benoit & Blais, 1988). Competing hypotheses suggest that mechanical defoliation of tamarack can increase concentrations of monoterpenes in oleoresin that hinder population growth rates of eastern larch beetle, for example (Werner, 1995). However, differences may exist among species of defoliators and between mechanical and herbivore-caused defoliation (Krause & Raffa, 1992).

In this work, we analyzed aerial survey data documenting patterns in defoliation and mortality from 2000-2017 on tamarack in Minnesota (Figure 6.2). To understand drivers of the anomalous dynamics of both larch casebearer and eastern larch beetle and quantify sensitivity of both species to climate, we conducted parallel analyses to elucidate 1) climatic predictors of spatiotemporal patterns of larch casebeaer and 2) the role of climate and defoliation by larch casebearer in spatiotemporal patterns of eastern larch beetle. Larch casebearer appears sensitive to climate, as cold temperatures in autumn and spring and wet springs are implicated in moderating the insect's population dynamics (Tunnock & Ryan, 1985) and greater accumulation of growing degree-days has been linked to greater annual survival (Chapter 5). Likewise, eastern larch beetle is highly responsive to temperature (Mckee, 2015; Mckee & Aukema, 2015), and defoliation by larch casebearer has been hypothesized to moderate beetle attack dynamics (Benoit & Blais, 1988). Understanding climatic drivers of these two disturbance agents will aid in forecasting outbreaks and, because both insects can cause and/or facilitate mortality, forecasting their outbreaks will aid in predicting vegetation dynamics of tamarack under global climate change scenarios.

Materials and Methods

Data collection and processing: disturbance agents

We analyzed spatial polygons of insect activity sketched by the USDA Forest Service and Minnesota Department of Natural Resources during annual aerial surveys conducted via fixed wing aircraft. Aerial surveys for several native and non-native forest disturbance agents have been conducted in Minnesota since the mid-1950s (Minnesota Department of Natural Resources, 2016). Larch casebearer has been detected each year since 2000, resulting in a time series from 2000-2016 with hectares of defoliation ranging from 672 to 10,382 ha per year. From 2002-2017, 519 to 85,444 ha of mortality attributed to eastern larch beetle has been mapped each year in Minnesota (Figure 6.1). The year of observed mortality by eastern larch beetle was lagged by one year to the previous year to reflect the ecology of eastern larch beetle, as there is a one year delay between beetle colonization of the tree and visually apparent crown fading noted in aerial surveys

(Meddens & Hicke, 2014; Mckee, 2015; Hart *et al.*, 2017). As a baseline estimate of spatial association between these two disturbance agents, percent overlap of spatial polygons attributed to the two agents was calculated via the gArea() command via the GISTools package in R (Brunsdon & Chen, 2014).

For each disturbance agent, all spatial polygons were rasterized using 250 × 250 m grid cells in a geographic information system as follows. The spatial polygons were overlaid separately onto 250 ×250 m grid cell rasters. Each cell was then subdivided into 100 smaller subcells. Cells were assigned a "1" if any of the centroids of the 100 subcells occurred within a mapped polygon. The rasterization step using small grid cells decreased the likelihood that any small polygons would occur between centroids and fail to be included in our final raster. Cells were then aggregated to produce approximately 4 × 4 km cells, which enabled us to interface the disturbance data with climate data (see below). The rasterization process was conducted separately for larch casebearer and eastern larch beetle each year, such that each cell had a 17-year (2000-2016) and 16-year (2001-2016) time-series for larch casebearer and eastern larch beetle, respectively. Polygons and rasters were both projected in North American Datum, 1983, Universal Transverse Mercator (UTM) Zone 15.

A layer of tamarack basal area for the United States was obtained from the USDA Forest Service Forest Inventory and Analysis database (Figure 6.2a; USDA Forest Service 2017). Tamarack is the only host for both larch casebearer and eastern larch beetle in Minnesota, and thus analyses were limited to areas with a mean basal area of 0.1 m^2 of tamarack per 4 × 4 km cell. This threshold was chosen to preclude inferences on regions that may be climatically suitable for outbreak initiation and persistence but lack suitable hosts.

Data collection and processing: climatic data and predictors

We obtained temperature and precipitation data from the PRISM climate database (PRISM, 2017). Data were downloaded and processed via the prism package in R (Hart & Bell, 2015). Both daily and monthly prism data from 1997-2016 were downloaded at a 4 × 4 km resolution. To ensure spatial agreement between the climate and disturbance agent grid cells, the prism cells were resampled using a 4 × 4 km raster layer equivalent to the biotic disturbance rasters described above using bilinear interpolation (Hijmans, 2016).

