

THE RESURGENCE OF LARCH CASEBEARER IN THE GREAT LAKES REGION

A THESIS SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF
MINNESOTA

BY

Spencer Paul Stout

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

Advisor:

Brian H. Aukema, MS, MS, PhD

June 2021

Acknowledgments

I would first like to thank my advisor, Dr. Brian Aukema, for his constant support and patience through this opportunity. I am grateful Brian fueled my passion for teaching and allowed me to become a better researcher than I ever thought possible. Thank you for setting me on the right path.

Thank you to my committee members: Dr. George Heimpel and Dr. Robert Venette. I will always cherish the intellectual and fun discussions of biological control with George as I prepared my study protocols. Robert's invaluable input strengthened the science of my thesis. I am a better scientist thanks to both their help.

Aubree Kees was a support system throughout my graduate experience. She gave countless hours to assist with protocol and data collection for both chapters of my thesis. Thank you for help as a colleague and as a friend.

I owe thanks to each member of the Aukema Lab: Zach Smith, Kevin Chase, Dora Mwangola, Jake Wittman, Marie Hallinen, Hailey Shanovich, and Grace Haynes. Whether they were road trip companions, helped with data collection, or data analysis, each member offered their time and help whenever I needed it.

I also want to thank Garrett and James Aukema for weeding the cold-frame with me. It was a constant battle to keep the area clean for experiments and I am glad that I had these two kids to help pass the time.

I was able to determine study sites thanks to assistance from Brian Schwingle of the Minnesota Department of Natural Resources and Paul Cigan of the Wisconsin Department of Natural Resources. Erika Eidson, of the Idaho Department of Lands, was key in the

implementation of protocol for Chapter 2 and provided larch casebearers from Idaho for use in the experiments.

Dr. John Luhmen's assistance in confirming the identification of parasitoids was invaluable. I greatly appreciate the conversations we shared while we identified wasps.

I want to thank my family for their emotional support when graduate school was especially difficult. Also, thank you all for putting up with my constant need for practice talks. Lastly, I want to thank my partner, Sarah Treuchel for their constant support through the writing of this thesis.

Dedication

This thesis is dedicated to my grandparents, Nate (1929 - 2020) and Dee Daum (1928 - 2020). Thank you for your love and support. I will carry your memories through the rest of my life.

Thesis Abstract

Larch casebearer, *Coleophora laricella* Hübner, (Lepidoptera: Coleophoridae), was discovered infesting eastern larch, *Larix laricina* (Du Roi) K. Koch, in North America in the 1880s. The insect was likely introduced from Europe on shipments of European larch, *Larix decidua* Mill., stock. Larch casebearer quickly spread through the range of eastern larch and eventually western larch in western North America. Since 2000, larch casebearer defoliation has increased on these two species of *Larix* across North America despite previous successful biological control by two introduced parasitoids; *Agathis pumila* Ratzeburg (Hymenoptera: Braconidae) and *Chrysocharis laricinellae* Ratzeburg (Hymenoptera: Eulophidae) in the Great Lakes region. I sampled eastern larch at multiple sites across Minnesota and Wisconsin, USA in 2018 and 2019, to determine if these parasitoids were still present. I observed the continued presence of *A. pumila* and *C. laricinellae* in both states along with 13 other parasitoid species associated with larch casebearer. *Cirrospilus pictus* Nees (Hymenoptera: Eulophidae), an historically rare parasitoid, was more abundant than the specialist *A. pumila*. I also examine whether host species affects the overwintering cold tolerance and spring activation of two allopatric populations of larch casebearer from Minnesota and Idaho. Through a common garden experiment, I determined that the supercooling points and the number of degree days accumulated for spring activation for populations of larch casebearer from Minnesota reared on eastern and western larch were similar, suggesting that differences are not likely due to host species. Instead, the different populations on eastern larch and western larch may be two different species or varying autumnal and winter conditions may affect thermal plasticity and adaptation.

Table of Contents

Acknowledgements.....	i
Dedication.....	iii
Thesis Abstract.....	iv
List of Tables.....	vi
List of Figures.....	vii
Thesis Introduction.....	ix
Chapter 1. Examining the larch casebearer and the associated parasitoid complex on eastern larch in the Great Lakes region.....	1
Chapter Summary.....	1
Introduction.....	2
Methods.....	6
Results and Discussion.....	8
Acknowledgements.....	12
Tables.....	14
Chapter 2. Physiological response of larch casebearer reared on eastern vs. western larch trees in a common garden experiment.....	17
Chapter Summary.....	17
Introduction.....	18
Materials and Methods.....	21
Results.....	25
Discussion.....	26
Acknowledgments.....	30
Figures.....	31
Thesis conclusions.....	35
References cited.....	36

List of Tables

Table 1.1 Sites for larch casebearer and parasitoid surveys and the number of cases collected in 2018 and 2019 in Minnesota and Wisconsin, USA. The total number of cases collected is reported along with the number of viable cases used in calculations. Cases in 2019 that were shriveled, grey, or moldy, and not attached to any green foliage were omitted from analyses. (Page 14).

Table 1.2 Total number and density of specimens reared from sampling in 2018 and the initial survey in 2019 in Minnesota and Wisconsin, USA. (Page 15).

Table 1.3 Density and percent parasitism for larch casebearer, *A. pumila*, *C. laricinellae*, and *C. pictus* in 2018. Metrics are not presented for 2019 due to low sample sizes (Table 1.1). (Page 16).

List of Figures

Fig. 2.1: Supercooling points for allopatric populations of larch casebearer sourced from eastern larch in Minnesota ($n = 9$ insects) and western larch in Idaho ($n = 11$ insects), February 2020. The “whiskers” of the box extend no more than 1.5 times from the interquartile range (i.e. the first and third quartiles). The dot represents a value larger than 1.5 times the interquartile range within the supercooling point data for casebearers from Minnesota. The thick line in the middle of the box is the median observation for the respective group. The top and the bottom of the box represent the first and third quartiles. (Page 31).

Fig. 2.2: Accumulated degree days to spring activation for allopatric populations of larch casebearer from sourced from eastern larch in Minnesota ($n = 30$ insects) and western larch in Idaho ($n = 30$ insects), June 2020. The “whiskers” of the box extend no more than 1.5 times from the interquartile range (i.e. the first and third quartiles). The thick line in the middle of the box is the median observation for the respective group. The top and the bottom of the box represent the first and third quartiles. (Page 32).

Fig. 2.3: Supercooling points for populations of larch casebearer sourced from Minnesota reared on eastern larch ($n = 11$ insects) and western larch ($n = 8$ insects) were similar, February 2020. The “whiskers” of the box extend no more than 1.5 times from the interquartile range (i.e. the first and third quartiles). The thick line in the middle of the box is the median observation for the respective group. The top and the bottom of the box represent the first and third quartiles. (Page 33).

Fig. 2.4: Accumulated degree days to spring activation for populations of larch casebearer sourced from Minnesota reared on eastern larch ($n = 20$ insects) and western

larch ($n = 20$ insects) were similar, June 2020. The “whiskers” of the box extend no more than 1.5 times from the interquartile range (i.e. the first and third quartiles). The thick line in the middle of the box is the median observation for the respective group. The top and the bottom of the box represent the first and third quartiles. (Page 34).

Thesis Introduction

Insects are consistently introduced to novel environments through human mediated means, such as transfers of infested firewood, furniture, or planting stock (Heimpel & Mills, 2017). While most introductions are insignificant and simply go unnoticed, some lead to ecological and/or economic issues (Heimpel & Mills, 2017). Various factors can facilitate the success of a new invader, such as the absence of natural predators, plasticity towards novel interactions with hosts and competitors, and variations in climate (Mattson & Haack, 1987; Agrawal, 2001). Determining what specific factors affect a particular invader can aid researchers in reducing the impact of the pest.

The larch casebearer, *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae), arrived in North America from Europe in the 1880s, likely on stock of European larch, *Larix decidua* Mill. (Ryan *et al.*, 1987). Larch casebearer was able to quickly spread to eastern larch, *Larix laricina* (Du Roi) K. Koch, a tree native to North America (Rush, 1972; Ward *et al.*, 2019). Larch casebearer was found in Ontario, Canada in 1905 and caused significant damage to eastern larch in the Great Lakes region by the 1920s (Graham, 1949; Cody *et al.*, 1967; Rush, 1972). Larch casebearer was found infesting western larch, *Larix occidentalis* Nutt. in Idaho in 1957, where it quickly spread throughout the distribution of western larch (Denton and Tunnock, 1968; Ryan *et al.* 1987). To date, alpine larch, *Larix lyallii* Parl., has not been infested by larch casebearer. This tree species is found at higher elevations and is subjected to cooler annual temperatures compared to western larch (Tunnock and Ryan, 1985).

The larch casebearer is a univoltine moth (Ryan *et al.* 1987). Adult moths emerge from their pupal cases in late spring to mate. Females will lay between ten to 100 eggs

per female depending on ambient temperature (Quednau, 1967; Denton, 1979). Newly hatched larvae mine directly into larch needles to feed. When caterpillars reach third instar, they construct a small silk-lined case from a hollowed needle (Ryan, 1975). These cases serve as protection against weather and predators while the caterpillars continue to feed on larch needles (Ryan, 1975). Upon the arrival of autumn, third instar caterpillar migrate to needle fascicles or twigs to overwinter; they then attach their case to the tree and seal it off with silk. Lagging spring needle flush by about 28 days, and after the caterpillars molt into fourth instar larvae, they continue to feed for a few weeks until they are ready to pupate for about two weeks within newly constructed silk-sealed cases (Ryan, 1975; Ward *et al.* 2019).

