Biology and population dynamics of the eastern larch beetle, *Dendroctonus simplex* LeConte, and its interactions with eastern larch (tamarack), *Larix laricina*.

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Dedication

To my nephew Liam, and nieces Clara, Reese, and Libby.

May you always achieve your goals.

Abstract

The range of the eastern larch beetle, *Dendroctonus simplex* LeConte (Coleoptera: Scolytinae), is concomitant with its primary host, eastern larch (tamarack), *Larix laricina* (Du Roi) K. Koch, throughout the North American boreal forest. Since 2000, an ongoing outbreak of eastern larch beetles in the south-central part of tamarack's range throughout the Great Lakes region has caused extensive mortality to mature tamaracks, affecting over 86,500 hectares of tamarack forest in Minnesota. Extended outbreaks in live trees are atypical of this insect, so the eastern larch beetle's biology and ecology were studied under laboratory and field conditions in Minnesota from 2011 – 2014 to decipher the factors contributing to this ongoing outbreak.

In the laboratory, the minimum and optimal developmental temperatures for eastern larch beetles were determined to be 7.5 and 27.9°C, respectively. Some progeny were able to reproduce in the absence of an overwintering period, suggesting that a reproductive diapause may not be obligate in all individuals. This was confirmed by field studies, which found that a second generation of eastern larch beetles successfully completed development during the summer and fall of 2012. Confirmation of two generations instead of three sibling broods established by re-emerging parents in one year was established by detailed phenological and physiological methods.

As beetle infestations progressed through tamarack stands, beetles initially preferred to attack the largest tamaracks before killing smaller hosts at random in successive years. Reproductive success of females increased in larger and older tamaracks, and those "challenged" by unsuccessful attacks in the recent past. Higher concentrations of resin pockets within the phloem consistently reduced beetle reproduction. The size of male and female beetle offspring, as well as the total lipid content of female offspring, increased with tamarack size and phloem thickness. Development within "challenged" tamaracks reduced both the total and proportional lipid contents of all beetle offspring.

New understandings of the population dynamics of eastern larch beetles are discussed. Expanding growing seasons, for example, may facilitate fractional voltinism, or, two generations in one year, among a portion of the population. Synchronous beetle emergence the following

spring – shown in phenological studies – would enhance host procurement, especially of the largest and most preferred hosts that produce the most vigorous offspring, thus exacerbating the outbreak.

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Introduction

Bark beetles (Coleoptera: Scolytinae) are a group of insects that are important biotic components of ecosystem function. Within forest ecosystems, bark beetles are one of the most important agents of disturbance and contribute to processes of nutrient cycling, fire ecology, hydrology, and forest succession dynamics (Amman and Baker 1972, Nealis and Peters 2008, Axelson *et al*. 2009). As phloeophagous herbivores, bark beetles spend the majority of their lifecycle beneath the bark, within the sub-cortical tissues of host trees. Adults emerge from natal hosts and undergo a brief dispersal period in order to colonize new hosts and acquire mates (Wood 1982b).

Bark beetles are generally classified as “aggressive” or “non-aggressive” species depending on their biology relating to host colonization behavior and population dynamics. The majority of bark beetles are non-aggressive species that attack trees that have recently died, are moribund, or weakened by injury or disease. Such species persist at chronic levels within forest ecosystems, rarely undergo population eruptions, and cause minimal damage to forest stands. In contrast, aggressive bark beetles undergo intermittent population eruptions during which they colonize healthy, vigorous hosts, and cause large-scale forest mortality (Wood 1982b).

The eastern larch beetle, *Dendroctonus simplex* LeConte, is a native bark beetle of North America (Wood 1982b). The geographic range of the eastern larch beetle extends from the Canadian Maritime provinces, across the Canadian boreal forest to British Columbia and includes the northeastern and midwestern United States of America (U.S.A.) as well as Alaska (Simpson 1929, Baker 1972, Furniss and Carolin 1977, Wood 1982b, Langor and Raske 1989a, Hiratsuka *et al*. 2004). The range of the eastern larch beetle closely matches that of its preferred host, eastern larch (tamarack), *Larix laricina* (Du Roi) K. Koch (Pinaceae) (Wood 1982b, Seybold *et al*. 2002). The eastern larch beetle is the only major bark beetle species that attacks and colonizes eastern larch (Bright 1976, Wood 1982b, Seybold *et al*. 2002).

Tamarack is a dominant plant species of the boreal and northern forest ecosystems, particularly in wet, low-lying areas (Duncan 1954, Burns and Honkala 1990). Tamarack is an

important species in northern forest ecosystems because it can influence hydrology, reduce soil erosion, and provide important wildlife habitat. Moreover, tamarack has important cultural significance and has some value as a commercial species (Burns and Honkala 1990). As such, large-scale mortality of tamarack due to eastern larch beetle activity may have important implications ecologically, culturally, and economically.

Eastern larch beetles have historically been acknowledged as "non-aggressive" bark beetles of low economic concern (Baker 1972, Bright 1976). Typically, eastern larch beetles prefer to colonize the tissues of recently dead tamaracks or those that are injured, stressed, or moribund due to flooding, drought, wind-throw, insect defoliation, old age, fire, or mechanical damage such as forest harvesting or road construction (Hopkins 1909, Simpson 1929, Dodge 1938, Roe 1962, Bright 1976, Werner *et al*. 1981, Langor and Raske 1989a, Seybold *et al*. 2002). Eastern larch beetles will readily colonize stressed host trees regardless of whether the trees remain standing or have fallen due to wind-throw events (Simpson 1929, Bright 1976, Wood 1982b). Minor and isolated events of eastern larch beetle activity have been recorded for over 100 years (Hopkins 1909, Langor and Raske 1988a). It is known, however, that in rare cases eastern larch beetles will attack green, healthy, tamaracks with no obvious predisposing factors for beetle attack. Such behavior occurs during favorable conditions; for example, during periods when adult beetle numbers exceed the carrying capacity of the focal (*i.e*., stressed) host tree(s) undergoing mass-attack (Hopkins 1909, Dodge 1938, Werner *et al*. 1981).

Eastern larch beetles have undergone population eruptions several times across eastern North America over the past 130 years. Outbreak activity has been recorded in the U.S.A. from Michigan (1888 and 1980s), West Virginia (1897), New York (1915), and Minnesota (1938 and 1980s), as well as Maine, New Hampshire, Vermont, and Maryland (Langor & Raske 1989b), and in Canada from the provinces of Ontario (1883 and 1960s), Quebec (1926), and Nova Scotia (1939) (Seybold *et al*. 2002). Tamarack mortality prior to 1970, while appreciable, tended to be isolated and scattered throughout the specis range in North America. Outbreaks of the 1970s and 1980s, however, marked the first recorded landscape-level outbreaks that affected large tracts of contiguous land throughout the north-eastern U.S.A, Alaska, and eastern Canada

(Langor and Raske 1989b). An extensive outbreak in the late 1970s and early 1980s throughout much of the north-eastern U.S.A., Quebec, and Maritime Canada resulted in the mortality of 1.4 million m^3 (600 million board feet) of timber (Seybold *et al*. 2002). The estimate of 1.4 million m^3 is certainly a large under-estimate of the loss of tamarack growing stock wood because damage estimates were not available for Quebec or any of the north-eastern U.S.A (Langor and Raske 1989a). During the same time period outbreaks of eastern larch beetle also occurred in Alaska that killed over 3.3 million ha of tamarack (Seybold *et al*. 2002). These severe outbreaks prompted forest researchers to argue that the status of eastern larch beetle as a non-aggressive forest pest should be upgraded to a bark beetle of primary concern to forest health (Langor and Raske 1989a, Langor and Raske 1989b).

Large-scale epidemics of eastern larch beetles that develop rapidly and cause the mortality of thousands of tamaracks over a large area were not previously reported to occur in Minnesota (Dodge 1938). However, since the year 2000, an ongoing beetle outbreak within Minnesota has caused tamarack mortality across approximately 86,500 ha of forested state land (J. Albers, Minnesota Department of Natural Resources, pers. comm., 2015). This affected area represents approximately 22% of the approximately 394,000 ha of tamarack forest cover within the state (Albers 2010). Unlike previous landscape-scale outbreaks in other areas of North America, predisposing factors have not been readily apparent: these large tracts of tamarack mortality within central and northern Minnesota have occurred in areas with no obvious history of defoliation, drought, or flooding injury (Seybold *et al*. 2002, Jones *et al*. 2011).

Because eastern larch beetles have not traditionally been considered pests of great economic importance, little research has been devoted to understanding their biology and ecology. To that end, this dissertation describes the biology and ecology of eastern larch beetles in Minnesota. It examines in detail the interactions between eastern larch beetle and its host, the eastern larch (tamarack). It quantifies how host characteristics, or quality, influence the host selection behavior, colonization dynamics, and reproductive success of adult beetles, and how host quality ultimately influences the fitness the beetle offspring. The over-arching goal of this dissertation is to increase the current understanding of the factors that regulate the population

dynamics of eastern larch beetles, and determine if the life-history characteristics of eastern larch beetles may be associated with the sustained activity in Minnesota and the Great Lakes region. By improving the current knowledge of the eastern larch beetle, forest researchers can better predict the population dynamics of eastern larch beetle and its future potential to cause widespread mortality to tamarack forests.

In Chapter 1, I provide a literature review of previous research conducted on the eastern larch beetle and summarize the present state of knowledge of the beetle up until the time that this dissertation was undertaken. As such, new information gained during the undertaking of this dissertation regarding the life-history of the beetle will conflict with the previous research presented within the literature review.

In Chapter 2, I examine how environmental temperature regulates the development of the eastern larch beetle by rearing eastern larch beetles in small sections of logs infested under controlled laboratory conditions and exposed to five different temperatures within growth chambers. The minimum temperature of eastern larch beetle development, as well as the temperature required for the optimal rate of development is described. In addition, the effect of temperature on the reproductive success of parent beetles is examined. Finally, I examine how the temperature that the sub-adult life-stages are exposed to during development influences the fitness of the emergent young adult beetles as interpreted by measures of beetle size and fat content. This chapter has been published as McKee and Aukema (2015) *Agricultural and Forest Entomology* 17: 102 – 122.