We defined a suite of climatic variables to investigate drivers of the presence/absence of disturbance agents at the grid cell level (Table 6.1). Climatic predictors included monthly minimum temperatures (September – May), degree-day accumulation, the onset and length of the growing season, and monthly, annual, and additive precipitation. For the model predicting the presence of mortality due to eastern larch beetle, three different defoliation variables were evaluated to provide insight into the potential for larch casebearer to facilitate outbreaks of eastern larch beetle: 1) presence of defoliation in the current year 2) presence of defoliation across the past two years (current and previous year) and 3) presence of defoliation across the past four years (current and previous three years). That is, for the third defoliation predictor, if a cell was positive for defoliation in the current year and three years prior, it would be assigned an integer value of two. All GIS analyses were completed using R statistical software (R Core Team, 2017), and several packages enabled the analysis and processing of spatial data: GISTools (Brunsdon & Chen, 2014), maps (Becker *et al.*, 2016), raster (Hijmans, 2016), and rgdal (Bivand *et al.*, 2016).

Statistical analyses

We approached data analysis with a three-step process. The response variable for all analyses was measured as the presence or absence of defoliation or mortality at the grid-cell level (coded as 1 = present, 0 = absent) and all models were logistic regressions fit with the glm() command in R (R Core Team, 2017). First, we investigated the optimal spatiotemporal neighborhood terms to include as covariates in further regression analyses investigating climatic effects (both insects) and predisposing defoliation effects (eastern larch beetle only). Terms for spatial neighborhoods (i.e., insects in the surrounding area in the current year) and temporal lag terms (i.e., insects in the same area in preceding time steps) contend with spatial and temporal dependence in the data (Aukema *et al.*, 2008; Sambaraju *et al.*, 2012). For spatial neighborhoods, we considered both first order (i.e., rook's case, four nearest cells) and second order (i.e., queen's case, 8 nearest cells) terms. That is, for a given cell with a first order neighborhood, the number of cells positive for defoliation or mortality out of the nearest four were recorded as an integer value. The temporal lag terms represented the presence of defoliation or mortality in a cell in the previous year or two years prior (i.e., first and second order temporal lag, respectively). Spatial and temporal terms were insect-specific such that only cells positive for the insect being investigated were included in a given analysis. In addition to an intercept only model, each first order or second order spatial term was fit with the first order temporal term and both the first and second order temporal terms, resulting in five autologistic regression models of insect activity; one set of five models for each insect.

The model with the lowest AIC was used in subsequent model development for each insect separately. To facilitate the use of Akaike's Information Criterion in comparing combinations of spatial neighborhoods and temporal lag terms, the time series for both insects was truncated to 2003-2016 for all analyses. The first year of mortality by eastern larch beetle was detected in 2001 (following the application of a one-year lag; see above). Thus, a two-year lag would result in cells from 2001 and 2002 being excluded from the final analysis: 2000 and 1999 were not in the window of observed activity for eastern larch beetle but would be needed for the first and second order lag terms for beetle mortality in 2001. Rather than assigning zeros to all values prior to 2000 and 2001 for eastern larch beetle, we elected to analyze data across a window of time when both agents were active on the landscape and detectable via aerial surveys (2003-2016). This resulted in 75,194 grid cells for analyses for each disturbance agent, equivalent to 5,371 4 × 4 km cells per year per insect.

After identifying spatial and temporal terms to contend with spatiotemporal dependence in the data, we moved to the second and thirds steps of model building to elucidate correlates of defoliation and mortality. First, climatic and defoliation predictors (the latter fit in models predicting presence of eastern larch beetle-caused mortality) were fit individually in addition to the best fitting terms for the spatial neighborhood and temporal lags, which were not necessarily equivalent between species. Next, all predictors were fit in a full model and a backwards elimination procedure was used to select variables in developing the best predictive models for landscape dynamics in defoliation and mortality. During backwards elimination, analyses were limited to predictors that, when fit individually as described above, had a *p*-value greater than 0.20.

This restriction reduced the number of potentially colinear climatic predictors considered and helped protect against overfitting. Models were developed by iteratively removing the variable with the highest *p*-value until all variables remaining had a *p*-value less than 0.05.

Results

Since the early 2000s, 672 to 10,382 ha of defoliation by larch casebearer have been detected each year via aerial surveys (Figure 6.1). Grid cells positive for defoliation were patchily distributed along the edge of tamarack's range in Minnesota (Figure 6.2b) and defoliation generally increased until 2009, when it was most widespread, but ranged from 4,615 to 7,464 ha through 2016 (Figure 6.1). Since 2001, 519 to 85,444 ha of mortality by eastern larch beetle have been mapped, peaking in 2016. Unlike defoliation by larch casebearer, mortality by eastern larch beetle has generally increased since the first aerial detection of beetle-killed trees in 2001 (Figure 6.1). Moreover, damage by eastern larch beetle was more widespread than larch casebearer, as 1,794 unique cells were positive for mortality attributed to eastern larch beetle (Figure 6.2c) compared to 494 unique cells positive for defoliation by larch casebearer (Figure 6.2b). Note that the same cells were often infested more than once. Only 4.9 % of the mapped mortality attributed to eastern larch beetle was also covered by defoliation, as determined by analyzing polygons before rasterization.