Severe infestations with multiple years of repeated defoliation caused stunted growth and subjects trees to potential secondary mortality factors such as eastern larch beetle (Denton, 1979; Ward & Aukema, 2019). Early surveys canvassed natural predators of larch casebearer. While these surveys found a variety of native parasitoids attacking larch casebearer, no suitable candidates for biological control emerged (Coppel and Shenefelt, 1960). Instead, eleven parasitoids of the larch casebearer were introduced to the eastern United States from Europe between 1932-1937 to determine potential biological control agents (Graham, 1948; Coppel and Shenefelt, 1960). Many of the species failed to establish or provide any control (Cody *et al.*, 1967). However, *Agathis pumila* (Ratz.) (Hymenoptera: Braconidae) and *Chrysocharis laricinellae* (Ratz.) (Hymenoptera: Eulophidae) both established successfully and became the focus of the biological control program in the United States (Cody *et al.*, 1967; Ryan, 1980).

Agathis pumila is a braconid wasp with a univoltine lifecycle and a feeding specialist on larch casebearer. Adults emerge in early to mid-summer to mate and oviposit on early needle-mining instars, where they remain dormant until the caterpillar reaches pupation the following spring (Quednau, 1970; Ryan, 1980). The host is then killed and *A. pumila* completes development (Cody *et al.*, 1967; Ryan, 1980).

Chrysocharis laricinellae is a generalist eulophid wasp with two or three generations per year depending on host densities and the densities of *A. pumila* (Graham, 1948; Cody *et al.*, 1967; Ryan, 1980). While it is known that *C. laricinellae* will oviposit into cases that have been previously parasitized by other parasitoid species, including *A. pumila*, it is not considered a hyperparasitoid (Graham, 1948; Ryan, 1980). As a result of this facultative relationship, *C. laricinellae* is dependent upon *A. pumila* to be able to provide successful control of larch casebearer (Graham, 1948; Ryan, 1980; Ryan, 1986).

Releases of both control agents were consistent through the mid-20th century in both eastern North America and western North America (Cody *et al.* 1967; Ryan, 1981; Miller-Pierce *et al.*, 2015). Before and after release studies demonstrated that control of larch casebearer was achieved when the parasitism rate of *A. pumila* reached 10%, with some control attributed to *C. laricinellae* (Ryan, 1997). Larch casebearer damage was undetectable at the landscape level by the 1990s and the biological control program was thus considered a success (Ryan, 1997).

Larch casebearer defoliation events have been increasing in number and frequency across North America since 2000 (Ward & Aukema, 2019). This change has prompted several studies evaluating the status of natural enemies and the introduced biological control agents (Miller-Pierce *et al.*, 2015; Miller and Finlayson, 2019). In

western North America, Miller-Pierce *et al.* noted the continued presence of *A. pumila* and *C. laricinellae*, with average parasitism rates of 19.3% and 2.8%, respectively (Miller-Pierce *et al.*, 2015). Parasitism rates for *A. pumila* need to be above 10% for effective biological control of larch casebearer in western North America (Ryan, 1997).

No similar surveys of biological control agents since Cody *et al.* (1967) and Rush (1972) in eastern or central North America have been performed, however. As such, Chapter 1 evaluates the status of the biological control agents in the Great Lakes region. I sampled eastern larch, *Larix laricina* (Pinaceae), at multiple sites across Minnesota and Wisconsin, USA in 2018 and 2019 to confirm the continued presence of *A. pumila* and *C. laricinellae*. I determined the densities of larch casebearer and the percent parasitism of several natural enemies present in the system.

Several studies investigating climatic aspects of larch casebearer's resurgence have also been conducted (Ward and Aukema, 2019; Ward *et al.*, 2019a, 2019b, 2020, Chapter 2). Ward *et al.* (2020) found variation between allopatric populations of larch casebearer infesting western larch vs. eastern larch in their overwintering cold tolerance and their spring activation. Larch casebearer from Minnesota infesting eastern larch appear cold hardier with a mean supercooling point 10°C lower than the supercooling points of larch casebearers collected in Oregon, Idaho, and Montana on western larch. In the spring, larch casebearer from Oregon, Idaho, and Montana needed to accumulate about one third the number of growing degree days compared to larch casebearer from Minnesota; 63 degree days vs. 172 degrees days, respectively, before the insects activate. Several hypotheses have been put forward to determine why these striking differences exist between eastern vs. western populations of larch casebearer. The origin of larch

casebearer in western North America is not confirmed and therefore, it is entire possible that the two populations are two different species. Differences in climate, specifically autumnal temperatures, can also affect the overwintering physiology of insects (Mousseau and Dingle, 1991; Ward *et al.* 2020). Finally, different host species can lead to rapid phenotypic changes in insects as they need to adapt to variation in host plant physiology (Singer and McBride, 2010).

In chapter 2, I confirm the difference in overwinter cold tolerance and spring activation of larch casebearer first noted by Ward *et al.* 2020 and investigate whether host species is responsible for these differences. Using a common garden experiment, I reared larch casebearer larvae from Minnesota from eggs on two different species of larch, eastern and western larch. Following the same protocol of Ward *et al.* 2020, I quantify the supercooling points and the number of growing degree days needed for spring activation of the experiment groups.

Chapters comprising this thesis 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 co-authors and/or committee members: Brian Aukema, George Heimpel, Robert Venette, Aubree Kees, Samuel Ward, and Erika Eidson. Therefore, I present the following research in the plural voice, rather than singular.

Chapter 1. Examining the larch casebearer and the associated parasitoid complex on eastern larch in the Great Lakes region

Chapter Summary:

In the past twenty years, populations of larch casebearer, *Coleophora laricella* Hübner, (Lepidoptera: Coleophoridae), have increased on *Larix* spp. across North America. The resurgence of defoliating activity is a departure from decades of successful biological control by two introduced parasitoids; *Agathis pumila* Ratzeburg (Hymenoptera: Braconidae) and *Chrysocharis laricinellae* Ratzeburg (Hymenoptera: Eulophidae) and has prompted renewed interest in the status of biological control in this system. While recent studies have observed continued presence of these parasitoids in the western United States, surveys of biological control agents of larch casebearer have not been conducted for more than 50 years in the Great Lakes region of the eastern United States and Canada. We sampled eastern larch, *Larix laricina* (Pinaceae), at multiple sites across Minnesota and Wisconsin, USA in 2018 and 2019. We confirmed the continued presence of *A. pumila* and *C. laricinellae* in both states along with 13 other parasitoid species associated with larch casebearer. Mean densities of larch casebearer ranged from 1.4 – 6.8 insects/100 buds in 2018 to <1 insect/100 buds in 2019. Total parasitism rates in 2018 ranged from 11.5 – 19.0%. *Chrysocharis laricinellae* was the most abundant parasitoid, with densities as high as 6.1 insects/100 buds in one site, followed by *Cirrospilus pictus* Nees (Hymenoptera: Eulophidae) at approximately half that density. The latter has never been reported in high numbers so its abundance, higher than the

specialist *A. pumila*, was surprising. Climatic changes that may be affecting trophic relationships in this system are discussed.

Introduction

The larch casebearer, *Coleophora laricella* Hübner (Lepidoptera: Coleophoridae), is native to the European Alps where it feeds on European larch, *Larix decidua* Mill. In the 1800s, larch casebearer arrived in North America, likely on a shipment of nursery stock, and quickly moved to eastern larch, *Larix laricina* (Du Roi) Koch. First documented in 1886 in North Hampton, Massachusetts, the first Canadian establishment was reported by Fletcher in Ottawa, Ontario in 1905 (Rush, 1972). By the 1920s, larch casebearer was causing heavy defoliation of eastern larch throughout the Great Lakes region (Cody *et al.*, 1967; Graham, 1949; Rush, 1972). In 1957, larch casebearer was discovered infesting western larch, *Larix occidentalis* Nutt., in Saint Marie, Idaho, from whence it spread rapidly across proximate western states in less than 10 years (Denton and Tunnock, 1968; Ryan *et al.* 1987).

This defoliator feeds on several species of larch or tamarack, *Larix* spp. (Ryan *et al.* 1987). Adult moths emerge in late spring, mate, and lay between ten and 100 eggs per female on the underside of needles depending on ambient temperature (Denton, 1979; Quednau, 1967). Newly hatched larvae mine directly into larch needles to feed. At third instar, they construct a one- to two-millimeter case carved from a hollowed needle and lines it with silk (Ryan, 1975). Wearing their cases as protection, larvae attach their cases to new needles on which they feed. In late summer, the case-bearing third instars migrate to needle fascicles or twigs, reattach their cases, and plug the openings with silk. The larvae overwinter inside their cases. Upon bud break in the spring, and after molting into

fourth instars, they continue to feed for a few weeks until they are ready to pupate within new silk-sealed cases (Ward *et al.* 2019a).

Repeated defoliation by casebearing larvae stunts tree growth and predisposes trees to secondary mortality factors such as fungi or eastern larch beetle, *Dendroctonus simplex* LeConte (Denton, 1979; Ward & Aukema, 2019). As such, several control strategies against larch casebearer were developed. Aerial spraying of insecticides DDT and malathion were highly effective but costly. Moreover, they necessitated precise timing to target the free-feeding fourth instars so were viewed as stop gaps until biological control programs would prove effective (Denton and Tunnock 1968). Early surveys found a variety of native parasitoids attacking larch casebearer, although few demonstrated promise as agents of control (Coppel and Shenefelt, 1960). A classical biological control program was implemented in the mid-20th century and at least eleven parasitoids of the larch casebearer were introduced to the eastern United States from Europe between 1932-1937 (Coppel and Shenefelt, 1960; Graham, 1948). Similarly, five parasitoids of larch casebearer were imported from England and released in Ontario in the mid-1930s, with additional releases of select agents continuing into the 1940s (Graham, 1948). Many of the species failed to establish or provide any control (Cody *et al.*, 1967). Two species, however, *Agathis pumila* (Ratz.) (Hymenoptera: Braconidae) and *Chrysocharis laricinellae* (Ratz.) (Hymenoptera: Eulophidae), originally released in Massachusetts in 1932, established successfully. These two species would dominate the focus of the biological control program against larch casebearer in the many years to come (Cody *et al.*, 1967; Ryan, 1980).

Agathis pumila is a braconid wasp with a univoltine lifecycle and is a feeding specialist on larch casebearer. Adults emerge in late June and July to mate and oviposit on early needle-mining instars. *Agathis pumila* is a koinobiont endoparasite. Infected hosts exhibit delayed development by as much as two weeks relative to unparasitized larvae (Quednau, 1970). At host pupation, the larvae of *A. pumila* kills the host and completes its own development (Cody *et al.*, 1967; Ryan, 1980).