For Chapter 3, I test whether eastern larch beetles uniformly possess an obligate reproductive diapause that must be broken by an overwintering period. In a controlled laboratory experiment I observed successful reproduction and the formation of two successive generations of eastern larch beetle offspring established by parent beetles that did not experience an overwintering period, or a chilling treatment of any kind meant to simulate overwintering conditions. Further, I examined the difference in reproductive ability of young non-wintered adults that emerge naturally from the pupal chamber *vs.* non-wintered young adults that had to be extracted manually from the pupal chamber to determine if the two behaviors correspond with any

reproductive advantage. This chapter is in press as McKee and Aukema (2015) *The Canadian Entomologist* DOI: http://dx.doi.org/10.4039/tce.2014.81.

In Chapter 4, I provide a detailed description of eastern larch beetle seasonal phenology in the Great Lakes region from 2011 – 2014 related to degree day accumulations. Utilizing 132 tamaracks from multiple field sites near Lake of the Woods, Minnesota, U.S.A., I describe patterns of beetle flight throughout the year, spring emergence patterns of adult beetles, periods of adult beetle attack on tamaracks, adult beetle re-emergence from fully colonized host trees, and establishment of the first, second, and third broods, in addition to development of each brood group including pre-winter emergence by the newly-formed young adults.

For Chapter 5, I interpret select phenology data from Chapter 4, with new field data of beetle physiology and physical characteristics as evidence that, in 2012, eastern larch beetles established, for the first time on record, a second generation of offspring within a single season under natural field conditions. Specifically, I present evidence indicating that young adults from the first brood of 2012 *(i.e.*, first generation) emerged from natal trees in late summer and proceeded to successfully attack living tamaracks and establish viable offspring (*i.e.*, second generation) prior to winter. The data presented in Chapter 5 indicates that eastern larch beetles are not limited to a single reproductive generation per year as previously thought.

In Appendix 1, I investigate the interactions between eastern larch beetles and tamarack. Selecting 132 apparently healthy tamaracks, I took measurements of tree diameter, phloem thickness, growth rate, age, phloem resin pocket density, competition from nearby trees, and history of previous unsuccessful beetle attack. First, I studied how these host traits influenced the host selection behavior of eastern larch beetles and determined the host attributes that were important to eastern larch beetles during host selection process. Also, I determined how the preferences of attacking beetles for certain host attributes changes once the highest quality hosts are killed and removed from the pool of potential host trees. Further, I determined how the colonization dynamics and attack behavior of eastern larch beetles is mediated by the traits of a host tree under attack. Moreover, I then examined how the host traits affected the reproductive success of the attacking beetles. Finally, I examined the emergent offspring that successfully

developed within a subset of the tamaracks that were attacked to investigate how host traits influence the fitness of the resultant offspring.

This dissertation is written in chapter format for future publication in peer-reviewed journals. As a result of this layout, a small amount of redundancy exists between chapters in order to preserve the independence of each work. Throughout this project I served as the principal investigator, however, invaluable advice was provided by my supervisor Dr. Brian Aukema, and by my dissertation committee members Dr. Anthony D'Amato, Dr. Stephen Kells, and Dr. Robert Venette. As such, I therefore use plural, rather than singular ownership when referring to the research presented within this dissertation.

Chapter 1.

Literature review of previous research on the eastern larch beetle *Dendroctonus simplex* LeConte (Coleoptera: Scolytinae)

1.1 Host species

Primarily restricted to eastern larch (tamarack) (*Larix laricina* (Du Roi) K. Koch) (Wood 1982b, Seybold *et al*. 2002), the eastern larch beetle (*Dendroctonus simplex* LeConte) (Coleoptera: Scolytinae) does not naturally colonize the other species of larch that are native to North America such as western larch (*L*. *occidentalis* Nutt.) and subalpine larch (*L*. *lyallii* Parl.) (Seybold *et al*. 2002). However, exotic *Larix* species are attacked and colonized when planted within the geographic range of the insect (Langor and Raske 1989a, Seybold *et al*. 2002). For example, eastern larch beetles will readily colonize Dahurian larch (*L. gmelinii* (Rupr.)), Japanese larch (*L. kaempferi* (Lamb.) Carr.), Siberian larch (*L*. *sibirica* Lebed.), and European larch (*L*. *decidua* Mill) (Seybold *et al*. 2002). In addition to *Larix* species, eastern larch beetles have also been reported to attack red spruce (*Picea rubens*) in the north-eastern United States (Baker 1972).

Under artificial conditions, eastern larch beetles will colonize and attempt reproduction in other tree species. When baited with combinations of frontalin-seudenol-ethanol or frontalin-methylcyclohexenol-ethanol, logs of western larch and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were both attacked by natural populations of eastern larch beetles in northern Minnesota (Dodds *et al*. 2010). Although the baited logs of western larch had higher numbers of attacks, successful parental galleries, larval galleries, and brood emergence relative to Douglas-fir, Douglas-fir was suitable for eastern larch beetle development (Dodds *et al*. 2010). Reproduction of eastern larch beetles has not been recorded in natural stands of western larch or Douglas-fir; however, neither tree species is found within the native range of the insect (Burns and Honkala 1990). Earlier laboratory studies also concluded that eastern larch beetles will successfully reproduce when introduced into logs of western and eastern larches, although reproduction in

logs of Douglas-fir was not successful (Furniss 1976). Beetles were most productive within their native host, eastern larch (Furniss 1976). Douglas-fir prevented eastern larch beetle reproduction via antibiosis because none but a single introduced female survived (Furniss 1976). The successful reproduction of beetles within Douglas-fir logs reported by Dodds *et al*. (2010) may have been due to desiccation and volatilization of a portion of the toxic resin components during transport from western North America or during the field trials, allowing the attacking beetles to survive within the logs. The studies of Furniss (1976) were executed under laboratory conditions using freshly cut Douglas-fir logs that may have contained higher residual concentrations of toxic resin components. The differences in the results of the previous studies are not clear, however.

Interestingly, although reproduction was higher in logs of eastern *vs.* western larch, introduced beetles were noted to act in a much more vigorous and aggressive manner while constructing ovipositional galleries within the western larch logs. This observation, combined with the high fecundity of the beetles within western larch, raised concerns that eastern larch beetles may behave in a much more aggressive and destructive manner than what is observed within stands of eastern larch if ever introduced into areas containing natural stands of western larch (Furniss 1976). Furniss (1976) cautioned that establishment of eastern larch beetles within western larch is likely if the beetle is able to bridge the geographic barrier that currently separates the distributions of the eastern and western larch.

1.2 Factors associated with the increased tree-killing activity of eastern larch beetles

The factors that reduce tamarack vigor and increase the risk of eastern larch beetle attack on individual trees or localized groups of tamaracks are well-known. At small scales, many eastern larch beetle infestations are less than a quarter hectare in size and appear to begin as a result of a localized stress impacting tree physiology such as insect defoliation, flooding, drought, fire, mechanical damage, natural senescence, snow damage, and wind-throw (Furniss and Carolin 1977, Langor and Raske 1989a, Langor and Raske 1989b, Seybold *et al*. 2002, Albers 2010).

The most common form of physiological stress thought to predispose tamaracks to eastern larch beetle attack is defoliation. Although many eastern larch beetle outbreaks have been associated with lepidopteran defoliators, the current eastern larch beetle outbreak in Minnesota does not appear to be related to defoliation since only approximately 5% of the area affected by eastern larch beetle have been defoliated by an outbreak of larch casebearer (*Coleophora laricella* (Hübner)) (Albers 2010, Jones *et al*. 2011). The species of defoliator most commonly associated with eastern larch beetle activity varies regionally (Langor and Raske 1989b). Defoliators include the larch sawfly (*Pristiphora erichsonii* (Hartig)), spruce budworm (*Choristoneura fumiferana* (Clemens)), larch casebearer, and larch budmoth (*Zeiraphera* spp.) (Werner 1986, Langor and Raske 1989b, Seybold *et al*. 2002). Defoliation typically occurs within 1 – 3 years preceding eastern larch beetle activity (Seybold *et al*. 2002). For example, repeated defoliation of tamaracks in Newfoundland, Canada by spruce budworm during the mid-1970s caused little direct tamarack mortality but did reduce tree growth and vigor. Trees with reduced growth were subsequently attacked by eastern larch beetles causing a large outbreak to occur (Langor and Raske 1989a, Langor and Raske 1989b). Similarly, an eastern larch beetle outbreak that began in Alaska in 1974 rapidly intensified across 240 000 ha after beetles began infesting tamaracks that had been defoliated for two successive years by the larch budmoth (Werner 1986). Tamaracks killed during the Alaskan outbreak from 1974 – 1977 had reduced radial growth for the three years prior to attack compared to trees that were not killed (Werner 1986). Similar patterns of multi-trophic interactions among species have also been observed in other bark beetle-host-defoliator systems. For example, severe defoliation of ponderosa pine (*Pinus ponderosa* Douglas ex. C. Lawson) by the pine looper (*Phaeoura mexicanaria* (Grote)) created a surplus of suitable breeding substrate for *Ips pini* (Say) and *I. calligraphus* (Germar), permitting *Ips* populations to increase beyond normal densities (Dewey *et al*. 1974). In some instances, tamaracks weakened by pinewood nematode (*Bursaphelenchus xylophilus*) (Steiner and Buhrer) Nickle have also been linked to localized eastern larch beetle outbreaks (Langor and Raske 1989b).