Prior to evaluating the explanatory power of climatic variables and defoliation, we identified appropriate spatial neighborhood terms and temporal lag terms unique to each disturbance agent (Table 6.2). For larch casebearer, presence of defoliating populations of larch casebearer within 4 km (i.e., the four surrounding grid cells) and in the immediate vicinity each of the previous two years was associated with a higher probability of detecting larch casebearer. The equivalent spatial neighborhood and temporal lag terms were also identified as the best fitting for eastern larch beetle (Table 6.2), with similar positive correlations between tree mortality and proximate tree-killing beetle populations in space and time.

After identifying the best-fitting spatial and temporal terms, we developed subsequent regression models by retaining those terms and adding each climatic or defoliation (model predicting mortality by eastern larch beetle only) predictor individually. For larch casebearer, the probability of defoliation occurring increased with higher minimum temperatures in the autumn and winter (September, October, December-February) (Table 6.3). Minimum temperatures in spring (Mar-May) appeared less important, as there was no relationship between these months the presence/absence of defoliation. The onset and intensity of the growing season also appeared important for larch casebearer dynamics, as earlier estimated emergence by larch casebearer in a given cell in the previous year and higher degree-day accumulation between February 20 and November 30 in the previous year's growing season were associated with a higher probability of detecting defoliation on the landscape. Spring precipitation appeared to be beneficial for larch casebearer, as total precipitation in both April and May was positively correlated with the presence of defoliation.

Our parallel analysis of the presence/absence of mortality attributed to eastern larch beetle likewise demonstrated that tree-killing activity by this insect was highly sensitive to climate (Table 6.4). Minimum temperatures appeared less important for landscape dynamics of eastern larch beetle than climatic trends during the growing season (approximately April-November). For example, monthly minimum temperatures in September, November, and May were the only months in which warmer minimum temperatures resulted in more tamarack mortality from eastern larch beetle. No other terms for monthly minimum temperatures were statistically significant, but late springs appeared beneficial for beetle populations. The estimated first day of flight, occurring at 120 cumulative degree-days (base = 5 °C), was significantly, positively correlated with tree mortality. Likewise, the first day that the minimum temperature was above 0 °C, a proxy for root thawing date, was also positively correlated with the presence of mortality. That is, when roots thaw out later in spring, beetle populations are expected be more abundant on the landscape. Warmer years, indicated by the degree-day accumulation across the entire year, were positively associated with the presence of mortality.

While springs and warmer growing seasons were positively associated with tree-killing activity by eastern larch, we also noted strong signals of drought and defoliation by larch casebearer predisposing trees to colonization by eastern larch beetle that ultimately caused mortality. Two variables representing total precipitation in the previous three and six years, respectively, were both highly and negatively correlated with mortality. Defoliation across several time scales was also associated with increased beetle activity, as defoliation in the current year, number of years of defoliation out of the current and previous year, and number of years of defoliation out of the current and previous three years were all positively correlated with mortality.

Finally, we identified several variables in multiple regression models of landscape dynamics to explain defoliation of tamarack by larch casebearer and mortality to tamarack by eastern larch beetle (Table 6.5). The most significant variables in explaining variation in the presence/absence of defoliation, as determined by the highest *Z*-ratios, were the presence of defoliation in the nearest 4 km in the current year and immediate vicinity the previous two years (i.e., the first order spatial neighborhood and first and second order temporal lag terms; Table 6.5). We found that minimum temperatures in October of the previous year, minimum temperatures in September of the previous year, degree-day accumulation from February 20 to November 30 in the previous year, and total precipitation in May were also positively correlated with the presence of defoliation (Table 6.5).

In the model predicting mortality by eastern larch beetle, similar to the model of defoliation, spatial and temporal terms were most important for predicting mortality (Table 6.5). In addition to these terms, several environmental and biotic predictors remained in the final model and reflected inferences drawn from models in which climatic and biotic predictors were fit individually. For example, monthly minimum temperatures in November and May were positively correlated with mortality. Both later emergence in the spring (i.e., median ordinal day of the first flight) and greater accumulation of degree-days per annum appeared to benefit beetle populations, as both variables were positively correlated with mortality. Annual precipitation across the previous three years was inversely correlated with mortality, suggesting that short periods of drought (3 years vs. 6 years) may be sufficient to modulate outbreak dynamics of eastern larch beetle. There again remained evidence of a landscape-scale association between eastern larch beetle and larch casebearer; defoliation by larch casebearer in both the current and the previous 3 yr was associated with increased probability of mortality caused by eastern larch beetle.