Chrysocharis laricinellae is a generalist eulophid wasp that paralyzes and kills the host upon oviposition. *Chrysocharis laricinellae* can have two or three generations per year depending on host densities and the densities of *A. pumila*, as *A. pumila* facilitates the success of *C. laricinellae* by delaying host development and enhancing phenological synchrony with *C. laricinellae* (Graham, 1948; Quednau, 1970; Cody *et al.*, 1967; Ryan, 1990). *Chrysocharis laricinellae* will oviposit into cases that have been previously parasitized by other parasitoid species, including *A. pumila*, although it is not considered a hyperparasitoid (Graham, 1948; Ryan, 1990). Due to the inability of *C. laricinellae* to parasitize certain life stages of larch casebearer, small changes in phenology can disrupt the ability of *C. laricinellae* to provide adequate control. As a result of this facultative relationship with *A. pumila* and poor synchronization, *C. laricinellae* is not generally considered to be an effective biological control agent by itself (Graham, 1948; Quednau, 1970; Ryan, 1986; Ryan, 1990).

Biological control often takes at least five years post-release in the larch casebearer system to take effect (Ryan, 1997). In the Great Lakes region, surveys across 21 sites in Michigan in 1954 revealed mean parasitism rates of 25.1% on larch casebearer with *C. laricinellae* contributing most of the overall parasitism (Cody *et al.* 1967). Nine

years later, Cody *et al.* repeated the study and observed two notable changes (Cody *et al.* 1967). First, the mean parasitism rate had increased to 51% across all sites. Second, all parasitism was attributed to *A. pumila* with a complete absence of *C. laricinellae*. During the 1963 collections, populations of larch casebearer were very low suggesting successful biological control (Cody *et al.*, 1967).

In the western United States, both species of parasitoids were released in 1960 and had established populations throughout the range of western larch by 1980 (Miller-Pierce *et al.*, 2015; Ryan, 1981). Studies prior to releases compared to studies following releases of parasitoids demonstrated that control of larch casebearer was achieved when the parasitism rate of *A. pumila* reached 10%, notwithstanding sporadic contributions from *C. laricinellae* (Ryan, 1990). By 1990, the biological control program was declared successful across North America as larch casebearer damage was undetectable at the landscape level populations of larch casebearer fell below one or two moths per 100 larch buds (Ryan, 1997).

Since 2000, however, there has been a national resurgence of sporadic defoliation events due to larch casebearer in both eastern and western larch (Ward & Aukema, 2019). Over the past two decades, up to 30,000 ha of defoliation has been detected in Washington and Oregon and up to 10,000 ha in Minnesota each year (Ward & Aukema, 2019). This resurgence has renewed interest in the current status and prevalence of parasitoids of larch casebearer, as few follow-up surveys were conducted following successful defoliator suppression. A recent study in the range of western larch noted that *A. pumila* and *C. laricinellae* were still present in Oregon, Washington, Idaho, and Montana, with average parasitism rates of 19.3% and 2.8% by *A. pumila* and *C.*

laricinellae, respectively (Miller-Pierce *et al.*, 2015). In the Great Lakes region, however, no follow-up surveys of parasitoids of larch casebearer have been conducted in the past fifty years since the work of Cody *et al.* (1967) and Rush (1972). As such, this note reports the results of surveys of biological control agents and their respective parasitism rates on larch casebearer in Wisconsin and Minnesota in the Great Lakes region in 2018 and 2019 and compares present parasitism to past levels.

Methods

Sampling was initiated on 1 June 2018 when larch casebearers were in spring-feeding larval and pupal life stages (Ward *et al.* 2019a). Two sites with known populations of larch casebearer were selected near Floodwood, Minnesota (Table 1). Five eastern larch trees were selected at random from each site. Four branches approximately 1 cm in diameter measuring 45 cm from the leading end of the branch were cut and placed into 3.8 liter plastic tubs with fine mesh lids. All accessory twigs were included. Samples were immediately returned to the laboratory and placed in a rearing room at a constant 23°C and 14L:10D light conditions. Larch casebearer moths and associated parasitoids were allowed to develop and emerge from the samples into gallon containers with fine mesh lids for six weeks. After six weeks, total branch length, the number of previously live buds, dead buds (i.e., those with no previous needles), cones, cases, and all species of Hymenoptera were recorded. Wasps were identified to lowest possible taxonomical level using Ryan (1980) and identifications were confirmed by Dr. J. Luhman of the Insect Museum of the University of Minnesota. Voucher specimens were placed in the University of Minnesota Insect Collection.

On 1 July 2018, a subset of $n=2000$ cases from the Floodwood, Minnesota site during field collections was placed into individual sealed 10ml vials and observed every day for the next 34 days for insect emergence to determine the prevalence of multiparasitism and hyperparasitism, i.e. multiple parasitoids emerging from one single case or the presence of multiple hymenopterous molts within the case. The vials were kept inside the laboratory at 20°C under long-day light conditions. A subset of 50 cases were dissected to search for evidence of hyperparasitism or multiparasitism.

In 2019, one of the two original sites was revisited along with nine additional sites across Minnesota and Wisconsin. Sites were selected from the Minnesota and Wisconsin Departments of Natural Resource database of known infestations of larch casebearer and followed old survey transects from Cody *et al.* (1967) (Table 1). Two separate surveys were timed based on degree-day estimates of relevant life stages (Ward *et al.*, 2019a). The first survey occurred during larch casebearer pupation between 19 and 22 June 2019. The second survey occurred after adult emergence and oviposition between 19 and 22 July 2019. For each survey, three eastern larch trees were randomly selected within a range of 2-15 m in height. Four branches were cut from each tree measuring 1 cm in diameter and 45 cm from the leading end of the branch; one from the upper crown, two from the mid-crown, and one from the lower crown, using a hand or pole pruner and following the four cardinal directions: north, east and west, and south, respectively (Miller-Pierce *et al.*, 2015). The branches, along with accessory twigs, were placed into one-gallon plastic tubs with fine mesh lids. Following the same protocols as 2018, samples were brought to the laboratory and processed after six weeks as previous. As in

2018, a subset of $n=2000$ pupal cases from the initial 2019 survey were collected and placed into individual sealed vials for daily monitoring for 34 days.

Following protocols of Cody *et al.* (1967) and Miller-Pierce *et al.* (2015), moth density was calculated as the number of moths per 100 larch buds. The rate of parasitism of larch casebearer by each parasitoid wasp was calculated by dividing the total number of wasps per site by the total number of cases per site (Miller-Pierce *et al.*, 2015).

Unfortunately, of the hundreds of pupal cases of larch casebearer collected in 2019, most of the cases were shriveled, grey, moldy, and attached to the twigs rather than the foliage; an indication that no emergence from the overwintering stage had occurred. We report, as stated in Table 1, but omit unviable cases from further analyses due to uncertainty whether such cases were from prior years or reflected recent winter mortality. We do not report parasitism rates in 2019 because we did not obtain a minimum of 100 viable cases at any site.

Results and Discussion

Larch casebearer moths were reared from samples across both sites in 2018 and seven of the ten Minnesota and Wisconsin sites in the first surveys of 2019 (Table 2). Only half of the sites across Minnesota and Wisconsin in 2019 contained potentially viable cases (i.e. large, cigar shaped, on foliage, and of a light brown colour) (Table 1). A total of 44 larch casebearer moths were reared from all sites in 2019, compared to almost 300 from two sites in Minnesota the previous year (Table 2). Moth density ranged from 4 moths/100 buds to 8 moths/100 buds in 2018 to <1 moth/100 buds in 2019. These herbivore densities were slightly lower than recent analogous surveys in the western United States where Miller-Pierce *et al.* (2015) observed densities of larch casebearer

ranging from 6.2 to 13.1 moths/100 buds. Prior studies in the Western United States found that historical outbreaking populations not under biological control averaged 50.0 moths/100 buds, but dropped to 1.6 moths/100 buds after biological control by *A. pumila* (Ryan, 1997).

Both *C. laricinellae* and *A. pumila* were both recovered from samples in 2018 (Table 2). *Chrysocharis laricinellae* ($n = 249$) was more abundant than *A. pumila* ($n = 31$) at both sites. *Agathis pumila* densities at both sites averaged <1 insect/100 buds, while *C. laricinellae* densities ranged between 2.5 - 6.1 insects/100 buds (Table 3). Similar to the trend of *C. laricella* results, fewer parasitoids were recovered in 2019 vs. 2018 (Table 2). One specimen of *A. pumila* was reared from one site in Minnesota and six *A. pumila* were reared from a site in Wisconsin (Table 2). The second survey of 2019 yielded only two wasps, one *A. pumila* and one *C. laricinellae*, reared from a total of 14 viable cases at the Clam Lake site and eleven *C. laricinellae* reared from a total of 15 viable cases collected at the Nelma site in Wisconsin.

Cirrospilus pictus Nees (Hymenoptera: Eulophidae) ($n = 177$) was reared in greater abundance than *A. pumila* ($n=31$) in 2018 (Table 2), despite being a historically rare parasitoid of larch casebearer (Ryan, 1979). Overall, mean *C. pictus* densities of approximately 2.8 insects/100 buds were lower than *C. laricinellae* but greater than *A. pumila* (Table 3). *Cirrospilus pictus* is a generalist parasitoid originally from Europe, and has been reported on lepidopterous, hymenopterous, and coleopterous leafminers, such as citrus leafminer (Bouček and Askew, 1968; Pinto, M. Lo, *et al.* 2005). This species has acted both as a primary and secondary parasitoid in various systems throughout Europe (Bouček and Askew, 1968). It has been recorded parasitizing Siberian larch casebearer,

Coleophora sibiricella Flkv. (Lepidoptera, Coleophoridae) on Siberian larch, *Larix sibirica* (Pinaceae) (Ermolaev, 2000), which was inadvertently introduced into Serbia with *Larix decidua* (Pinaceae). *Cirrospilus pictus* has been observed as a parasitoid of larch casebearer within Serbia, but little is known about its role in biological control (Tabakovic-Tosic, 2011).