Localized flooding events caused from road construction or beaver dams are a common cause of tamarack stress and mortality. Trees killed by either agent can provide substantial amounts of substrate for beetle breeding and reproduction (Seybold *et al*. 2002). Tamaracks killed in the current beetle outbreak in Minnesota have been killed within a variety of growing conditions that range from upland to lowland sites and within dry and saturated soils, making it difficult to associate patterns of site-specific growing conditions with observed tree mortality (Albers 2010).

At landscape-level scales, factors that are responsible for stressing tamaracks physiologically and enhancing the potential for outbreaks of eastern larch beetles also include the age and composition of tamarack stands, geographic location, and long-term climatic patterns. Langor and Raske (1989b) note that stands of over-mature tamaracks commonly support outbreaks of eastern larch beetles, possibly as a result of mature trees experiencing decreased vigor and defensive capacities relative to younger trees. The extent of the eastern larch beetle outbreaks that occurred in northeastern North America and Alaska during the 1970s may have been exacerbated by increased maturity of tamaracks within the affected regions (Langor and Raske 1989a, Langor and Raske 1989b). Moreover, although tamaracks in both mixed and pure stands are susceptible to attack by eastern larch beetles, stands that exhibit an even-age distribution and/or low species diversity may be at an increased risk of beetle attack (Seybold *et al*. 2002).

The shallow root system of tamaracks renders the trees susceptible to physiological stress in saturated as well as dry soils (Burns and Honkala 1990). In northern areas of North America where permafrost or semi-permafrost is present year-round, tamaracks may be stressed by cold, nutrient-poor soils with poor drainage through the frost layer within the soil. Cold and wet soils may be especially detrimental to larger trees that have more extensively developed root systems. These roots remain in permanent contact with frozen soils and water that accumulates above the permafrost layer (Werner 1986). Conversely, the shallow root systems of tamaracks have difficulty absorbing soil moisture when drought conditions lower the subterranean water table. In Minnesota, drought conditions and fluctuating water levels over the last 7 – 9 years have

been suggested to have reduced the fitness and defensive abilities of tamaracks across large tracts of land (Jones *et al*. 2011). Albers (2010) notes that although drought over the last 8 – 9 years may have allowed the outbreak to continue, drought is not suspected to have triggered the initial outbreak.

As ectothermic organisms, much of the development and biology of insects is tightly regulated by ambient environmental conditions (Bale *et al*. 2002). Therefore, short- and long-term climatic patterns may be the most important aspect governing eastern larch beetle population dynamics across broad geographic scales. For example, in Minnesota, climate modeling has predicted that warmer winter temperatures over the past 40 years now allows 25 – 30% more adult eastern larch beetles to survive the over-wintering process, emerge, mate, and produce offspring in the following year (Venette and Walter 2008). A one-quarter fold increase in the number of reproductively viable beetles is suggested to have had important implications for the population dynamics of eastern larch beetle in Minnesota (Jones *et al*. 2011).

1.3 Eastern larch beetle identification

The eggs of eastern larch beetles are white, oval, and approximately 0.9 mm long and 0.5 mm wide (Fig1.1A) (Prebble 1933, Werner 1986, Seybold *et al*. 2002). Larvae are soft-bodied, slightly "C" shaped, creamy-white or yellowish, legless grubs, with a distinct, hard, round head-capsule that is amber to light brown in color (Fig. 1.1B) (Thomas 1965, Werner 1986, Seybold *et al*. 2002, Hiratsuka *et al*. 2004). Larval size is dependent upon growth stage or instar. Overall, the body lengths of larvae range from ~ 1 mm for first instars to ~ 4.5 mm for fully mature fourth instars (Seybold *et al*. 2002). Measurements of the rigid, heavily sclerotized head-capsule are required to accurately determine instar. First instars have a mean head capsule width of 0.41 mm (range = 0.32 – 0.45), while the head-capsules of second, third, and fourth instars average 0.56 (0.51 – 0.60), 0.76 (0.68 – 0.84), and 0.99 (0.92 – 1.12) mm, respectively, based on measurement of eastern larch beetle larvae in New Brunswick, Canada (Prebble 1933). Eastern larch beetles occupying different geographic areas may have slight differences in body size due

to environmental factors, or host-specific attributes such as nutrient content (Reid and Robb 1999). For example, Langor and Raske (1987b) determined that eastern larch beetle larvae from Newfoundland had mean (range) head capsule widths of 0.48 (0.38 – 0.56), 0.67 (0.62 – 0.74), 0.88 (0.78 – 0.98), and 1.13 (1.04 – 1.26) mm for first through fourth instars, respectively, and are considerably larger than those reported by Prebble (1933). The variation between the two sets of measurements is sufficient to allow certain larvae assigned to an instar by Prebble (1933) to be included in a different instar class as defined by Langor and Raske (1987b). For a taxonomic description of eastern larch beetle larvae, refer to Thomas (1965), who provides a detailed taxonomic key for the identification of the various species of *Dendroctonus* based on larval characteristics.

Pupae of the eastern larch beetle can be found in oval chambers at the terminal end of larval feeding galleries (Fig. 1.1C) (Seybold *et al*. 2002). The average size of pupae are 4.5 (± 0.91) mm long and 1.8 (± 0.56) mm wide (Werner 1986). Pupae initially are white to creamy-yellow in color but darken to a grayish hue before adult eclosion (Werner 1986, Langor and Raske 1987b, Seybold *et al*. 2002). Eastern larch beetles have exarate pupae in which the thoracic appendages, developing elytra, bodily contours, and head are clearly visible (FRM, pers. obs.). The sex of pupae of eastern larch beetles can be determined by a small lobe on the abdomen between the eighth sternite and ninth tergite of female specimens. Males lack this lobe (Schofer and Lanier 1970).

Newly eclosed teneral (*i.e.*, callow) adults are initially white or creamy-yellow but darken to a light brown during sclerotization (Fig. 1.1D). When fully sclerotized, the head, pronotum, thorax, legs, and abdomen of the adults are robustly built and are black and shiny. The elytra of fully sclerotized adults are reddish-brown or maroon (Fig. 1.1E&F) and covered with tiny punctures and crenulations, giving the elytra a textured appearance that contrasts with the relatively smooth appearance of the body. Adults are generally cylindrical in form and measure 3.4 – 5.0 mm in length. Adult females are typically larger than males, with average lengths of 4.4 (± 0.31) and 4.1 (± 0.29) mm, respectively (Simpson 1929, Baker 1972, Bright 1976, Wood 1982b, Werner 1986, Hiratsuka *et al*. 2004).

The sex of adult beetles can be reliably determined by an examination of the 7th abdominal tergite. The posterior margin of the 7th abdominal tergite of a female beetle is uniformly smooth, formed by a constant arc to the lateral margins of the tergite. In contrast, the posterior margin of the 7th abdominal tergite of the male possesses two distinct, medial teeth used to stridulate (via rubbing against the "file" on the underside of the elytra) during courtship behaviors. Additionally, the posterior edge of the male tergite is bicurved, creating an obtuse point near each lateral margin of the tergite (Lyon 1958, Furniss 1976). The posterior edge of the male tergite appears darker and more melanized than that of the female (FRM, pers. obs.), possibly reflecting structural reinforcement along the edge of the tergite used to stridulate.

1.4 Life cycle

Several aspects of the biology of the insect have been studied in Alaska, and in Newfoundland, Canada (Werner *et al*. 1981, Werner 1986, Langor 1987, Langor and Raske 1987a, b, 1988b, Langor 1991, Werner 1995). Here, I summarize what is known from these past studies, although in some instances subsequent chapters in this dissertation describe new knowledge regarding the ecology of eastern larch beetles.

The large geographic distribution of the eastern larch beetle in North America dictates that the lifecycle varies in response to location, long-term climatic patterns across regions, as well as yearly climatic trends within regions. Geographic location influences the number of broods established in a given year by the parent adults. A brood is the collective group of offspring that developed from eggs that were laid within a common egg gallery by a single pair of adult eastern larch beetles. Although establishing two "sibling" or "sister" broods per season is common for the same group of parent beetles, a third sibling brood may be established in warmer climates or years (Baker 1972, Langor and Raske 1987a, b, Seybold *et al*. 2002, Hiratsuka *et al*. 2004). Conversely, only a single brood may be established in more northern climates (Werner 1986, Langor and Raske 1989b). Additionally, the number of broods established per season may be related to the abundance of suitable breeding material. Simpson (1929) observed that one and

three broods were produced in years of low and high abundance of breeding material, respectively. Additionally, Langor and Raske (1987a) state that host material selected by parent beetles for the second brood is most likely extremely weakened trees, stumps, or logging slash within close proximity to tamaracks colonized for the first brood. Regardless of the number of larval broods established, it is thought that eastern larch beetles exhibit only a single reproductive generation per year (*i.e.*, the spring-emergent parent beetles) (Werner 1986, Langor and Raske 1987a, b, Seybold *et al*. 2002, Hiratsuka *et al*. 2004). Historically, this has been true of eastern larch beetles in Minnesota (Dodge 1938). Based on field observations of beetle behavior, the eastern larch beetle is considered throughout most of its range to be uni-voltine, but may be semi-voltine in northern climates (Bright 1976).

In the Great Lakes Region, reproductively mature parent beetles emerge from over-wintering hosts between April and June (Seybold *et al*. 2002). Beetles in Minnesota may begin to emerge in the first week of May (Albers 2010). In the Canadian Prairie Provinces, emergence begins in May and lasts until June (Hiratsuka *et al*. 2004). In the Canadian Maritime Provinces, adult emergence usually occurs from April to May (Langor and Raske 1989b) but may begin in early-May, peak in late-May, and last until mid-June (Langor and Raske 1987a, 1989a). Emergence may also be delayed, and occur from June through to July (Langor 1987). For Alaskan eastern larch beetle populations, spring emergence occurs near the beginning of May, peaks from mid to late-May, and continues until June (Werner 1986, Langor and Raske 1989b). Due to the geographic variation in the phenology of eastern larch beetles emerging from over-wintering hosts, and the scarcity of comprehensive lifecycle studies of the insect, the lifecycle described below is largely based on studies from Newfoundland, Canada (Langor and Raske 1987a, b).