Discussion

Native insect defoliators can act as predisposing agents to endophytic feeding guilds (Wright *et al.*, 1984; Raffa *et al.*, 1998; Wallin & Raffa, 2001) and such interactions can manifest at large spatial scales (Goodsman *et al.*, 2015). Our analyses identified a significant landscape-scale association between an invasive, non-native defoliator and native bark beetle. We caution that the association of landscape-scale dynamics between larch casebearer and eastern larch beetle does not necessarily indicate that larch casebearer has facilitated the outbreak of eastern larch beetle in Minnesota, as aerial survey patterns cannot prove of disprove interactions occurring at individual tree levels at finer spatial scales. We expect, however, that the association between these agents in 4 × 4 km grid cells results from trees being rendered more susceptible to both herbivores due to regional stressors such as a changing climate. Further work is needed to elucidate differences in host preference, as each herbivore may select hosts of different age or size classes in stands, reflective of niche partitioning. For example, eastern larch beetles appear to preferentially colonize larger hosts (Mckee, 2015; Crocker *et al.*, 2016), whereas younger trees along edges may be most susceptible to larch casebearer (Tunnock & Ryan, 1985).

Defoliation by larch casebearer is spatiotemporally synchronous up to a minimum of 50 km in Minnesota (Chapter 1), and thus lower densities are likely present but not accounted for in our analysis. Crown discoloration by larch casebearer, which is correlated with larval density on western larch (Ciesla & Bousfield, 1974) and eastern larch (Chapter 2), may only be detectable by aerial surveys at extremely high larval densities. As such, trace defoliation by larch casebearer likely existed within grid cells that may have been sufficient to predispose trees to infestation by eastern larch beetle while escaping detection via aerial surveys. Moreover, there may exist upper and lower thresholds of defoliation by larch casebearer, only between which trees are susceptible to colonization by eastern larch beetle, perhaps due to induced defensive systems within tamarack. Such an interaction may explain the small percentage overlap of disturbance polygons and the positive association between the agents at the grid cell level. That is, there may exist a nonlinear or quadratic relationship between colonization by eastern larch beetle and the density of larch casebearer, similar to that observed in other defoliator-bark beetle systems such as the two-year cycle spruce budworm (*Choristoneura biennis* Freeman; Lepidoptera: Tortricidae) and the spruce beetle (*Dendroctonus rufipennis* Kirby; Coleoptera: Curculionidae) (Goodsman *et al.*, 2015).

Irrespective of landscape interactions between these disturbance agents, several other climatic factors were associated with increases in the relative abundance of both disturbance agents. Analyses suggested that larch casebearer may be sensitive to fewer climatic drivers than eastern larch beetle (Table 6.5). The finding that defoliation is more likely with greater degree-day accumulation in previous year's growing season is consistent with previous estimates that warmer growing seasons increase the proportion of the population that reach the overwintering stage each autumn (Chapter 5). Phenology models developed in Chapter 5 suggested that the proportion of insects reaching the overwintering stage has increased through time. The association between warming October and September minimum temperatures with defoliation by larch casebearer may be indicative of either decreased mortality due to warmer absolute minimum temperatures, or simply reflect a positive correlation between minimum temperatures and average temperatures in those months. That is, warmer Octobers and Septembers may benefit larch casebearer populations by enabling late developing larvae to reach the overwintering stage. Thus, our three predictors (OctMin, SepMin DegD-LCB, Table 6.5) may be indicative of the same mechanism. Our finding that defoliation increased with spring precipitation (MayPPT, Table 6.5) may suggest that drought negatively impacts casebearers, either due to desiccation of larvae feeding in or on desiccating needles, premature needle drop by hosts in response to water stress (i.e., larvae would be shed with needles), or some other unknown mechanism.

In addition to the spatial and temporal terms, two of the most important drivers of tamarack mortality by eastern larch beetle were minimum temperatures in November and degree-day accumulation across the entire year. Warmer Novembers could lead to decreases in cold-driven mortality (Venette & Walter, 2008) and/or permit further development of larvae or sclerotization of teneral beetles in autumn that provides greater opportunity for successful development of two generations per year that emerge the following spring. Synchronized emergence facilitates host procurement by beetles attacking en masse (Mckee, 2015), consistent with our finding that estimated delayed flight in spring increases tree mortality (Table 6.5). In Minnesota, increases in the number of broods produced per annum and a shift to fractional or bivoltinism have been documented by eastern larch beetle in warmer years (Mckee, 2015). Our model provides evidence that warmer years, which likely increase beetle pressure, have contributed to the landscape scale outbreak of eastern larch beetle.