Thirteen other parasitoids were reared from samples in 2018 and 2019. All other species were reared out in low numbers (<10 specimens) and at low densities (<1 wasp/100 buds) across both years of the surveys. *Conura albifrons* Walsh (Hymenoptera: Chalcididae) was the most common parasitoid native to North America, although only six specimens were noted in total. This generalist parasitoid was observed to be the most dominant native parasitoid of larch casebearer in Idaho and Washington as well (Hansen, 1980). There was hope that *C. albifrons* would provide control over larch casebearer, but surveys have never noted high densities or parasitoid rates. Historically, other native parasitoids have been rare within this system and our results showed that this is still the case in 2018 and 2019 (Ryan, 1997).

Total parasitism from all parasitoids ranged from 11.5% to 19.0% across both sites in 2018. In 2018, *C. pictus* and *C. laricinellae* contributed the most parasitism at 5.4% and 12.5%, respectively, depending on site (Table 3). *Agathis pumila* only contributed approximately 1% parasitism at both sites. The second survey of 2019 observed a combined parasitism rate of 3.5% at the Clam Lake site by *A. pumila* and *C. laricinellae*. The 2.6% parasitism rate observed at the Nelma site in Wisconsin was due entirely to *C. laricinellae*.

We did not find any incidences of hyperparasitism or multiparasitism in the collections of cases stored and examined individually. Of the 2000 vials collected and checked daily in 2018, 291 *C. laricinellae* individuals, 37 *A. pumila*, and 257 *C. pictus* were reared, but never multiple individuals out of a single case. Of the 2000 vials collected and checked daily in 2019, one *C. pictus*, and 32 *A. pumila* were reared. No adult moths were reared from any of the samples collected during the second survey of 2019. Following the dissections of cases, no evidence of hyperparasitism or multiparasitism was noted.

Our surveys confirmed that both *A. pumila* and *C. laricinellae* are still present and active in forests of eastern larch in sites within the Great Lakes Region. Cody et al. (1964), surveying in same regions in the 1950s and 1960s, hypothesized that “when both parasitoids are present and larch casebearer populations are high, *Chrysocharis laricinellae* is most abundant. When larch casebearer populations are at a low level, *Agathis pumila* is most abundant.” Poor sample quality in 2019 precluded larger numbers of insects needed for detection of year-to-year changes in growth rates, such as delayed density dependence between *A. pumila* and its host. We remain unsure why sample quality was so poor in 2019 and investigated whether a lethal overwintering cold temperature event could have decreased populations from 2018 to 2019. The mean supercooling point of larch casebearer is -43.4 ± 1.3 °C (Chapter 2; Ward *et al.*, 2019b). While some individuals in the population may have experienced overwintering mortality, temperature records at two recording stations proximate to our field sites (NOAA Station ID Bemidji 3.5 WSW, Minnesota, US US1MNB0010 and NOAA Station ID Eagle River, Wisconsin, US USC00472314), demonstrated that temperatures did not fall below

-40 °C at any point during that winter. We thus view it as unlikely that mass mortality was due to winter temperatures conditions. Nevertheless, our results in areas revisited more than 50 years later support Cody's hypothesis; our 2018 survey observed higher moth density and higher overall parasitism by *C. laricinellae* and our 2019 observed lower moth density and the presence of only *A. pumila* in the initial survey.

Given the combined presence of *A. pumila* and *C. laricinellae* on the landscape, there remain unanswered questions about why larch casebearer has been noted with higher magnitude and frequency in the Great Lakes region in the past 20 years after decades of successful biological control (Ward and Aukema, 2019). This resurgence is likely due to several factors, such as increased spring warming permitting more casebearers to successfully reach fall overwintering stages prior to needle abscission on the deciduous conifers (Ward *et al.*, 2019a). Moreover, Ward *et al.* (2020) observed that warming temperatures are increasing the population growth of larch casebearer, while simultaneously decreasing the population growth rates of *A. pumila* and *C. laricinellae*. Finally, the effects of *C. pictus* in this system are largely unknown but may be an additional destabilizing force, especially if *C. pictus* is acting as a hyperparasitoid on *A. pumila* or *C. laricinellae*.

Acknowledgements

We thank Elgin Lee, Kristine Jecha, Mara Short, and Nicole Witt (University of Minnesota) for their assistance in data collection; George Heimpel (University of Minnesota), Samuel Farhner Ward (Mississippi State), and Aubree Kees (University of Minnesota) for assistance with methodology; John Luhmen (University of Minnesota) for assistance with parasitoid identification, and Paul Cigan (Wisconsin Department of

Natural Resources) and Brian Schwingle (Minnesota Department of Natural Resources) for assistance in finding sites. Funding was provided by USDA Forest Service Award 15-DG-1142004-237 and Minnesota Agricultural Experiment Station Project MIN-17-095.

Table 1: Sites for larch casebearer and parasitoid surveys and number of cases collected in 2018 and 2019, Minnesota and Wisconsin, USA

Site	State	Coordinates (Lat, Long)	Description	Casebearer cases collected *					
				June 2018		June 2019		July 2019	
				Total	Viable	Total	Viable	Total	Viable
Floodwood	MN	47.001661, -93.042495	Black spruce/eastern larch swamp	1297	1297	1131	30	690	23
Wawima	MN	46.996509, -93.169525	Roadside stand of eastern larch	2177	2177				
Pine River	MN	46.730262, -94.464411	Black spruce/eastern larch swamp			71	71	11	5
Cass Lake	MN	47.340353, -94.600994	Black spruce/eastern larch swamp			0	0	1	1
Bagley	MN	47.496920, -95.262631	Black spruce/eastern larch swamp			3	3	3	1
Canyon	MN	47.033537, -92.569891	Black spruce/eastern larch swamp			107	51	27	18
Clam Lake	WI	46.166311, -90.908495	Black spruce/eastern larch swamp			15	9	58	14
Land O' Lakes	WI	46.097407, -89.192511	Black spruce/eastern larch swamp			0	0	4	0
	WI	46.068335, -89.054330	Maintained turf grass lawn with eastern			9	4	0	0
Phelps			larch						
Nelma	WI	46.001335, -88.934434	Boreal forest with intermittent eastern larch			98	55	424	15
Harshaw	WI	45.646097, -89.699252	Roadside stand of eastern larch			109	64	36	18

*The total number of cases collected is reported along with the number of viable cases used in calculations. Cases in 2019 that were shriveled, grey, or moldy, and not attached to any green foliage were omitted from analyses.

Table 2: Total number and density of specimens reared from sampling in 2018 and the initial survey in 2019, Minnesota and Wisconsin, USA

Species (Family)	Site											
	2018		2019									
	Floodwood	Wawima	Pine River	Cass Lake	Bagley	Floodwood	Canyon	Clam Lake	Land O' Lakes	Phelps	Nelma	Harshaw
Larch casebearer (Coleophoridae)	51	234	3	0	0	11	2	5	0	3	6	9
<i>Agathis pumila</i> (Braconidae)	10	21	1	0	0	0	0	0	0	0	6	0
<i>Chrysocharis laricinellae</i> (Eulophidae)	162	87	0	0	0	0	0	0	0	0	0	0
<i>Cirrospilus pictus</i> (Eulophidae)	72	105	0	0	0	0	0	0	0	0	0	0
<i>Conura albifrons</i> (Chalcidae)	0	3	0	1	0	0	0	1	0	0	1	0
<i>Bracon</i> sp. (Braconidae)	0	3	1	0	0	0	0	0	0	1	0	0
<i>Gelis</i> sp. (Ichneumonidae)	1	0	0	0	1	0	0	0	0	0	1	0
<i>Pteromalus</i> sp. (Pteromalidae)	0	1	0	0	0	0	0	0	0	0	0	0
<i>Euderus</i> sp. (Eulophidae)	1	1	0	0	0	0	0	0	0	0	0	0
<i>Spalangia</i> sp. (Pteromalidae)	0	1	0	0	0	0	0	0	0	0	0	0
<i>Elachertus</i> sp. (Eulophidae)	1	3	0	0	0	0	0	0	0	0	0	0
<i>Mastrus</i> sp. (Ichneumonidae)	0	0	0	0	0	0	0	0	0	0	1	0
<i>Polynema</i> sp. (Mymaridae)	0	0	0	0	0	0	0	0	0	0	1	0
Unknown Mymaridae	0	0	0	0	0	0	0	0	0	0	1	0
Unknown Encyrtidae	0	0	0	0	0	0	0	0	0	0	1	0
Unknown Diapriidae	0	0	0	0	0	0	0	0	0	0	2	0

Table 3: Density and percent parasitism for larch casebearer, *A. pumila*, *C. laricinellae*, and *C. pictus* in 2018. Metrics are not presented for 2019 due to low sample sizes (Table 1).

Site	Density (number of insects/100buds)				Percent Parasitism				
	Larch casebearer	<i>Agathis pumila</i>	<i>Chrysocharis laricinellae</i>	<i>Cirrospilus pictus</i>	<i>Agathis pumila</i>	<i>Chrysocharis laricinellae</i>	<i>Cirrospilus pictus</i>	Other	Total
Floodwood	1.4	0.4	6.1	2.7	0.8	12.5	5.6	0.2	19.0
Wawima	6.8	0.6	2.5	3.0	1.1	4.5	5.4	0.6	11.5

Chapter 2. Physiological response of larch casebearer reared on eastern vs. western larch trees in a common garden experiment

Chapter Summary:

1. Larch casebearer, *Coleophora laricella* Hübner, was introduced into North America from Europe in the 1880s. By the 1960s, this defoliating caterpillar was found infesting two geographically isolated species of larch; eastern larch, *Larix laricina* (Du Roi) K. Koch and western larch, *Larix occidentalis* Nutt..
2. A recent study demonstrated that allopatric populations of larch casebearer from eastern larch in Minnesota and western larch in Oregon, Idaho, and Montana, U.S.A. differ significantly in their overwintering cold tolerance and the number of accumulated degree days until larval activation in the spring. The species of host tree upon which larvae were feeding was posited as a potential reason for the observed differences.
3. We collected larch casebearers in Minnesota and caged them separately on eastern larch vs. western larch in a common garden experiment in Minnesota to determine if feeding on different a host tree species affects the cold tolerance and the accumulated degree days until spring activation.
4. We found that supercooling points and the accumulated degree days until activation of progeny sourced from eastern populations of larch casebearer feeding that fed on two different species of larch were similar. Thus, it is unlikely that previously observed differences in cold tolerance and phenology were due to host species.
5. Our results suggest that cryptic species differences and/or climatic variations between western and midwestern North America that influence overwintering

physiology could be responsible for the observed differences. Thermal plasticity and adaptation across a range of winter conditions may facilitate invasion success of this invasive herbivore.