1.4.1 First brood

Parent beetle emergence from over-wintering hosts begins in the first week of May, peaks during the third week of May, and lasts until mid-June. Attack on tamaracks by parent beetles begins mid-May and lasts until the last week of June. It is during this period that the first

brood of the year is established. Parent beetles typically spend 30 d under the bark establishing the first brood (Langor and Raske 1987a).

Eggs belonging to the first brood are present within the ovipositional galleries constructed by parent females from late-May to mid-July. The incubation period of eastern larch beetle eggs can range from 4 – 6 (Werner 1986) or 8 – 13 d (Prebble 1933). After hatching, the larvae feed and mature through four instars. First instars are present from mid-June to mid-July, second instars from late-June until late-July, and third instars from early-July until early-August. Fourth instars are present from the second week of July until mid to late-August and can be found while many third instars are still present. First, second, and third instars require 4 – 7 d of feeding before molting into the successive instar. The fourth instar requires 7 – 14 d. Pupae incubate for 7 – 8 d before eclosing as callow young adults (Prebble 1933). Pupae can be found from mid-July to late-August. Young adults are present within the host tree from mid-July until May of the following spring. Young adults that eclose from the pupa prior to winter either remain within the pupal chamber to over-winter *in situ*, or will emerge from the pupal chamber, relocate to the base of the host tree, and construct a new gallery within the bark in which to overwinter (Dodge 1938, Werner 1986, Langor and Raske 1987a, Hiratsuka *et al*. 2004). The total time required for the first brood to develop from eggs to teneral young adults is approximately 47 d (range = 41 – 54) (Simpson 1929, Prebble 1933) and approximately 60 d from eggs to fully sclerotized, mature adults (Langor & Raske 1987b, Seybold *et al*. 2002, but see Werner (1986).

1.4.2 Second Brood

Following the establishment of the first brood, male and female parent beetles may re-emerge from colonized tamaracks and establish the second brood if suitable host material is available (Simpson 1929, Langor 1987, Langor and Raske 1987a). The number of parent beetles participating in re-emergence is reported to vary considerably from 15% (Langor and Raske 1987b) to 90% (Baker 1972, Seybold *et al*. 2002). Re-emergence by parent beetles begins late in June and is nearly complete by mid-July. However, re-emergence can continue until the end of

July. The second brood is established throughout July. Parent beetles spend approximately 32 d under the bark establishing the second brood (Langor and Raske 1987b).

Eggs of the second brood are typically present from mid-July to early-August, though may be found until the end of August. First instars are present from the third week of July until the end of August. The second instars develop from late-July until mid-September. Similar to the first brood, the presence of the third and fourth instars overlaps considerably and each are present from the first week of August until early-November. Young adults from the second brood are present in the host tree from mid-September until the following spring (Baker 1972, Langor and Raske 1987a). Young adults of the second brood are not reported to emerge from natal hosts (Langor and Raske 1987a). Often, many larvae from the second brood (and third brood, when applicable) do not develop to maturity prior to the onset of cold weather and over-winter as immature life-stages (Seybold *et al*. 2002). The immature larval stages of second and third broods can occur into June of the following year (Seybold *et al*. 2002). The second brood requires approximately 70 d to develop from eggs to sclerotized adults (Langor and Raske 1987b).

1.4.3 Third brood

Following the establishment of the second brood, parent beetles may re-emerge for a second time to attack new host material and establish the third brood. This second period of re-emergence occurs throughout August (Simpson 1929). In colder climates, parent beetles are reported to die following the second re-emergence and do not attack additional hosts (Langor & Raske 1987b). However, a third brood may be established during favorable conditions, or, when severely weakened host material is locally abundant (Simpson 1929). In the only reported instance of a third brood being established, parent beetles laid eggs by the end of August, and larvae were present by mid-September. Larvae did not complete development to young adults prior to winter and overwintered in their galleries (Simpson 1929).

1.5 Host colonization

At the onset of warmer spring temperatures, previous research indicates that overwintered adult eastern larch beetles are not yet sexually mature and have small, undeveloped gonads (Langor and Raske 1987b). Additionally, the thoracic flight muscles are not completely formed and render the beetles incapable of flight (Langor and Raske 1987b). Over-wintered adults become fully mature following a 10 – 15 day feeding and maturation period (Furniss and Carolin 1977, Langor and Raske 1987a; but see Chapters 3 & 5). During the period of maturation feeding, the flight muscles and fat bodies of both sexes and the male testes increase greatly in size. The size of the female ovarioles appear to be unaffected by maturation feeding (Langor and Raske 1987a). Eastern larch beetle brood adults are not known to mate prior to emergence from the natal host tree (Furniss 1976, Langor and Raske 1987b), however, empirical studies have not specifically addressed this phenomenon.

Eastern larch beetle adults begin to emerge from host trees to disperse and colonize new host material following maturation feeding and the development of the flight muscles and sexual organs. Females are the host-selecting sex (Wood 1982b). Female beetles emerge from over-wintering hosts prior to males and can comprise as much as 70% of the beetles captured within the initial 8 d of emergence (Langor and Raske 1987a). Eastern larch beetles begin emerging and dispersing from over-wintering hosts when air temperatures reach 5°C and engage in peak emerge and host colonization activities at temperatures above 10°C (Baker *et al*. 1977, Langor and Raske 1987a). The mean density of emergent over-wintered adults was found to be 8.2, 13.0, 29.1, and 11.6 adults per 100 cm^2 of bark at bole heights of 0.5, 1.0, 2.5, and 5.0 m from the tree base (Langor and Raske 1987a). During dispersal flights, eastern larch beetles appear to fly within 3 m of the ground, although the distance that beetles fly during the dispersal phase is not known (Werner 1986).

Following dispersal and host selection, females begin colonizing hosts by boring entrance holes through the outer bark of the tree in order to gain access the underlying phloem layer. In a behavior that is unique among the *Dendroctonus*, eastern larch beetles commonly share entrance holes. In 60, 35, and 5% of cases, a single entrance hole will serve one, two, or three – four pairs

of beetles, respectively. Although several beetle pairs may use the same entrance hole, the female of each beetle pair will construct an independent ovipositional gallery. The male of each beetle pair aids the female by clearing the frass (*i.e.*, boring dust) and packing it into the proximal end of the egg gallery as well as overtop of the eggs deposited by the female within niches cut into the margins of the egg gallery. Males are also construct ventilation holes at 4 cm intervals along the length of the egg gallery. Additionally, males will carve turning niches into the side of the ovipositional gallery at 5 cm intervals for use during frass-clearing and mating activities. The rate of gallery elongation increases in response to the presence of a male beetle and with increasing ambient temperature. (Furniss and Carolin 1977, Langor and Raske 1987b).

When tamaracks are being attacked by eastern larch beetles in the spring of the year for the first brood, the main stem of tamarack is colonized, in addition to any exposed roots and larger branches (Seybold *et al*. 2002). During this attack period the bole of tamaracks can be attacked to great heights. For example, the lower 8 m of the bole (or tree trunk) may be attacked on a tree measuring 10 – 11 m in height. Generally, beetle colonization of the host bole begins at heights of 2.5 – 4.5 m with the lower portion attacked soon thereafter. Bole heights of 4.5 – 6.5 m are usually attacked after approximately 2 d, with portions above 6.5 m being attacked last, generally 12 – 18 d following the period of initial attack (Langor and Raske 1987a). As beetles attack the upper regions of the bole progresses, the density of attack declines. An average attack density of 1.1 (± 0.7 SD) attacks per 100 cm^2 was recorded at heights above 6.5 m when the average attack density of the entire bole measured 2.4 (± 1.2 SD per 100 cm^2 (Langor and Raske 1987a). Similarly, Werner (1986) found that although attacks could be as high as 4.9 m on the bole of trees ≥ 14 cm dbh, attacks were rare above 3 m and were the most dense on the lowest 1.5 m of the bole. Eastern larch beetle attack densities on second brood trees may be quite low compared to first brood trees and is usually confined to the lower 3 m of bole. Additionally, second brood trees are usually located within close proximity to the first brood trees (*i.e.*, 2 – 3 m) (Langor and Raske 1987a).

Female eastern larch beetles release aggregation pheromones when boring in the bark and phloem, and while excavating ovipositional galleries. This serves to attract male and female

conspecifics to the focal tree under attack (Werner 1986, Prendergast 1991, Seybold *et al*. 2002). Despite a poor understanding of eastern larch beetle chemical ecology, it is known that females are generally joined by males within 2 d of boring into host tissue (Langor and Raske 1987b) after the females have excavated approximately 10 cm of ovipositional gallery (Werner 1986). Upon locating the entrance hole to a female ovipositional gallery, males will remain on the bark and stridulate to the resident female for up to 10 minutes prior to entering the gallery. Male beetles will not enter ovipositional galleries that are too short in length (*i.e.*, < 6 – 8 mm) but will wait for the female to elongate the gallery prior to entering. When a male arrives at a gallery already containing a male, the males will stridulate to one another for a length of time before the newcomer vacates the gallery.

The process of host colonization also triggers physiological alterations within the male and female beetles. Soon after host colonization the flight muscles of both sexes rapidly degenerate by as much as 50% during the initial 2 – 3 d of host colonization. Moreover, host colonization stimulates the reduction of the male and female fat bodies. A massive and rapid enlargement of the female ovarioles also occurs. The size of the male testes remains unchanged (Langor 1987).

1.6 Mating behavior

Mating occurs at the distal end of the ovipositional gallery 5 – 30 min after the male enters. Initial courtship behaviors involve the male jostling the female with his head and forelegs. The male then reverses direction within the ovipositional gallery by turning around within turning niches and stroking the female with his hind legs for 10 – 30 s prior to copulation. Females begin ovipositing 1 – 2 d post-mating (Langor and Raske 1987b). Females continue to elongate the ovipositional gallery between bouts of mating (Werner 1986, Langor and Raske 1987b). Only females participate in elongating the ovipositional gallery.