Drought may act alone as a largescale disturbance (Hanson & Weltzin, 2000) in addition to facilitating disturbances by insects (Mattson & Haack, 1987). Our analysis of single predictors for larch casebearer suggested that more precipitation in spring may lead to increased defoliation (Table 6.3), although analysis of all predictors via backwards selection did not identify precipitation as a significant driver (Table 6.5). The relationship between drought and eastern larch beetle outbreaks is not well understood, though the initiation and spread of outbreaks by spruce beetle, a congener that also attacks trees early in spring, is similarly facilitated by drought (Hart *et al.*, 2014, 2017). We found that total annual precipitation in the previous three years is negatively correlated with mortality by eastern larch beetle (Table 6.5), indicating that drought may indeed facilitate outbreaks of eastern larch beetle in Minnesota. In Minnesota, tamarack occurs in bogs, conifer swamps, and/or fens with complex hydrology (Tilton, 1978) that moderates growth of resident larches (Girardin *et al.*, 2001). Thus, we caution that annual precipitation may not accurately reflect the level of water stress. Soil moisture levels, a more reliable proxy for plant water stress, are not available on an annual basis for the time scales investigated here.

Our parallel analyses elucidated a landscape-scale interaction between the invasive larch casebearer and native eastern larch beetle. Both insects appear to be undergoing anomalous outbreaks driven, in part, by a warming climate. The ability to forecast future outbreaks of both non-native and native insects is critical for managing forests under global climate change scenarios (Millar *et al.*, 2007). Biotic disturbances by native agents are important parts of ecosystem functioning, though significant deviations from historical patterns make managing such disturbances, and understanding their drivers, paramount for predicting changes in forest structure and function (Dale *et al.*, 2001). Moreover, the global challenge of newly arriving and establishing non-native insects may also present novel disturbance regimes in forest ecosystems (Kenis *et al.*, 2009; Clark *et al.*, 2010). Our work demonstrates the potential for such agents to interact with native biotic disturbances agents at large spatial scales. In this system, further research is also needed to understand how natural enemies released as part of the importation biological control program against larch casebearer, once considered a resounding success (Graham, 1948; Webb & Quednau, 1971; Otvos & Quednau, 1981), respond to a changing climate. Developing correlative landscapes models can facilitate forecasting and also help identify avenues of future research to elucidate the mechanisms underpinning these landscape-scale patterns of tree mortality.

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Tables

Table 6.1 Descriptions of predictors considered in statistical models predicting the probability of defoliation by larch casebearer or mortality by eastern larch beetle on tamarack across 4×4 km grid cells in Minnesota, USA (2003-2016).

Variable type	Insect	Variable	Explanation and rationale
Spatial	Both	LCB-NN$_4$ ELB-NN$_4$	First-order neighborhood; total number of cells out of the nearest four (i.e, first cell in each cardinal direction) that are positive for the disturbance agent.
	Both	LCB-NN$_8$ ELB-NN$_8$	Second-order neighborhood; total number of cells out of the nearest eight that are positive for the disturbance agent;
Temporal	Both	LCB-Lag1 ELB-Lag1	Presence/absence of a disturbance agent in a cell the previous year.
	Both	LCB-Lag2 ELB-Lag2	Presence/absence of a disturbance agent in a cell two years prior.
Climatic	Both	SepMin	Monthly minimum temperatures (°C). Acute cold exposure can moderate insect survival (Bale & Hayward, 2010). Mortality due to cold temperatures may be important for both larch casebearer (Tunnock & Ryan, 1985) and eastern larch beetle (Venette & Walter, 2008). For September-December, monthly temperatures from the previous year were used. For example, mortality due to cold in December 2002 would modulate insect pressure in spring/summer of 2003.
	Both	OctMin	
	Both	NovMin	
	Both	DecMin	
	Both	JanMin	
	Both	FebMin	
	Both	MarMin	
	Both	AprMin	
	Both	MayMin	
	LCB	DegD-LCB	Degree-day accumulation (base = 5 °C) between February 20 and November 30 of the previous year. Greater degree-day accumulation may result in a greater proportion of larvae reaching the overwintering stage (Chapter 5).
	LCB	MarPPT	

	LCB LCB	AprPPT MayPPT	Total precipitation (mm) in March, April, or May. Extremely wet or dry springs may negatively impact larch casebearer populations due to fungal outbreaks or premature needle drop, respectively.
	ELB	Flightday	Estimated first day of flight for eastern larch beetle based on degree-day models (base = 5 °C). Earlier spring emergence may result in a longer growing season, and thus more time for the parents and offspring to develop through the life cycle.
	ELB	Frostday	First day that the daily minimum temperature was above 0 °C. The earlier that roots begin to thaw in spring, the more capable the tree may be of defending against bark beetles (e.g., resin flow).
	ELB	DegD-ELB	Degree-day accumulation (base = 5 °C) across the entire year
	ELB	PrecipPrev3	Total annual precipitation (mm) in previous 3 years
	ELB	PrecipPrev6	Total annual precipitation (mm) in previous 6 years
Biotic	ELB	Defol	Presence/absence of defoliation by larch casebearer in current year
	ELB	DefolPrev	Presence/absence of defoliation by larch casebearer in current and previous year; integer
	ELB	DefolPrev3	Presence/absence of defoliation by larch casebearer in current and previous three years; integer