Introduction:

Establishment of insects in novel environments subjects them to a variety of selective pressures that can induce trait differences unique from populations in the native range (Moran and Alexander, 2014). Unique selective pressures experienced by geographic separation of adventive populations in the new range can further accentuate these differences. Selective forces may be pronounced; insects may need to rapidly adapt to disparate thermal cues in their novel environment through phenotypic plasticity or face local extinction, for example (Bradshaw and Holzapfel, 2006; Moran and Alexander, 2014). Similarly, host species can mediate several aspects of insect life histories, including oviposition, clutch size, dispersal, diapause, and spring emergence (Singer and McBride, 2010). Over time, phenotypical differences in allopatric populations may start to express in most of the respective population (Thorpe *et al.*, 2008; Moran and Alexander, 2014).

The larch casebearer, *Coleophora laricella* Hübner, is a needle-feeding defoliator that was introduced into the United States in the 1880s likely when shipments of European larch, *Larix decidua* Mill., were brought to Massachusetts for planting stock (Ryan *et al.*, 1987). The insect then spread to tamarack/eastern larch, *Larix laricina* (Du Roi) K. Koch, and began spreading westward in North America. In 1957, larch casebearer was detected for the first time infesting western larch, *Larix occidentalis* Nutt., in St. Marie's, Idaho. From there, it spread throughout much of the extent of the

range of that host (Ciesla and Bousfield, 1973; Ryan *et al.*, 1987). To date, alpine larch, *Larix lyallii* Parl., has not yet been affected by larch casebearer, likely because this tree species experiences colder temperatures at high elevations (Tunnock and Ryan, 1985).

Throughout its range, the larch casebearer has a univoltine lifestyle with adult emergence in late spring to early summer (Denton, 1979). Upon hatching, larvae mine the needles until they grow too large to fit inside them. At this point, they construct a silk-lined case out of a hollowed needle to continue feeding externally. In late autumn, before needle drop, the casebearers migrate to the fascicles or twigs to overwinter within the cases. Activation of larch casebearer in the spring lags behind bud break by about 28 days, when larvae will resume feeding and molt into their final instar (Ward *et al.*, 2019). After pupation, adults emerge from the posterior end the case to search for mates. Gravid female moths oviposit individual eggs onto the underside of needles, laying up to 100 eggs over their lifetime (Denton, 1979).

Trees suffer little to no growth loss during light infestations with limited defoliation (Alfaro *et al.*, 1991). However, in severe infestations, trees may respond to complete defoliation by prematurely dropping their remaining needles to reflush within the same growing season (Tunnock, 1969; Alfaro *et al.*, 1991). Multiple years of continuous defoliation and subsequent reflushing is energetically costly, which can weaken the hosts and predispose them to secondary mortality factors such as eastern larch beetle (Benoit and Blais, 1988; Alfaro *et al.*, 1991; Ward and Aukema, 2019). Due to the extensive impacts of introduced larch casebearer on eastern and western larch in North America, a biological control program was developed and implemented against larch casebearer. Two non-native parasitoids, *Agathis pumila* (Ratz.) and *Chrysocharis*

laricinellae (Ratz.), emerged as the most promising biological control agents (Ryan, 1990). *Agathis pumila* is a braconid wasp with a univoltine lifecycle that specializes on larch casebearer; adults emerge in late June and July to mate and oviposit on early needle-mining instars. *Chrysocharis laricinellae* is a generalist eulophid wasp with a multivoltine lifecycle exhibiting up to three generations per year. The combined parasitism of these two agents reduced populations of larch casebearer such that defoliation from larch casebearer disappeared from the landscape by the 1980s, prompting the program to be considered a success (Ryan, 1990; Ward and Aukema, 2019).

Since 2000, however, there has been a resurgence of defoliation events from larch casebearer nationally. This activity has prompted several studies evaluating the current status of natural enemies (Miller-Pierce *et al.*, 2015; Miller and Finlayson, 2019; Chapter 1), as well as investigations on climatic aspects associated with the insect's resurgence (Ward and Aukema, 2019; Ward *et al.*, 2019a, 2019b, 2020). In one of these studies, Ward *et al.* (2020), created a seasonal phenology model to predict degree days required for the development of each life stage. While the model worked well for populations of larch casebearer in the Great Lakes region of North America, it failed to capture substantial variation exhibited by populations of larch casebearer developing on western larch in the western United States. For example, in Minnesota, larch casebearers activated from their overwintering stage after accumulating 172 ± 19 degree days, with a 5°C base temperature, in the spring. In the western US, comparative thermal requirements for emergence were approximately 63 degree days. Moreover, mean supercooling points of overwintering populations of larch casebearers collected in Minnesota from eastern larch

were 10°C lower than the supercooling points of larch casebearers collected in Oregon, Idaho, and Montana on western larch (Ward *et al.*, 2020). The supercooling point is the lowest temperature reached prior to the insect freezing (Wilson *et al.*, 2003).

These physiological differences between eastern and western populations could arise from a variety of causes such as genetic differences, differences in thermal regimes influencing diapause intensity and termination, and/or host species (Singer and McBride, 2010; Ward *et al.* 2020). In the present work, we focused on testing the host species explanation. We conducted two experiments. In the first, we tested whether the differences observed in supercooling points and the number of degree days in the spring for larval activation between eastern vs. western populations of larch casebearer were still present (Ward *et al.*, 2020). We treated this experiment as a control and measured supercooling point and the number of degree days in the spring for larval activation following similar protocols to Ward *et al.*, 2020. In the second experiment, we conducted a common garden experiment to test if supercooling points and the number of degree days in the spring for larval activation differed for populations of larch casebearers sourced from Minnesota reared on two different larch species: eastern larch vs. western larch.

Methods:

Experiment 1: Differences between eastern vs. western larch casebearers

Overwintering cases of larch casebearer representing an eastern population were collected from a site located near Floodwood, Minnesota, USA (47.001661, -93.042495) on 11 February 2020. We collected approximately 200 cases by cutting twigs and branches to which cases were attached and placed them into large plastic containers. The

plastic containers were brought back to a greenhouse cold-frame at the University of Minnesota. Approximately 200 overwintering larch casebearer cases representative of a western population were collected similarly on stock of western larch and sent from the Idaho Forest Service Nursery on 10 February 2020. These cases were sent overnight in a plastic cooler with several icepacks surrounding the branches. Upon arrival at the University of Minnesota, the branches with larch casebearer cases were placed into a large plastic container and placed in the greenhouse cold-frame.

Beginning on 10 February of 2020, random larch casebearer cases were selected from the wild Minnesota and Idaho populations to perform supercooling assays. Unfortunately, many cases were grey and shriveled, which indicated they were older and/or not viable. Pandemic restrictions precluded additional collections. Nonviable cases were not used in assays. In total, 18 and 14 insects from Minnesota vs. Idaho populations were tested, respectively. Following the same protocol as Ward *et al.* (2020), cases were carefully picked from the trees and placed into a 1.5 ml microcentrifuge tube. 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). The thermocouple was adjusted to make contact with the case in the bottom of the tube. Each sealed tube was placed individually into the center of a 20-cm polystyrene cube and then 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). Supercooling points were measured at the moment of the exothermic release of heat caused by freezing. The cases were removed after each trial and placed into a rearing chamber with short day light cycles, 8L:16D, and at a

temperature of 23°C to monitor for survival, which was determined by movement of the case. Four trials were performed over a two week period.

A Hobo-sensor was placed on 15 February 2020 among the remaining overwintering casebearers in the cold-frame to measure local temperatures every 15 minutes. An examination of NOAA weather data from the University of Minnesota Saint Paul weather station (ID: USC00218450) between 01 Jan and 20 Feb 2020 showed that no degree days were accumulated prior to placement of the temperature loggers. Starting 1 April 2020, remaining cases from the wild populations ($n = 30$ each from Minnesota and Idaho, respectively) were monitored daily for spring activation, indicated by the caterpillars moving around on the twig or fascicle. Degree days were calculated above the 5 °C threshold for larch casebearer starting 20 February 2020.

ANOVAs were used to determine the relationship between the supercooling points or accumulated degree days until activation as separate response variables and larch casebearer source location as the categorical variable using the “nlme” package in R (Pinheiro *et al.*, 2020). A random effect was inserted into the models. “Batch number,” i.e. supercooling group, was placed in the analysis of the supercooling points. Model assumptions of normality and homoscedasticity of residuals were assessed by visual inspection of residual plots. All data analysis was conducted in R statistical software version 3.6.1 (R Core Team, 2019).

Experiment 2: Evaluation of casebearer differences on eastern larch vs. western larch.

Seven eastern larch trees and seven western larch trees were utilized for the experiment, purchased in Minnesota on 25 May 2019 and in Washington on 3 June 2019, respectively. All trees were four years old. Upon receipt, trees were repotted from 22.7-

liter pots into 37.9-liter pots 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. The trees were stored at the University of Minnesota, St. Paul campus in a greenhouse cold-frame with natural light and temperature conditions. Trees were watered four times a week with approximately 3 liters of water to ensure the soil was thoroughly soaked.