1.7 Oviposition and brood development

Ovipositional galleries are vertical, slightly sinuous, oriented with the wood grain, and lightly etched into the surface of the underlying sapwood (Baker 1972, Bright 1976, Furniss and Carolin 1977, Langor and Raske 1987b, Hiratsuka *et al*. 2004). The sinuous pathway of the ovipositional galleries may be in response to beetles avoiding unsuitable patches of phloem or areas with high resin concentration. Often, females will construct a 1 – 3 cm angled portion at the base of the ovipositional gallery prior to tunneling vertically, resulting in a hook-shaped gallery (Langor and Raske 1987b). During the initial stages of host colonization when intraspecific competition for phloem is minimal, adjacent ovipositional galleries will only occasionally intersect (Seybold *et al*. 2002). As host colonization continues and attack densities increase, ovipositional galleries begin to cross and become greatly intertwined (Langor and Raske 1987b). The length of ovipositional galleries can vary considerably and has been noted to be dependent on the reproductive attempt (*i.e.*, brood) of the parent beetles. For example, ovipositional galleries average 42 cm (range = 20 – 85 cm) in brood one trees versus 26 cm (range = 16 – 36 cm) in brood two trees. Shorter gallery lengths in brood two trees is possibly due to reduced female fitness and/or a greater capacity for host resinosis defensive responses to beetle attack during the later months of the season (Langor and Raske 1987b). Other sources report that the ovipositional galleries range in length from 15 – 45 cm (Bright 1976, Furniss and Carolin 1977, Seybold *et al*. 2002), although the specific brood cohort that these values refer to is not provided. Galleries may possess one to several side branches originating from the main vertical gallery (Bright 1976, Hiratsuka *et al*. 2004). Side branches generally do not exceed 8 cm in length but are used for oviposition in a manner identical to the main gallery (Langor and Raske 1987b).

During gallery excavation the females carve niches into the sides of the gallery in an alternating sequence (Baker 1972, Furniss and Carolin 1977). These niches are used for egg deposition. Niches have a width and depth of approximately 2 mm and are spaced at approximately one niche per cm of ovipositional gallery length. The spacing of egg niches does not change with beetle infestation height on the bole, sample tree, or brood (Langor and Raske 1987b). Each niche contains one to four eggs (Seybold *et al*. 2002), although three to six is also

reported (Baker 1972, Furniss and Carolin 1977). Egg niches contain 1, 2, 3, or 4 eggs 17, 42, 26, and 11% of the time, respectively, with an average of 1.4 eggs per niche (Langor and Raske 1987b). Ovipositional galleries average 1.2 eggs per cm gallery length and are consistent across various infestation heights on the bole, sample trees, or brood number. No eggs are laid within the initial 2 – 4 cm of ovipositional gallery length. The number of eggs per gallery averages 48 (range = 24 – 93) and 31 (range = 20 – 41) for brood one and brood two trees, respectively. Following oviposition, the eggs are covered as the niches become packed with frass by the gallery-excavating adults (Langor and Raske 1987b).

Upon hatching, larvae feed on the phloem tissue of the host. During feeding, the larvae create galleries that are oriented perpendicularly to the ovipositional gallery. Larval galleries tend to be quite short (Baker 1972). Throughout feeding and maturation the larvae also consume the hyphae of symbiotic fungi that extend into the larval galleries (Langor and Raske 1987b). Once the larvae have matured through to the fourth instar they construct pupal chambers at the terminal end of the feeding galleries in which to pupate. Prior to pupation, the entrance to the pupal chamber is sealed with frass (Furniss and Carolin 1977, Langor and Raske 1987b, Seybold *et al*. 2002, Hiratsuka *et al*. 2004). After eclosion from the pupa, most brood adults remain within pupal chambers for the winter (Simpson 1929). However, 30 – 40% of brood adults emerge from host trees between mid-August and late-October and migrate down the bark to the base of the tree. These beetles then excavate tunnels under the bark at the base of the tree in which to spend the winter (Werner 1986, Langor and Raske 1987a, Seybold *et al*. 2002, Hiratsuka *et al*. 2004). These tunnels are known as hibernal galleries. Emergent beetles will either construct their own exit hole, or utilize one already constructed by a previously emerged individual (Langor and Raske 1987a).

The density of adult brood produced per unit area within host trees is reported as 50 adults per 100 cm^2 within brood one trees and 23 adults per 100 cm^2 within brood two trees (Langor and Raske 1987b). Beetles established on the south aspect of host trees develop more rapidly than beetles on the other aspects. Up to 77% of the brood adults captured in the initial 10 d of emergence originated on the southern aspect of the bole (Langor and Raske 1987a).

Phloem that is less than 2 mm thick typically does not contain many successful broods, while phloem 3.5 mm thick is marginal for reproduction, with thicker phloem being optimal (Langor and Raske 1987a).

1.8 Over-wintering biology

Langor and Raske (1987a) report that brood adults are the only cold-tolerant life-stage of the eastern larch beetle, with larvae and pupae being killed by low temperatures as early in the year as October. Adults demonstrate increased cold tolerance as winter progresses, however, which may explain the results of Venette and Walter (2008) who found survival of adults and larvae down to – 42 and – 49°C, respectively. As such, other reports state that larvae and pupae are routinely able to successfully over-winter in areas where mild winter temperatures and/or snow accumulation permit survival (Baker 1972, Langor and Raske 1987a, Seybold *et al*. 2002, Albers 2010, FRM pers. obs.). Adults, pupae, and larvae can each successfully over-winter via the method of super-cooling (Jones *et al*. 2011). Generally, males suffer significantly higher rates of winter mortality relative to females, however, within either sex, larger beetles have higher winter survival relative to smaller beetles (Langor and Raske 1987a).

A portion of eastern larch beetles spend the winter in large aggregations within the phloem of the lower bole and roots beneath the level of the snowline while the remaining individuals remain in the pupal chambers to overwinter (Werner 1986, Langor and Raske 1987a). The density of beetles in over-wintering aggregations can be extremely high. The lower bole of the first set of brood trees can contain aggregations of beetles with average densities of 29 (± 7.5), 19 (± 5), and 11 (± 4) beetles per 100 cm^2 of bark at heights of 0 – 20, 40 – 60, and 80 – 100 cm, respectively (Langor and Raske 1987a). Over-wintering at the base of host trees beneath the snowline provides thermal protection from freezing temperatures as the snow cover accumulates (Langor and Raske 1987a, Seybold *et al*. 2002). The snowpack is highly insulating with temperatures beneath the snowline as much as 20°C warmer than the ambient air. This thermal protection helps adult beetles to survive when air temperatures reach as low as – 52°C

(Werner 1986). However, the extreme cold hardiness of eastern larch beetles is also highly important for increasing beetle survival (Venette and Walter 2008). The over-wintering mortality of adult beetles ranges between trees from 2.6 – 10.7% with mortality being half as severe on the southern relative to the northern aspects of the bole (Langor and Raske 1987a). The duff layer at the base of host trees does not seem to be a protective over-wintering environment since no live adults have been recovered from this habitat (Langor and Raske 1987a). Winter mortality may vary with time to complete development. Greater mortality of the second relative to the first brood have been observed with a 14% *vs.* 0% mortality level, respectively (Langor and Raske 1988b).

1.9 Chemical ecology

There are many studies that examine primary attraction of bark beetles to potential hosts through the detection of, and orientation to, volatile host monoterpenes (Borden and Stokkink 1973, Moeck and Simmons 1991, Tunset *et al*. 1993, Pureswaran and Borden 2005, Saint-Germain *et al*. 2007). In many instances, host volatiles have been shown to significantly increase bark beetle attraction to pheromone components by acting as synergists (Borden *et al*. 1983, Conn *et al*. 1983, Frank 1997, Erbilgin *et al*. 2003, Reddy and Guerrero 2004, Pureswaran and Borden 2005). To date, the chemical and olfactory cues of tamaracks that are utilized by eastern larch beetles during host selection have not been well studied.

Likewise, the chemical ecology of eastern larch beetles requires study and is currently not well-known (*e.g.*, Werner *et al*. 1981, Prendergast 1991, Francke *et al*. 1995, Werner 1995, Barkawi *et al*. 2003, Dodds *et al*. 2010). There have been few studies examining primary attraction of eastern larch beetle to the volatile compounds found in the resin of potential tamarack hosts. In a test of the attractiveness of tamarack resin monoterpenes, *α*-pinene, *β*-pinene, camphene, *δ*-3-carene, limonene, myrcene, 4-allylanisole, and *β*-phellandrene were all found to be equally attractive to eastern larch beetles when presented individually, although *α*-pinene tended to attract the greatest number (Werner 1995).

After boring into the bark and phloem of a host tree, pioneering females initiate the mass-aggregation and host colonization process via pheromone-mediated attraction of male and female conspecifics to focal trees undergoing the initial stages of attack (Prendergast 1991). Data suggest that pheromone production by pioneering females does not proceed immediately following the colonization of host tissues since conspecific attraction to tamarack logs increases after females have been excavating ovipositional galleries for approximately 24 h (Baker *et al*. 1977), or when the female has constructed approximately 10 cm of egg gallery (Werner 1986). Male beetles are suggested to contribute pheromones that increase conspecific attraction to the focal tree (Seybold *et al*. 2002), although no pheromones have yet been isolated from male beetles (Barkawi *et al*. 2003). The semiochemical system employed may reflect that utilized by other *Dendroctonus*, where female-produced aggregation pheromones attract males to a tree undergoing attack. Pheromones released from newly-recruited male beetles then act to attract additional male and female beetles, resulting in a positive-feedback circuit of beetle recruitment and a mass-attack event on a focal host tree (Safranyik and Carroll 2006). In order to avoid over-crowding and intraspecific competition within successfully colonized host trees, eastern larch beetle likely employ anti-aggregation pheromones; however, the chemistry and source (*i.e.*, male and/or female beetles) of these semiochemicals is not yet known. Aggregation within a host tree is suggested to be terminated by the utilization of female-produced anti-aggregation pheromones once males join females within their ovipositional galleries (Prendergast 1991). The attraction of male beetles to females declines significantly once a female has been joined by a male. However, the residual attractiveness of larch logs containing paired females is still significantly greater than logs containing only male beetles (Prendergast 1991).