Table 6.2 Coefficient estimates and AIC metrics from models evaluating the combination of spatial neighborhood and temporal lag terms for larch casebearer and eastern larch beetle in Minnesota, USA, 2003-2016.

Model	Intercept	Spatial neighborhood		Temporal lag (year)		AIC	ΔAIC
		NN_4	NN_8	1	2		
Larch casebearer							
1	-4.16					11,916	0
2	-5.57	2.62		2.98		5,812	-6,104
3	**-5.59**	**2.56**		**2.46**	**1.29**	**5,733**	**-6,183**
4	-5.46		1.40	3.00		6,288	-5,628
5	-5.48		1.36	2.47	1.34	6,194	-5,722
Eastern larch beetle							
1	-2.71					42,191	0
2	-3.93	1.50		2.06		23,423	-18,768
3	**-3.97**	**1.43**		**1.68**	**1.17**	**22,927**	**-19,264**
4	-3.93		0.81	1.98		23,733	-18,458
5	-3.97		0.77	1.61	1.13	23,268	-18,923

Table 6.3 Models predicting defoliation by larch casebearer when each predictor was fit individually in addition to the best combination of terms for spatial neighborhoods and temporal lags (Table 6.2). Model intercept terms not presented.

Variable	MLE	SE	*Z*-ratio	*P*
SepMin	0.0386	0.0223	1.73	0.08
OctMin	0.1446	0.0289	5.00	<0.0001
NovMin	0.0284	0.0170	1.67	0.09
DecMin	0.0232	0.0095	2.44	0.0146
JanMin	0.0152	0.0059	2.56	0.0103
FebMin	0.0292	0.0108	2.71	0.0067
MarMin	0.0135	0.0095	1.42	0.16
AprMin	-0.0037	0.0097	-0.38	0.71
MayMin	0.0079	0.0123	0.64	0.52
DegD-LCB	0.0015	0.0002	6.43	<0.0001
MarPPT	0.0007	0.0017	0.39	0.70
AprPPT	0.0038	0.0014	2.71	0.0067
MayPPT	0.0025	0.0009	2.83	0.0047

Table 6.4 Models predicting mortality by eastern larch beetle when each predictor was fit individually in addition to the best combination of terms for spatial neighborhoods and temporal lags (Table 6.2). Model intercept terms not presented.

Variable	MLE	SE	*Z*-ratio	*P*
SepMin	0.0466	0.0818	4.63	<0.0001
OctMin	0.0022	0.0125	0.17	0.86
NovMin	0.0735	0.0081	9.05	<0.0001
DecMin	-0.0019	0.0042	-0.45	0.65
JanMin	0.0012	0.0028	0.42	0.68
FebMin	0.0031	0.0051	0.61	0.54
MarMin	-0.0008	0.0044	-0.19	0.85
AprMin	-0.0042	0.0045	-0.93	0.35
MayMin	0.0153	0.0055	2.76	0.0057
Flightday	-0.0043	0.0017	-2.49	0.0129
Frostday	0.0021	0.0010	2.18	0.0293
DegD-ELB	0.0008	0.0001	7.43	<0.0001
PrecipPrev3	-0.0009	0.0001	-9.45	<0.0001
PrecipPrev6	-0.0006	6.0×10^{-5}	-9.16	<0.0001
Defol	0.7932	0.1079	7.35	<0.0001
DefolPrev	0.4287	0.0690	6.22	<0.0001
DefolPrev3	0.2778	0.0404	6.87	<0.0001

Table 6.5 Best fit logistic regression models as determined via backwards selection for larch casebearer and eastern larch beetle with the best fit combination of spatial neighborhood and temporal lag terms (Table 6.2). See Table 6.1 for an explanation of predictors.