We collected casebearers in Minnesota, allowed them to mate, and established their progeny for study on the above two species of trees as follows. On 1 June 2019, pupating larch casebearer were collected near Floodwood, MN (47.001661, -93.042495) from which to rear out larch casebearer moths (Ward *et al.*, 2019). Branches and twigs of eastern larch with high concentrations of larch casebearer cases were placed into large plastic containers and brought back to the laboratory at the University of Minnesota. Cases were carefully plucked from the needles and placed into individual sealed vials. Each vial was observed daily from 1 June 2019 to 15 July 2019 for moth emergence. Vials were kept in a vented plastic container in a greenhouse cold-frame with natural light and temperature conditions.

Ten moths were placed together in a fine mesh bag, approximately 30 cm long by 15 cm wide, and left overnight in the lab for the moths to mate. While adult moths are not sexually dimorphic, field collected moths have assumed a 50:50 M:F distribution in the past (Ward and Aukema, unpublished data). The fine mesh bags along with the moths were secured with zip-ties and a cotton ball seal onto a larch branch with at least 500 larch needles for oviposition of eggs. These bags were placed onto the trees on 5, 6, 7, 8, 9, 11, 13, and 18 June 2019. A total of 140 larch casebearer moths were evenly placed

onto eastern and western larch trees with 70 larch casebearers per host species. The trees were monitored extensively until dormancy November 2019. The mesh bags were left on the trees to protect the larch casebearer cases from natural predators.

Beginning in February 2020, one half of the cases from each tree were randomly selected for supercooling assays as described in experiment 1, with the remaining cases left on the trees and within the mesh bags. In total, 17 and 15 insects from eastern and western larch were placed in supercooling assays, respectively. Starting 1 April 2020, the remaining cases from the common garden experiment were monitored daily for spring activation, indicated by the caterpillars moving from the twig or fascicles to recently flushed needles. Degree days were calculated above the 5°C threshold for larch casebearer starting 20 February 2020 as previous.

Two separate ANOVAs were created to determine the relationship between the mean supercooling points or accumulated degree days until activation as response variables on host species as the categorical variable using the “nlme” package in R (Pinheiro *et al.*, 2020). For the model examining the effect of host species on supercooling points, a random effect term for processing batch was incorporated. For the model examining the effect of host species on degree days to activation, a random effect term for tree was incorporated into the model. Models assumptions were assessed as previous.

Results

Experiment One:

The mean (\pm SE) supercooling points for larch casebearer collected from Idaho in February of 2020 were -29.6 ± 0.7 °C. The supercooling points of larch casebearer from

Minnesota exhibited mean supercooling points 13.9 °C lower than those sourced from Idaho, at -43.4 ± 1.3 °C. These numbers were significantly different (Fig. 1; $F_{1,16} = 94.37$, $P < 0.0001$). None of the individuals in the supercooling experiment eventually activated. Larch casebearer collected from Idaho and Minnesota required mean (\pm SE) accumulated degree days of 67 ± 10 degree days and 174 ± 20 degree days, respectively, before spring activation. Similar to the supercooling points, these differences were statistically significant (Fig. 2; $F_{1,58} = 2772.50$, $P < 0.0001$).

Experiment Two:

The supercooling points for larch casebearer from Minnesota reared on eastern larch, -41.5 ± 0.9 °C, vs. western larch, -39.2 ± 0.8 °C, were similar (Fig. 3; $F_{1,16} = 3.52$, $P = 0.08$). None of the cases used in the supercooling assays activated. The mean accumulated degree days until activation for larch casebearer from Minnesota reared on eastern larch, 175 ± 11 degree days, and western larch, 171 ± 13 degree days, were also similar (Fig. 4; $F_{1,31} = 1.39$, $P = 0.25$).

Discussion

The results of our common garden experiment showed that while there are pronounced differences in overwintering cold tolerance and the amount of warmth needed to activate to resume feeding in the spring between eastern (i.e., Minnesota) and western (i.e., Idaho) populations, these differences are not likely due to the species of tree (eastern larch versus western larch) on which the insects were feeding. When phytophagous insects use different resources host species, traits will eventually diverge and both cold tolerance and emergence timing can be affected by host interactions (Berlecher and Feder, 2002; Singer and McBride, 2010; Rosenberger *et al.*, 2017).

Disparate phenologies across different host species can incite changes within insect species to allow synchronization with the host plant, for example (Dambroski and Feder, 2007; Singer and McBride, 2010). In the case of larch casebearer, its resilience to a wide variety of conditions in synchrony between larval activation and spring foliage flush, its most critical life period, has been contributed to its widespread invasion success and colonization of multiple *Larix* spp. native to North America (Ward *et al.*, 2019).

Insects that feed on different hosts may experience phenotypical changes that can affect their cold hardiness (Hunter and McNeil, 1997; Feng *et al.*, 2016). Terpenoids can affect free-feeding defoliators on *Larix* spp. (Rudloff, 1987; Powell and Raffa, 1999). High concentrations of diterpenes in eastern larch deter feeding and decrease weight of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) Linnaeus, for example, likely decreasing growth rate (Powell and Raffa, 1999). Terpenoids found in trees of Pinaceae play important roles in defense against defoliating insects and different species of the same genus have variations in these terpenoids (Rudloff, 1987; Powell and Raffa, 1999). Western larch and eastern larch differ significantly in the concentrations of major terpenes. Eastern larch has lower concentrations of α -pinene, β -pinene, β -phellandene, limonene, cadinene isomers, cadinol isomers, and monool compared to western larch, but has higher concentrations of δ -3-carene, sabinene, terpinolene, camphene, bornyl acetate, nerolidol, and fornesol isomers compared to western larch (Von Rudloff, 1987). It is not entirely known how terpenoids affect larch casebearer, but it has been suggested that monoterpenes may delay the timing of activation of larch casebearer to when monoterpene concentrations decrease (Ward *et al.*, 2019). The lack of differences between species in this study, however, and previous studies indicate that larch

casebearer appears to be robust to variation in needle monoterpenes in general (Figs. 3,4; Ward *et al.*, 2019).

Differences in cold tolerance or spring emergence cues may instead be due to the disparate thermal regimes in Idaho versus Minnesota. Temperature and photoperiod are important factors in the development and initiation of insects and changes in these factors can quickly affect insects and their relationship with their host (Mattson & Haack, 1987; Peterson, 1994; Košťál, 2006). Minnesota and Idaho share similar latitudes and thus photoperiods throughout the year, so phenotypic differences are assumed to be caused by other thermal conditions. Autumnal factors can greatly affect overwintering diapause (Mousseau and Dingle, 1991). Warming autumns lead larch casebearer larvae from Minnesota to activate more than 100 degree days later in the subsequent spring, with 34% fewer individuals activating compared to groups at normal autumnal temperatures (Ward *et al.*, 2019). Idaho is generally warmer throughout the winter compared Minnesota with average low temperatures between -4.4 °C to 1.1 °C and -17.7 °C to -12.2 °C from December to March, respectively (Vose *et al.*, 2014). Thus, larch casebearer populations in Idaho need not be as cold hardy as larch casebearer populations in Minnesota, as supported by the results of our study. Larch casebearer populations from Idaho also accumulate fewer degree days to activate earlier in the spring than larch casebearer populations in Minnesota (Fig. 2). Temperatures suitable for growing accumulate earlier in Idaho and larch casebearer must activate earlier to keep synchronization with their hosts.

A third, but not mutually exclusive explanation for the differences noted in the two populations of larch casebearers is that one region may have an unidentified cryptic

species. Within the family Coleophoridae, the taxonomic structure down to genus and species levels is considered unstable as the minute characteristics of the casebearing moths lead to difficulty in identification at a morphological and molecular level (Anikin *et al.*, 2016). Ward *et al.* (2020) observed a 10 °C lower mean supercooling point for larch casebearers from Minnesota against larch casebearers from widely separated populations in Idaho, Oregon, and Montana while we observed a 13 °C difference between Minnesota and Idaho. Likewise, Ward *et al.* (2020) observed that larch casebearers from Minnesota needed about 109 degree days more to activate in the spring than larch casebearers populations in Idaho, Oregon, and Montana, while we observed a similar difference of 107 degree days. The consistency in the differences noted across time and space despite putatively different seasonal thermal regimes between 2017 (Ward *et al.*, 2020) and 2019 (present study) suggest that these two populations might be different species. There are about 1350 described species of moths within the genus *Coleophora* with most found in the Nearctic and Palearctic regions, although many species are disputed (Pitkin and Jenkins, 2004). When larch casebearer was discovered in Idaho in 1957, it was assumed it was brought from the east and was therefore the same species. It is also possible that Siberian larch casebearer was introduced into Idaho, however. Siberian larch casebearer, *Coleophora sibiricella* Flkv. (Lepidoptera: Coleophoridae), is a common pest of Siberian larch, a tree species that has been imported into North America (Shepherd and Ross, 1973; Tkacz, 1991; Ermolaev, 2004). Since we did not note any morphological differences between the Minnesota and Idaho populations, genetic and molecular examinations of the different populations must be performed to determine their relationship.

Our study suggests that varying overwintering physiology and spring activation of populations of larch casebearer from Idaho and Minnesota do not differ due to host effects and must be driven by another factor. Populations of larch casebearer could have expressed rapid adaptation to varying climatic pressures between western and eastern North America (Ward *et al.*, 2019). This ability to adapt rapidly has contributed to larch casebearer's widespread distribution across two native larch species in North America. Alternately, larch species of western and eastern North America could be hosts to different cryptic species of larch casebearer (Shepherd and Ross, 1973; Pitkin and Jenkins, 2004). Confirmation or refutation of this hypothesis through genetic and molecular analyses are needed to further determine the drivers of the variation between larch casebearer populations and understand whether such differences are contributing to the resurgence of this invasive defoliator in North America (Ward and Aukema, 2019).

Acknowledgements

Funding was provided by USDA Forest Service Award 15-DG-1142004-237 and Minnesota Agricultural Experiment Station Project MIN-17-095. Thanks to Kevin Chase, Dorah Mwangola, and Grace Haynes (University of Minnesota) for their assistance with transporting trees.