Laboratory studies have extracted a suite of air-borne chemical compounds from aerations of the air column surrounding gallery-excavating female eastern larch beetles. Francke *et al*. (1995) identified four pheromone compounds produced by female beetles: frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1] octane), 6-methyl-6-hepten-2-one, 6-methyl-5-hepten-2-one, and 6-methyl-3(*E*), 5-heptadien-2-one. In addition to frontalin, two unidentified compounds have also been isolated from volatile collections of gallery-excavating females in addition to female

abdominal tissue extracts (Barkawi *et al*. 2003). Frontalin was not found to be produced by female eastern larch beetles during ovipositional gallery excavation within logs of hybrid Japanese x European larch (*L. kaempferi* (Lambert) Carrière x *L. decidua* Miller). Additionally, seudenol (3-methylcyclohex-2-en-1-ol), MCH (3-methylcyclohex-2-1-one), and 1,2-MCH-ol (1-methylcyclohex-2-en-1-ol) were isolated from aerated extracts of excavating females within Japanese x European larch logs (Prendergast 1991).

The production of pheromones by eastern larch beetles likely occurs in part via the conversion of host-derived defensive chemical compounds since females have demonstrated the ability to convert C^{14} radio-labeled acetate to frontalin. Frontalin was not isolated from male beetles (Barkawi *et al*. 2003). Regarding seudenol, MCH, and 1,2-MCH-ol, the source of these compounds was not conclusively determined and may be the result of *de novo* synthesis by the female beetles, associated microorganisms, and/or oxidation of the monoterpenes of the larch logs (Prendergast 1991). As of 2002, seudenol had not been unequivocally isolated from adult eastern larch beetles (Seybold *et al*. 2002).

While all chemicals isolated from the air column of female beetles are presumed to be semiochemicals that serve various roles in the ecology of eastern larch beetle, only a few have been tested for bioactivity. Results indicating the bioactivity of frontalin with respect to eastern larch beetle behavior are mixed. Frontalin is reported to be the main pheromone component released by female eastern larch beetles with 95% of the frontalin produced by females being (-)-frontalin (Francke *et al*. 1995). Pure (-)-frontalin was found to be highly attractive to eastern larch beetles during field test conditions; however, the sex-ratio of the attracted individuals was not determined (Francke *et al*. 1995). Conversely, Baker *et al*. (1977) found frontalin to be non-attractive to eastern larch beetles either alone, or in combination with, *α*-pinene. In Alaska, frontalin appears to inhibit eastern larch beetle attraction, eliciting an 86% reduction in eastern larch beetle capture when added to traps baited with seudenol and *a*-pinene (Werner *et al*. 1981). Similarly, eastern larch beetle trapping studies in Minnesota revealed that beetles were repelled from seudenol-baited traps in the presence of frontalin (Albers 2010). Frontalin also eliminated

the attraction of male and female eastern larch beetles to tamarack logs paired with *α*-pinene baits (Werner *et al*. 1981).

Seudenol is attractive to eastern larch beetles (Werner 1995, Albers 2010). In Minnesota, both male and female beetles are highly attracted to traps baited with (-)-seudenol (Albers 2010). When paired with the monoterpene *α*-pinene, seudenol becomes significantly more attractive due to a synergistic effect between the pheromone and the monoterpene (Werner *et al*. 1981). Baker *et al*. (1977) also found that pairings of seudenol and *α*-pinene were highly attractive to eastern larch beetles. In Minnesota, seudenol and *α*-pinene lures are used with funnel traps to monitor beetle activity and flight periods (Seybold *et al*. 2002). Seudenol may be more attractive to males than to females since combinations of seudenol and *α*-pinene captured two-fold more male than female beetles (Werner *et al*. 1981). When seudenol was paired with *α*-pinene and *trans*-verbenol, the combination was highly attractive to eastern larch beetles (though less so than seudenol and *α*-pinene alone), but captured three-fold more males than females (Werner *et al*. 1981). However, the bias in the number of males captured in traps using synthetic pheromone components may be a result of unnaturally high chemical release rates as tamarack logs seeded with unmated female beetles captured an equal number of males and females (Baker *et al*. 1977). Also, seudenol and *α*-pinene have been found to be more attractive to eastern larch beetles than tamarack logs that were seeded with unmated females (Baker *et al*. 1977, Werner *et al*. 1981). Again, this may be due to the release rate of the synthetic seudenol lures being significantly greater than that of naturally produced seudenol released from female beetles.

Monitoring programs often pair seudenol with *α*-pinene due to the synergism in eastern larch beetle attraction resulting from the combination of the two compounds (Baker *et al*. 1977, Werner *et al*. 1981, Werner 1995, Seybold *et al*. 2002). There are, however, other tamarack monoterpenes that appear to be superior synergists with seudenol. When the tamarack monoterpenes *δ*-3-carene, *α*-pinene , and *β*-pinene were tested for their synergistic potential with seudenol, Prendergast (1991) found that *δ*-3-carene was the most efficacious synergist, followed by *β*-pinene. In other studies, *δ*-3-carene and camphene were equally efficacious as synergists

and were superior to *β*-pinene, limonene, and *α*-pinene, the second most efficacious group. *β*-phellandrene was the least efficacious. Conversely, the attractiveness of seudenol to eastern larch beetles declined by 73% when paired with myrcene, and 16% when paired with 4-allylanisole (Werner 1995).

3-methylcyclohex-2-1-one (MCH) appears to be highly repellent to eastern larch beetles. When added to traps baited with seudenol or *α*-pinene, MCH reduced beetle attraction of each by 92% and 83%, respectively (Baker *et al*. 1977, Werner *et al*. 1981). Similarly, conspecific attraction to tamarack logs seeded with unmated female eastern larch beetles is also severely reduced following the addition of MCH (Baker *et al*. 1977). The repellency of MCH was not reversed when tested in combination with *α*-pinene (Prendergast 1991).

1-methylcyclohex-2-en-1-ol (1,2-MCH-ol) is not attractive to eastern larch beetles, and does not synergize with seudenol. However, this compound has been suggested as having implications in controlling the pattern of host colonization by the recruitment rate and ratio of males to females (Prendergast 1991).

Pheromones that are associated with other bark beetles have also been tested for bioactivity in eastern larch beetles. *Trans*-verbenol, an aggregation pheromone of the mountain pine beetle *D. ponderosae* Hopkins (Safranyik and Carroll 2006) was not attractive to male or female beetles when added to traps baited with tamarack logs and *α*-pinene (Werner *et al*. 1981), or in combination with *α*-pinene alone (Baker *et al*. 1977, Werner *et al*. 1981). Additionally, *trans*-verbenol reduced eastern larch beetle attraction to combinations of seudenol and *α*-pinene by 36% (Werner *et al*. 1981) and may perform an anti-aggregation function in eastern larch beetles.

1.10 Fungal associates

Eastern larch beetles may be associated with two species of fungi, *Ophiostoma simplex* Jacobs et M.J. Wingfield and *Graphium simplex* Jacobs et M.J. Wingfield (Jacobs *et al*. 1997). The function of these fungi has not been determined. However, associations of similar fungi isolated from other species of bark beetles have demonstrated that such fungi are involved with

overcoming host defenses, detoxifying and conditioning host phloem resources, larval nutrition, semiochemical signaling to conspecifics, and mediating interactions between interspecific invertebrate associates (*e.g.*, Baker and Norris 1968, Barras 1973, Coppedge *et al*. 1995, Six and Paine 1998, Ayers *et al*. 2000, Kopper *et al*. 2004, Bentz and Six 2006, Adams and Six 2007, Bleiker and Six 2007, Adams and Six 2008). It is possible that *O. simplex* and *G. simplex* perform similar roles within the ecology of eastern larch beetles.

1.11 Competitors of eastern larch beetles

The eastern larch beetle is the major bark beetle associated with tamarack, and one of the few bark beetle species associated with tamarack. Other bark beetles that may associate with eastern larch beetles within tamarack hosts include the four-eyed spruce bark beetle (*Polygraphus rufipennis* (Kirby)), the spruce engraver (*Scolytus piceae* (Swaine)), *Crypturgus atomous* LeConte, and *Orthotomicus caelatus* (Eichhoff) (Coleoptera: Curculionidae) (Dodge 1938, Wood 1982b, Hiratsuka *et al*. 2004). Additionally, the red turpentine beetle (*D. valens* LeConte), the balsam fir bark beetle (*Pityokteines sparsus* (LeConte)), and *Pityophthorus opaculus* LeConte and very rarely, the Douglas-fir beetle (*D. pseudotsugae* Hopkins) are also found sharing tamarack with eastern larch beetles (Seybold *et al*. 2002). The associations between the eastern larch beetle and these other bark beetle species are not well known; however, it is possible that differences in body size dictate resource partitioning of a host tree. For example, the four-eyed spruce bark beetle, which is significantly smaller than the eastern larch beetle, is typically found inhabiting the upper portions of infested tamarack where phloem is too thin to support the development of the larger-bodied eastern larch beetle (Rose and Lindquist 1980).

A potentially important competitor of eastern larch beetles is *Stictoleptura canadensis* (Olivier) (Coleoptera: Cerambycidae) whose larvae consume the phloem and outer sapwood of tamarack and therefore compete for resources with eastern larch beetle larvae. Additionally, the larvae of *S. canadensis* are significantly larger than eastern larch beetle larvae and may also

facultatively prey upon the eastern larch beetle larvae when the two species co-occur within the same area of infested tamarack. An extensive list of other arthropod species and their potential association with eastern larch beetles can be found in Langor (1991).