Response[1]	Predictor	MLE	SE	*Z*-ratio	*P*
Defoliation by LCB	Intercept	-8.9729	0.5389	-16.65	<0.0001
	OctMin	0.0489	0.0237	2.06	0.0389
	SepMin	0.0680	0.0312	2.18	0.0295
	DegD-LCB	0.0015	0.0003	5.81	<0.0001
	MayPPT	0.0028	0.0009	3.04	0.0023
	NN_4	2.5274	0.0498	50.78	<0.0001
	Lag1	2.4230	0.1227	19.74	<0.0001
	Lag2	1.2977	0.1378	9.41	<0.0001
Mortality by ELB	Intercept	-8.8602	0.7479	-11.85	<0.0001
	NovMin	0.0997	0.0097	10.26	<0.0001
	MayMin	0.0258	0.0060	4.31	<0.0001
	Flightday	0.0313	0.0033	9.53	<0.0001
	DegD-ELB	0.0020	0.0002	10.63	<0.0001
	PrecipPrev3	-0.0012	0.0001	-11.66	<0.0001
	Defol	0.4163	0.1571	2.65	0.0081
	DefolPrev3	0.1815	0.0580	3.13	0.0018
	NN4	1.3373	0.0190	70.55	<0.0001
	Lag1	1.6597	0.0482	34.46	<0.0001
	Lag2	1.1315	0.0511	22.16	<0.0001

Figures

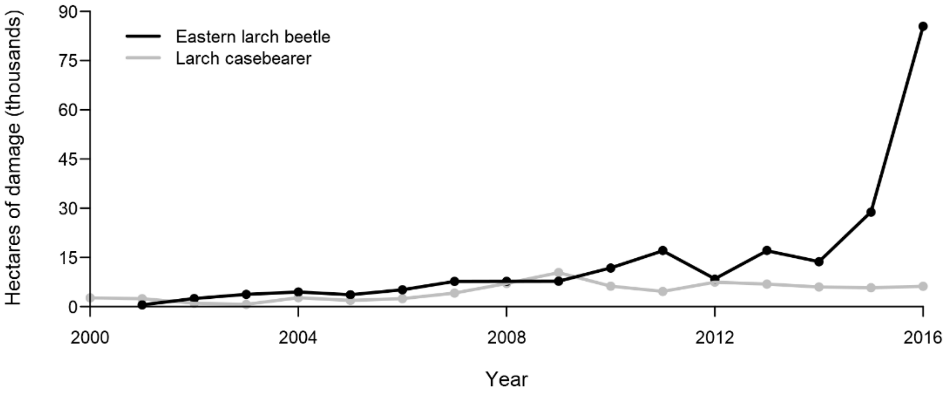

Figure 6.1 Annual hectares of defoliation by larch casebearer and mortality by eastern larch beetle in Minnesota, USA, (Minnesota Department of Natural Resources).