Fig. 1

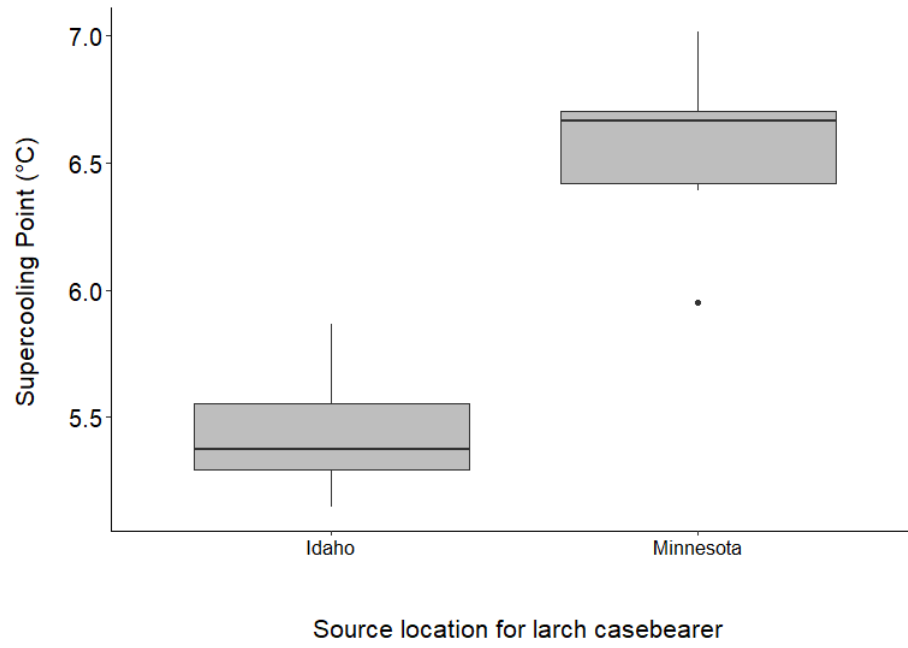


Fig. 2

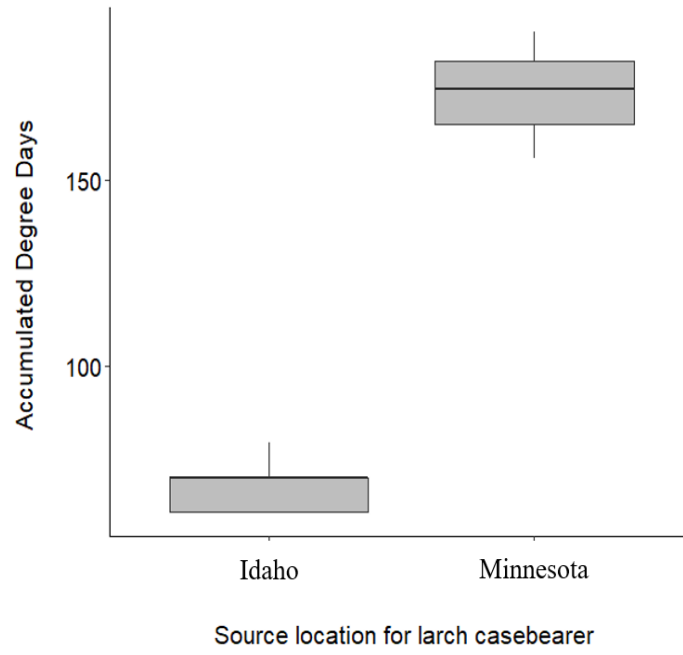


Fig. 3

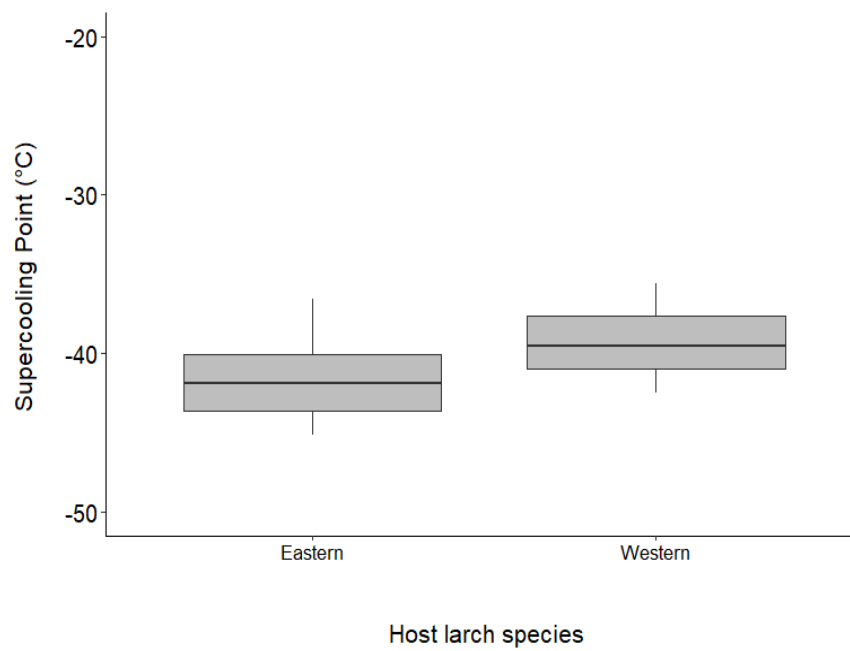
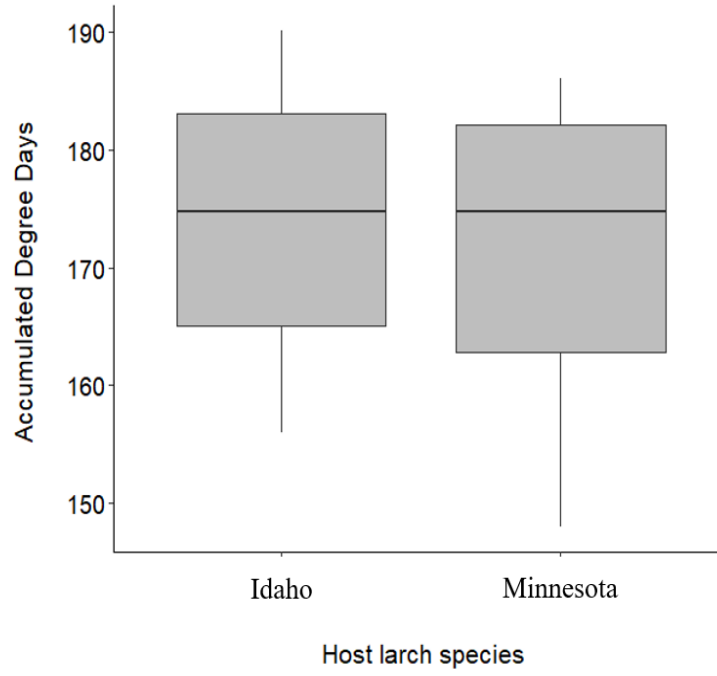


Fig. 4



Thesis Conclusions

- 1) **The introduced parasitoids, *A. pumila* and *C. laricinellae*, are still present within the Great Lakes region, along with 13 other parasitoid species.** We observed higher moth densities and higher overall parasitism by *C. laricinellae* in 2018 and lower moth densities and only parasitism by *A. pumila* in the initial survey of 2019, supporting the hypothesis that when larch casebearer populations are high, *C. laricinellae* is most abundant and when larch casebearer populations are low, *A. pumila* is most abundant (Cody *et al.* 1964).
- 2) **Despite being considered an historically rare parasitoid within this system, *Cirrospilus pictus* was more abundant than the specialist *A. pumila*.** *Cirrospilus pictus*, which has been observed as a hyperparasitoid in other systems, may be acting as a destabilizing force.
- 3) **Differences in the supercooling points and the number of degree days until activation in the spring between allopatric populations are not due to differences in host species, eastern larch vs. western larch.** These differences may exist due to two different cryptic species of larch casebearer or differences in thermal plasticity and adaptation across varying autumnal and winter conditions.

References:

- Alfaro, R. I., Qiwei, L., and Vallentgoed, J. (1991) Diameter growth losses in western larch caused by larch casebearer defoliation. *Western Journal of Applied Forestry*, **6**(4), 105-108.
- Anikin, V. V., Dyomin, A. G., and Knushevitskaya, M. A. (2016) Phylogeny and taxonomy of casebearer moths (Lepidoptera, Coleophoridae) based on morphological and molecular genetic data. 1. Reconstruction of phylogeny of Coleophoridae using analysis of COI gene variability. *Entomological Review*, **96**(1), 15-27.
- Benoit, P., and Blais, R. (1988) The effects of defoliation by the larch casebearer on the radial growth of tamarack. *The Forestry Chronicle*, **64**(3), 190-192.
- Berlocher, S. H., and Feder, J. L. (2002) Sympatric speciation in phytophagous insects: Moving beyond controversy? *Annual Review of Entomology*, **47**(1), 773-815.
- Bouček, Z., and Askew, R. (1968) *Index of Entomophagous insects. Palearctic Eulophidae (excl. Tetrastichinae)*. Ed. Delucchi and Remardiere, **3**: 254.
- Bradshaw, W. E., and Holzapfel, C. M. (2006) Evolutionary response to rapid climate change. *Science (Washington)*, **312**(5779), 1477-1478.
- Ciesla, W. M., and Bousfield, W. E. (1974) Forecasting potential defoliation by larch casebearer in the northern Rocky Mountains. *Journal of Economic Entomology*, **67**(1), 47-51.
- Cody, J. B., Knight, F. B., & Graham, S. A. (1967) The hymenopterous parasites *Agathis*

- pumila* (Braconidae) and *Epilampsis laricinellae* (Eulophidae) on the larch casebearer (Lepidoptera: Coleophoridae) in the northern lake states. *The Great Lakes Entomologist*, **1**(5): 159-167.
- Coppel, H.C., Shenefelt, R.D. (1960) Parasites introduced to help control the European Larch Casebearer - a progress report. Forestry Research Notes. Wisconsin University College of Agriculture and Conservation Department, **53**: 3.
- Dambroski, H. R., and Feder, J. L. (2007) Host plant and latitude-related diapause variation in *Rhagoletis pomonella*: a test for multifaceted life history adaptation on different stages of diapause development. *Journal of evolutionary biology*, **20**(6), 2101-2112.
- Denton, R. E. (1979) *Larch casebearer in western larch forests* (Vol. 55). Intermountain Forest and Range Experiment Station, Forest Service, US Department of Agriculture.
- Denton, R. E., Tunnock, S. (1968) Low-Volume Application of Malathion by Helicopter for Controlling Larch Casebearer. *Journal of Economic Entomology*, **61**(2): 582-583.
- Ermolaev, I. V. (2000) Evaluation of mortality factors of Siberian larch casebearer, *Coleophora sibiricella*, Flkv. (Lepidoptera, Coleophoridae) within sustained outbreak of leafminer. Biodiversity and Dynamics of Ecosystems in North Eurasia. Abstracts of the First International Conference, **4**: 1.
- Ermolaev, I. V. (2004) Ecological consequences of transient outbreaks of Siberian larch casebearer, *Coleophora sibiricella* Flkv., in the Udmurt Republic. *Russian Journal of Ecology*, **35**(4), 254-258.