1.12 Natural enemies

In addition to woodpeckers, predators associated with eastern larch beetles include *Platysoma* spp. (Coleoptera: Histeridae) and *Thanasimus dubius* (Fabricius) (Coleoptera: Cleridae) which prey upon the egg, pupal, and adult life-stages of eastern larch beetles (Seybold *et al*. 2002). The principal predators of eastern larch beetles in Newfoundland are *Medetera* spp. (Diptera: Dolichopodidae), *Zabrachia* spp. (Diptera: Stratiomyidae), and *Rhizophagus* spp. (Coleoptera: Rhizophagidae), with *M*. *gaspensis* Bickel being the most abundant (Langor and Raske 1988b, Langor 1991). The clerid beetle *T. undatulus* (Say) may be an important predator of immature and adult eastern larch beetles (Langor 1991). Additional predators of eggs and larvae include *Scoloposcelis flavicornis* (Reuter) (Hemiptera: Anthocoridae) and *Rhizophagus dimidiatus* Mannerheim (Coleoptera: Rhizophagidae). Potential, but unconfirmed predators include four species of staphylinid beetles (Coleoptera: Staphylinidae) (Langor and Raske 1988b). Predation is highest for on first and second instars and pupae, and is not dependent on bark thickness (Langor and Raske 1988b).

Parasitoids (Hymenoptera) are also important associates of eastern larch beetles. Parasitoids can affect up to 30% of the eastern larch beetle populations in Newfoundland and tend to be more abundant on the upper bole of infested tamaracks (Langor 1991), killing twice as many larvae on the upper portions of the bole than the lower (Langor and Raske 1988b). Eastern larch beetle larvae developing within the thinner bark and phloem of the upper bole of tamarack are more often within bark that can be penetrated by the ovipositors of parasitoids,, allowing a greater level of parasitism to occur on the upper bole relative to the thicker bark of the lower bole. Hymenopteran parasitoids cause the greatest mortality among the 3rd and 4th instars (Langor and Raske 1988b). In Newfoundland, four species of ectoparasitoids were found; *Spathius*

canadensis Ashmead, *Coeloides rufovariegatus* (Provancher) (Braconidae), *Rhopalicus tutela* (Walker) (Pteromalidae), and *Roptrocerus xylophagorum* (Ratzeburg) (Torymidae) (Langor 1991).

1.13 Characteristics of tamaracks infested by eastern larch beetles

Tamaracks that have been attacked by eastern larch beetles will exhibit adult entrance holes on the outer bark surface of the bole (Seybold *et al*. 2002). Adult entrance holes are not always obvious, particularly at low attack densities, because the beetles begin boring into the bark within bark crevices or under bark scales. As such, tamaracks colonized by eastern larch beetles are not immediately apparent from a distance and must be inspected at close range (*i.e.*, < 1 m) in order to observe frass accumulations (FRM, pers. obs.). Entrance, exit, and ventilation holes chewed in the bark of tamaracks by eastern larch beetles are easily distinguished. Entrance holes (Fig. 1.2A-D) measure approximately 2 mm in diameter, corresponding with the diameter of the attacking beetle. Entrance holes are also circular and symmetrical. Entrance holes are constructed by beetles chewing the bark from the outer surface and as such the edges of entrance holes tend to be beveled inward, creating a somewhat “soft”, rounded edge. In addition, because beetles use bark crevices or the undersides of bark flaps as leverage points to begin chewing through the bark, the beetles must enter the bark at an angle. This angular entry results in a very distinctive and characteristic shallow trough or groove 3 – 4 mm long, and 2 – 3 mm wide that enters the bark on a declined plane relative to the bark surface and connects with the opening of the entrance hole proper. The entrance hole will typically be plugged with hard-packed, resin-soaked boring frass, or, by liquid resin. Finally, entrance holes will most often be located under bark flaps, or within bark crevices (FRM, pers. obs.).

Beetle exit holes (Fig. 1.2E) are also circular and measure approximately 2 mm in diameter to allow beetles to pass through. However, the bark edges surrounding exit holes are very smooth, “crisp”, and clean in appearance, being somewhat different from the rounded edges of entrance holes. Also, because exit holes are chewed by beetles from the inside of the tree, exit holes will penetrate through the surface of bark flaps, and the bark surface in general and will

not be concentrated within crevices or under bark flaps. More importantly, exit holes do not possess the characteristic trough or groove. Because exit holes are generally constructed after a tree has been mass attacked, these holes are not filled with resin. Similarly, because beetles use these holes to exit the host, they will not possess a frass plug (FRM, pers. obs.).

Ventilation holes (Fig. 1.2F) resemble exit holes but with some slight differences. Ventilation holes are less circular in appearance, sometimes ovoid in shape, with a slightly smaller diameter (~1.5 mm) compared to exit holes. The smaller diameter of ventilation holes is presumably because they are not intended as pass-through points for the beetles. Because ventilation holes are also constructed by beetles chewing from the inside of the bark, they also do not possess the characteristic groove or trough associated with entrance holes. However, the edges of ventilation holes are much different than those of exit holes and serve as a diagnostic cue. Rather than being crisp and clean, the edges of ventilation holes are jagged and "ragged" in appearance. In addition, ventilation holes will often contain very loosely packed frass. Ventilation holes will penetrate the main surface of the bark, but do not pass though bark flaps (FRM, pers. obs.)

Mass accumulations of frass on the outside of attacked trees during the spring and summer are generally rare since most of the boring dust is left within ovipositional galleries by tunneling beetles (Seybold *et al*. 2002). Localized accumulations of frass can be found within the bark fissures, pockets formed by bark scales on the bole, the upper surfaces of branch bases, and at the base of attacked trees (Seybold *et al*. 2002). Also, spider webs are effective at capturing minute quantities of frass. It is useful to examine any spider webs located on the lower branches of a tree for accumulations of frass in the event that none can be located elsewhere on the tree, particularly if a tree is suspected of being only lightly attacked (FRM, pers. obs.). Additionally, the furrowed bark at the bases of any dead lower branches appears to be a preferred location for beetle entry during the early stages of host attack and is worth examining on trees that are suspected of being lightly attacked or in the initial stages of an attack. This method has allowed lightly attacked trees to be identified when no obvious signs of attack existed on the main bole (FRM, pers. obs.).

The color of the frass is useful for helping to determine the time-since-attack for a given tree. For example, freshly excavated frass has a bright reddish-orange color that contrasts strikingly with the grey bark of the tree. Older accumulations of frass fade to a dull tan-brown color that is not as visually apparent (FRM, pers. obs.). During the late summer and fall months, boring frass will accumulate to appreciable levels at the bases of heavily infested trees as mature brood adults migrate to the base of the natal host and re-enter the tree in preparation for over-wintering beneath the snowline (FRM, pers. obs.).

In Minnesota, eastern larch beetles that emerge from over-wintering hosts in the spring begin to attack tamaracks just as the new season foliage is beginning to flush (*i.e.*, needle length approximately 3 – 5 mm). Attacked trees are able to completely flush a set of foliage that appears normal despite extensive destruction of the phloem by attacking beetles and the developing larvae. As such, throughout the spring and summer months (until July) attacked trees appear to be healthy when the crowns are compared to non-attacked trees (FRM, pers. obs.). By late-July, early-August, or early-September, the foliage of attacked tamaracks begins to turn a lighter shade of green and then obtains a chlorotic yellow tint. Foliar chlorosis of attacked tamaracks usually begins approximately 3 weeks prior to the natural needle senescence of healthy, non-attacked tamaracks (Furniss and Carolin 1977, Langor and Raske 1989b, Hiratsuka *et al*. 2004). Foliage begins to fade from the bottom of the crown and progresses upwards to the upper portions (Albers 2010). However, the foliage from the bottom half of the crown will often fade, turn yellow, brown-off, and then fall while the top portion of the crown remains green. Individual trees that retain a green upper crown after the bottom portion has dropped its needles can make aerial detection of attacked tamaracks difficult (Seybold *et al*. 2002, Albers 2010). Also, it is important to note that only about 50% of killed tamaracks will display early signs of needle senescence (Langor and Raske 1989b, Albers 2010).

Conversely, some tamaracks killed by eastern larch beetles will begin to drop the needles while the needles are still pale green and will completely shed the foliage by the time other attacked tamaracks have started to display indications of needle chlorosis (FRM, pers. obs.). Such trees, when viewed from a distance, can be mistaken for trees killed the previous year.

Therefore, it is important to look into the crown to view the small twigs to note their condition and if intact fascicles remain. Weathering appears to remove the smaller twigs and fascicles from the crown within a couple of years of tree death and can provide clues as to the length of time a tree may have been dead (FRM, pers. obs.). All tamaracks killed by eastern larch beetles, regardless of the timing of foliage yellowing and needle drop, will fail to flush a new set of foliage during the spring following the year of attack (Seybold *et al*. 2002, Albers 2010).

Throughout the late fall and winter months tamaracks killed by eastern larch beetles often have much of the bark removed by woodpeckers, such as the American three-toed (*Picoides dorsalis*), black-backed (*P. arcticus*), and hairy (*Dendrocopos villosus*) that forage for over-wintering larvae and adult beetles. The degree of bark removal can be extensive, leading to an almost complete denudation of the bole from the snowline to the top of the tree (Furniss and Carolin 1977, Seybold *et al*. 2002). Bark removed by woodpeckers often accumulates in large piles at the bases of tamaracks containing overwintering larvae and adults (Albers 2010).