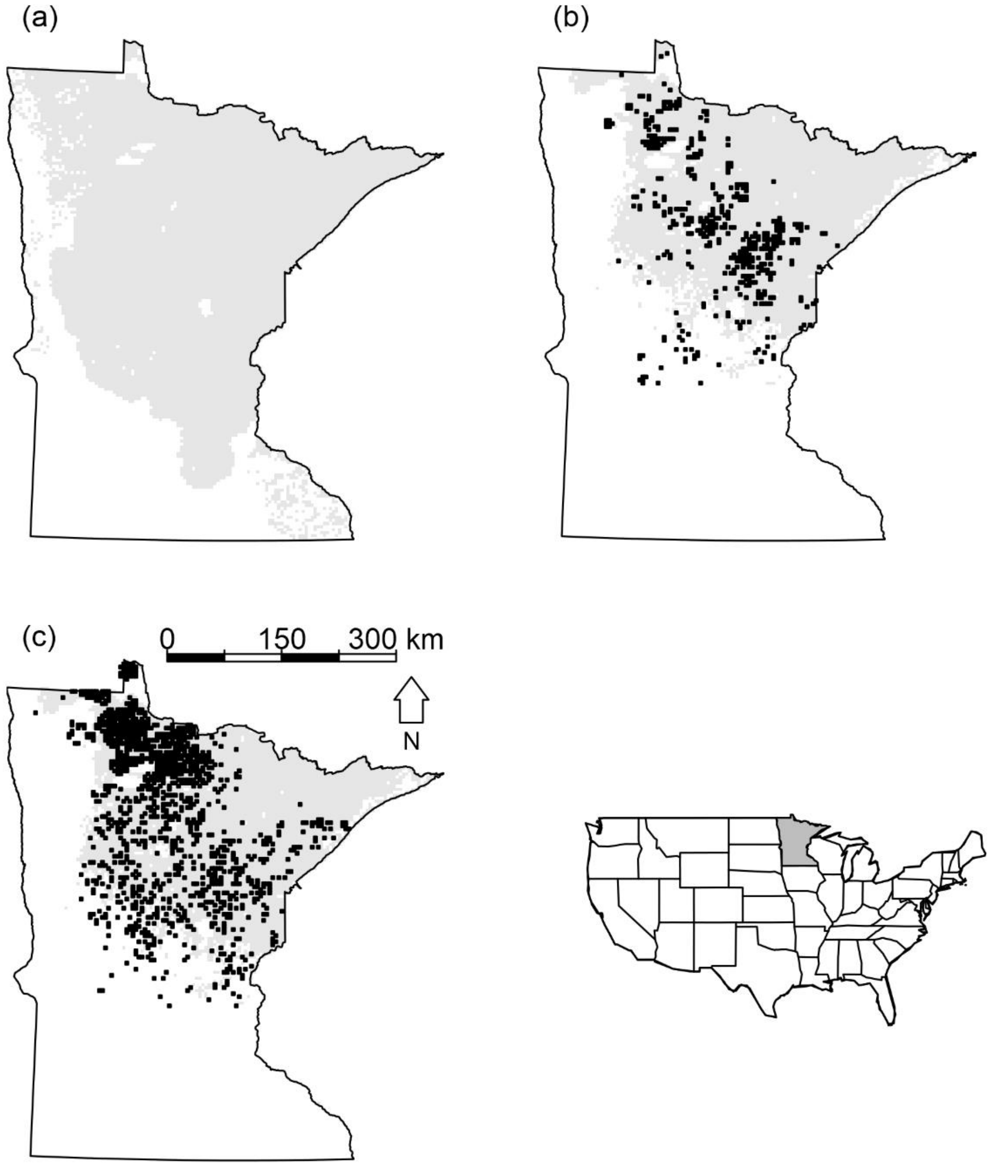

Figure 6.2 Depicts the (a) distribution of tamarack (b) cumulative defoliation by larch casebearer and (c) cumulative mortality due to eastern larch beetle in 4 × 4 km grid cells (which merge together in figure) in Minnesota, USA. Panel a is comprised of grid cells with non-zero values for basal area of tamarack. In panels b and c, grey area indicates grid cells with at least a mean basal area of tamarack of 0.1 m^2 per ha. Black grid cells indicate areas where defoliation or mortality was detected for at least 1 year between

2000-2016. All maps projected in UTM Zone 15 NAD 83. Inset map shows location of Minnesota in United States.

Dissertation conclusions

1) **Defoliation by larch casebearer was synchronous at scales up to 331 km in western larch forests and up 108 km in eastern larch forests. Defoliation appeared to fluctuate synchronously between western larch forests of Oregon/Washington and eastern larch forests of Minnesota.** Synchronous climatic patterns across our study region may have driven the synchronous resurgence of larch casebearer.
2) **Neither drought nor water-logging treatments to eastern larch trees appeared to influence larch casebearer population dynamics.** Defoliation by larch casebearer, drought, and water-logging significantly decreased growth of juvenile eastern larch trees. Juvenile trees did not appear to mount a defensive response to challenge by larch casebearer, as eastern larch trees did not alter their monoterpene production when challenged with casebearer larvae.
3) **Cold tolerance models based on our data suggest that larval survival has increased over the previous ~50 years in Minnesota, and may have contributed to the resurgence of larch casebearer.** Larch casebearer larvae are extremely cold tolerant in mid-winter and cold tolerance is significantly reduced in autumn and spring. Larch casebearer larvae freeze at -44 °C in January, and freezing appears to be a reliable proxy for mortality. Cold hardiness is reduced in autumn and spring, as larvae froze at mean temperatures of -36.5 °C and -30.3 °C in November and April, respectively.
4) **Larch casebearer is extremely robust to spring phenological synchrony with host bud break. Thus, changes in phenological synchrony with host eastern larch**

trees has not likely driven the resurgence of larch casebearer. Larch casebearer and eastern larch have similar lower developmental thresholds around 5 °C. Larch casebearer larvae rely on photoperiod and temperature cues to time the onset of spring feeding, whereas eastern larch relies on temperature, including a response to chilling, to time bud break. These disparate responses to environmental cues, and greater degree-day accumulation requirements by larch casebearer, interact such that foliage is always available for consumption by the time spring feeding larvae are active.

5) **The length of the growing season may be an important moderator of larch casebearer population dynamics and may have contributed to the resurgence of larch casebearer.** The proportion of larvae estimated to reach the overwintering stage each year is determined by degree-day accumulation across the growing season. Increases in degree-day accumulation per year over the last half century has resulted in greater estimated proportions of larvae that complete their life cycle, potentially facilitating outbreaks.
6) **Outbreaks of both larch casebearer and eastern larch beetle were significantly correlated with several climatic variables.** For larch casebearer, degree-day accumulation by larvae during the growing season was positively correlated with the occurrence of defoliation at the landscape-scale. Moreover, defoliation by larch casebearer was associated with increased prevalence of mortality of tamarack by eastern larch beetle. In addition to defoliation by larch casebearer, eastern larch beetle appeared highly responsive to climate. Mortality of eastern larch attributed to eastern larch beetle generally increased with warmer annual temperatures and droughts.

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