- Feng, Y. Q., and Tursun, R. (2016) Effect of three species of host tree on the cold hardiness of overwintering larvae of *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *European Journal of Entomology*, **113**, 212-216.
- Graham, A. R. (1949) Developments in the control of the larch casebearer, *Coleophora laricella* (Hbn.). *Rep. ent. Soc. Ont.*, **79**: 45–50.
- Hansen, J. D. (1980) The life history and behavior of *Spilochalcis albifrons* (Hymenoptera: Chalcididae), a parasite of the larch casebearer, *Coleophora laricella* (Lepidoptera: Coleophoridae). *Journal of the Kansas Entomological Society*, **1**: 553-566.
- Hunter, M. D., and McNeil, J. N. (1997) Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. *Ecology*, **78**(4), 977-986.
- Košťál, V. (2006) Eco-physiological phases of insect diapause. *Journal of insect physiology*, **52**(2), 113-127.
- Mattson, W. J., and Haack, R. A. (1987) The role of drought in outbreaks of plant-eating insects. *Bioscience*, **37**(2), 110-118.
- Miller, G. E., and Finlayson, T. (2019) Parasites of the larch casebearer, *Coleophora laricella* (Lepidoptera: Coleophoridae), in the West Kootenay area, British Columbia. *Journal of the Entomological Society of British Columbia*, **74**, 16-22.
- Miller-Pierce, M., Shaw, D. C., Demarco, A., & Oester, P. T. (2015) Introduced and native parasitoid wasps associated with larch casebearer (Lepidoptera: Coleophoridae) in western larch. *Environmental Entomology*, **44**(3): 920-920.
- Moran, E. V., and Alexander, J. M. (2014) Evolutionary responses to global change: lessons from invasive species. *Ecology Letters*, **17**(5), 637-649.

- Mousseau, T. A., and Dingle, H. (1991) Maternal effects in insect life histories. *Annual review of entomology*, **36**(1), 511-534.
- Peterson, M. A. (1995) Phenological isolation, gene flow and developmental differences among low-and high-elevation populations of *Euphilotes enoptes* (Lepidoptera: Lycaenidae). *Evolution*, **49**(3), 446-455.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team (2020) `_nlme: Linear and nonlinear mixed effects Models_`. R package version 3.1-151, <URL: <https://CRAN.R-project.org/package=nlme>>
- Pinto, M. L., Salerno, G., and Wajnberg, E. (2005) Biology and behaviour of *Cirrospilus diallus* and *Cirrospilus pictus*, parasitoids of *Phyllocnistis citrella*. *BioControl*, **50**(6): 921-935.
- Pitkin, B. and Jenkins, P. (2004) Butterflies and moths of the world. Generic names and their type-species. The Natural History Museum, London.
(<http://www.nhm.ac.uk/entomology/butmoth/>)
- Powell, J. S., and Raffa, K. F. (1999) Effects of selected *Larix laricina* terpenoids on *Lymantria dispar* (Lepidoptera: Lymantriidae) development and behavior. *Environmental Entomology*, **28**(2), 148-154.
- Quednau, F. W. (1967) Notes on mating behavior and oviposition of *Chrysocharis laricinellae* (Hymenoptera: Eulophidae), a parasite of the larch casebearer (*Coleophora laricella*). *The Canadian Entomologist*, **99**(3): 326-331.
- R Core Team. (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>

- Rosenberger, D. W., Aukema, B. H., and Venette, R. C. (2017) Cold tolerance of mountain pine beetle among novel eastern pines: A potential for trade-offs in an invaded range? *Forest Ecology and Management*, **400**, 28-37.
- Rush, P. A. (1972) The larch casebearer (*Coleophora laricella*) population (Lepidoptera: Coleophoridae) and its associated parasite complex on the Newcomb tract. M.Sc. (For.) Thesis, University of Michigan, Ann Arbor: 61.
- Ryan, R. (1975) *Additional releases of larch casebearer parasites for biological control in the western United States*. Pacific Northwest Forest and Range Experiment Station, Forest Service, US Department of Agriculture.
- Ryan, R. (1979) *Illustrated key to introduced and common native parasites of larch casebearer*. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station.
- Ryan, R. (1981) *Recent (1977-1980) releases of imported larch casebearer parasites for biological control*. US Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station.
- Ryan, R. (1986) Analysis of life tables for the larch casebearer (Lepidoptera: Coleophoridae) in Oregon. *The Canadian Entomologist*, **12**: 1255-1263.
- Ryan, R. B. (1990) Evaluation of biological control: introduced parasites of larch casebearer (Lepidoptera: Coleophoridae) in Oregon. *Environmental Entomology*, **19**(6), 1873-1881.
- Ryan, R. (1997) Before and after evaluation of biological control of the larch casebearer (Lepidoptera: Coleophoridae) in the Blue Mountains of Oregon and Washington, 1972–1995. *Environmental Entomology*, **26**(3): 703-715.

- Ryan, R. B., Tunnock, S., and Ebel, F. W. (1987) The larch casebearer in North America. *Journal of Forestry*, **85**(7), 33-39.
- Shepherd, R.F. and Ross, D.A. (1973) *Problem analysis: larch casebearer in B.C.* Pacific Forest Research Centre Canadian Forestry Service Victoria, British Columbia Internal Report BC-37, Department of the Environment.
- Singer, M. C., and McBride, C. S. (2010) Multitrait, host-associated divergence among sets of butterfly populations: implications for reproductive isolation and ecological speciation. *Evolution: International Journal of Organic Evolution*, **64**(4), 921-933.
- Tabakovic-Tosic, M., Tosic, D., Rajkovic, S., Golubovic-Curguz, & V., Rakonjac, L. (2011) Invasion species *Coleophora laricella* - One of the main limiting factor of *Larix decidua* during the forest afforestation and recultivation. *African Journal of Agricultural Research*. **6**(4): 866-872.
- Thorpe, R. S., Surget-Groba, Y., and Johansson, H. (2008) The relative importance of ecology and geographic isolation for speciation in anoles. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**(1506), 3071-3081.
- Tkacz, B. (1991) *Pest risk assessment of the importation of larch from Siberia and the Soviet Far East* (Pub. 1495). Forest Service, US Department of Agriculture.
- Tunnock, S. (1969) *Larch casebearer and other factors involved with deterioration of western larch stands in northern Idaho* (Vol. 68). Intermountain Forest & Range Experiment Station, Forest Service, US Department of Agriculture.
- Tunnock, S. and Ryan, R. (1985) Larch casebearer in Western Larch. *Forest Insect and Disease Leaflet 96*, USDA Forest Service, Washington D.C.

- Von Rudloff, E. (1987) The volatile twig and leaf oil terpene compositions of three western north American larches, *Larix laricina*, *Larix occidentalis*, and *Larix lyallii*. *Journal of natural products*, **50**(2), 317-321.
- Vose, R.S., Applequist, S., Durre, I., Menne, M.J., Williams, C.N., Fenimore, C., Gleason, K., Arndt, D. (2014) Improved historical temperature and precipitation time series for U.S. Climate Divisions *Journal of Applied Meteorology and Climatology*. DOI: <http://dx.doi.org/10.1175/JAMC-D-13-0248.1>
- Ward, S. F., & Aukema, B. H. (2019) Anomalous outbreaks of an invasive defoliator and native bark beetle facilitated by warm temperatures, changes in precipitation and interspecific interactions. *Ecography*, **42**(5): 1068-1078.
- Ward, S. F., and Aukema, B. H. (2019) Climatic synchrony and increased outbreaks in allopatric populations of an invasive defoliator. *Biological Invasions*, **21**(3), 685-691.
- Ward, S., Aukema, B., Fei, S., & Liebhold, A. (2020) Warm temperatures increase population growth of a nonnative defoliator and inhibit demographic responses by parasitoids. *Ecology (Durham)*, **101**(11), E03156-N/a.
- Ward, S. F., Eidson, E. L., Kees, A. M., Venette, R. C., and Aukema, B. H. (2020) Allopatric populations of the invasive larch casebearer differ in cold tolerance and phenology. *Ecological Entomology*, **45**(1), 56-66.
- Ward, S., Moon, R.; Aukema B. (2019) Implications of seasonal and annual heat accumulation for population dynamics of an invasive defoliator. *Oecologia*, **190**(3): 703-714.
- Ward, S. F., Moon, R. D., Herms, D. A., and Aukema, B. H. (2019) Determinants and

consequences of plant–insect phenological synchrony for a non-native herbivore on a deciduous conifer: implications for invasion success. *Oecologia*, **190**(4), 867-878.

Ward, S. F., Venette, R. C., and Aukema, B. H. (2019) Cold tolerance of the invasive larch casebearer and implications for invasion success. *Agricultural and Forest Entomology*, **21**(1), 88-98.

Wilson, P. W., Heneghan, A. F., & Haymet, A. D. J. (2003) Ice nucleation in nature: supercooling point (SCP) measurements and the role of heterogeneous nucleation. *Cryobiology*, **46**(1), 88-98.