1.14 Host selection with respect to tree size

Eastern larch beetles will attack tamaracks of almost any bole diameter within stands that range from wet, boggy lowlands to dry, upland sites and that constitute stands of mixed tree species or pure tamarack (Seybold *et al*. 2002, Albers 2010). As with other bark beetle species, visual cues and host silhouettes may be important during the host selection process. In general, trees that are attacked by eastern larch beetle tend to be the largest in the stand (Langor and Raske 1989b). Werner (1986) found that the number of attacks per unit area increased significantly with tree sizes ranging from 5 – 14+ cm diameter at breast height (dbh) but did not indicate whether the increase in attack density was proportional to the increase in tree size (*i.e.*, the amount of surface area available to flying beetles). Therefore, this result cannot conclusively be considered as evidence indicating that larger trees are preferred for colonization by eastern larch beetles. However, other studies also support the theory that larger tree size is advantageous regarding host attack, reproduction, brood survivorship, and subsequent emerge of

brood adults. Eastern larch beetles apparently require phloem that is at least 2 mm thick in order to make a successful colonization attempt since no successful attacks were found on areas of trees with phloem ≤ 2 mm thick (Langor and Raske 1987a). Moreover, the number of eggs per gallery increase significantly with tree dbh in size classes ranging from 6 – 14+ cm (Werner 1986). Additionally, Langor and Raske (1988b) found that total developmental mortality was significantly lower within the lower portions of hosts, where the bark and phloem are the thickest, relative to the upper portions of hosts. Similarly, larval development to the fourth instar and pupal stages was found to be the lowest in trees ≤ 4 cm dbh and significantly increased with tree dbh to sizes ≥ 14 cm (Werner 1986). Over-wintering emerge of brood adults in the spring also increases significantly with tree size (Werner 1986), suggesting that the thicker bark and phloem layers may help insulate over-wintering beetles and protect them from freezing temperatures.

During outbreaks, eastern larch beetles attack trees with dbh measurements of 8 – 48 cm (Langor and Raske 1989b). Although trees with a dbh of less than 12 cm are rarely attacked, trees as small as 2 – 4 cm dbh have been recorded as being attacked (Werner 1986, Langor and Raske 1989b). During outbreak conditions, 70 – 99% of the tamaracks killed by the eastern larch beetle were in diameter classes greater than 10 cm (Seybold *et al*. 2002). In the current beetle outbreak in Minnesota, tamaracks most often killed have dbh measurements of 10 cm or greater and are 40 years of age or older (Albers 2010). The wide range of trees that are targeted for attack during beetle outbreaks may indicate that under epidemic conditions beetles are less likely to demonstrate high selectivity and may attack most available tamaracks 10+ cm in dbh.

1.15 Impacts of eastern larch beetles on tamarack stand structure

Eastern larch beetles can influence the structure of tamarack stands at both endemic and epidemic population levels. At low population densities, eastern larch beetles remove small patches of stressed trees and create openings in the canopy that allow the recruitment of younger understory trees (Baker 1972, Bright 1976, Furniss and Carolin 1977). Additionally, endemic beetle populations attack and kill the larger, less vigorous trees within a stand and remove the

dominant canopy trees (Werner 1986, Langor and Raske 1989b). Species composition within tamarack stands containing multiple tree species may also be altered by infestations of eastern larch beetles that remove tamaracks within the stand (Seybold *et al*. 2002). During outbreaks, the overall stand density may be reduced by up to 50%, with the density of the largest tree sizes being reduced by 70 – 99% (Werner 1986). In stands with severe beetle infestations, up to 95% of the tamaracks may be killed and removed from the population, although levels of tree mortality are typically lower (Langor and Raske 1989a). Thus, eastern larch beetles have the ability to adjust not only the age- and size-class distribution of tamaracks within a stand, but can also be important agents in adjusting the species composition of mixed tamarack stands.

1.16 Tree defense

Detailed studies of the interactions between eastern larch beetles and tamaracks are lacking, particularly concerning the defensive response of tamaracks to eastern larch beetle attack. However, some information has been gathered during studies of beetle reproduction and development.

Many tamaracks do not exhibit an obvious qualitative defensive resinosis response to attack by eastern larch beetles. Tamaracks do not appear to have a great capacity to form prominent “pitch tubes” as in other conifer-bark beetle systems (*e.g.*, Lodgepole pine (*Pinus contorta*) / mountain pine beetle (*D. ponderosae*)) where pressurized resin encapsulates the invading beetles and physically removes the beetles from the host tissues (Safranyik and Carroll 2006). Although the frass that is removed from ovipositional galleries by male beetles is often saturated with host resin, tamaracks only rarely produce small pitch tubes in response to beetle attack (FRM, pers. obs.). Some trees will release copious amounts of resin in an exaggerated resinosis response following beetle attack (Hiratsuka *et al*. 2004). Resin flow in such trees may be conspicuous and cover much of the bole, branches, and surrounding vegetation; collecting in small pools on the ground beneath the trees during the summer of attack (Seybold *et al*. 2002, Albers 2010). Heavy flows of resin appear to originate from the mid- and upper regions of the

bole rather from portions within 2.5 m of the ground (FRM, pers. obs.). Hiratsuka *et al*. (2004) suggest that resin may flow copiously from the entrance holes of attacking beetles. Although this has not been observed on the lower portions (*i.e.*, ≤ 2.5 m) of the bole (FRM, pers. obs.), it may occur in the upper regions of the tree where observation is difficult. Tamaracks in Newfoundland, Canada, are reported to produce the most resin in response to eastern larch beetle attack during the initial days of beetle colonization during the spring (Langor and Raske 1988b). However, tamaracks in Minnesota generally were observed to generally induce a heavy resin response to beetle attack 2 – 3 weeks after being fully colonized (FRM, pers. obs.). Similarly, Seybold *et al*. (2002) report that tamaracks attacked by eastern larch beetles may exhibit a large resin response during the summer months following the spring attack period.

The timing and extent of the defensive resinosis response likely depend on tree physiology (Langor and Raske 1987a), attack density, tree vigor, tree genetics, and/or local climate. For example, a delayed resinosis response may be due to the beetles attacking tamarack early in the year while temperatures are still too cold to allow a tree to up-regulate defensive physiological processes following the winter dormancy period. Eastern larch beetles are considered to be a semi-aggressive species and may have evolved the behavior to concentrate their effort and colonize the majority of their host trees early in the spring while the soil remains cold or frozen from the previous winter. Beetles attacking tamaracks in the early spring may encounter a host that has not fully completed its winter dormancy and has reduced metabolic function and limited defenses. As such, early attacking beetles may avoid the full extent of the induced defensive response. Such a host colonization strategy is demonstrated by the spruce beetle (*D. rufipennis*) (Schmid and Frye 1977).

The defensive resin response of tamarack does reduce the survivorship of eastern larch beetle offspring. Generally, the resinosis response of tamaracks appears to only affect eggs as well as first and second instars. Seven and 13% of the eggs from the first and second broods were non-viable due to resin inundation (Langor and Raske 1988b). These observations suggest that tamaracks colonized early in the spring for the establishment of the first brood have a

reduced capacity to induce a resinosis response relative to the tamaracks colonized in the summer during the establishment of the second brood.

Some analyses on the effects of tamarack resin constituents on eastern larch beetle mortality have been conducted (Werner 1995). Exposure to 80 ppm of *α*-pinene, limonene, myrcene, *β*-phellandrene, or 4-allylanisole for 24 h resulted in complete adult beetle mortality. A similar assay of 80 ppm of 3-carene and camphene killed 77 – 89% of adult beetles. At 20 ppm, limonene, *β*-phellandrene, and 4-allylanisole killed 51 – 59% of adult beetles in 24 h. The apparent toxicity of tamarack monoterpenes to eastern larch beetle adults is: limonene = myrcene = 4-allylanisole > *β*-phellandrene > *α*-pinene > *β*-pinene > 3-carene > camphene at 60 ppm (Werner, 1995). Studies testing the toxicity of tamarack monoterpenes to larvae have not been reported (Werner 1995).

1.17 Management of eastern larch beetles

Mapping tamarack mortality due to eastern larch beetles in order to obtain an accurate estimate of the size and severity of an outbreak is challenging for several reasons. Within many forest types, tamaracks are often a subsidiary component of the forest cover and are scattered widely throughout a stand. Also, the crowns of approximately half of all killed tamaracks remain green throughout the year of attack and do not “flag” yellow. Such trees are often not detected until the aerial surveys of following year when the attacked trees fail to flush new foliage. The temporal delay between beetle attack and detection, in addition to the often scattered nature of tamarack make it difficult for aerial survey crews to locate killed trees, delineate the boundaries of various infestations, determine the cause of tamarack mortality, and make it difficult to construct estimates of timber volume losses (Langor and Raske 1989a).

There are currently no rating systems that can be used to assess the susceptibility of a tamarack stand to eastern larch beetle attack and identify stands with a high-risk of supporting beetle outbreaks. Langor and Raske (1987a) state that a hazard rating system for tamaracks needs to be developed in order to identify high-risk stands, aid the implementation of control

measures, prevent widespread damage, and allow for a more effective stand recovery. An effective hazard rating system should incorporate the impacts of defoliation and drought as well as an index of tree growth using radial stem growth as a proxy for stand stress (Langor and Raske 1987a).

For protecting specific high-value trees, chemical insecticides applied to the tree bole prior to beetle infestation have been shown to be effective in other bark beetles systems. However, widespread insecticide application for forest management is impractical (Seybold *et al*. 2002).

Management practices for physically controlling infestations of eastern larch beetles have not been developed. Herrick (1935) states that the only method of controlling eastern larch beetles is to cut and remove infested material and processed or burn it. General sanitation practices during logging, such as the clearing of slash piles and the removal log decks prior to the arrival of warm spring weather when beetles emerge will also minimize the number of beetles available to attack remaining tamaracks (Seybold *et al*. 2002). In addition, methods that promote tree health should increase tree defensive abilities and reduce the likelihood of beetles establishing an outbreak. However, studies examining the responses of tamaracks to various silvicultural treatments have not been undertaken (Seybold *et al*. 2002). For example, stand thinning to promote tree growth has not been attempted with tamaracks but is widely known to decrease the risk of insect outbreaks in other bark beetle systems (Fettig *et al*. 2007).

1.18 Figures

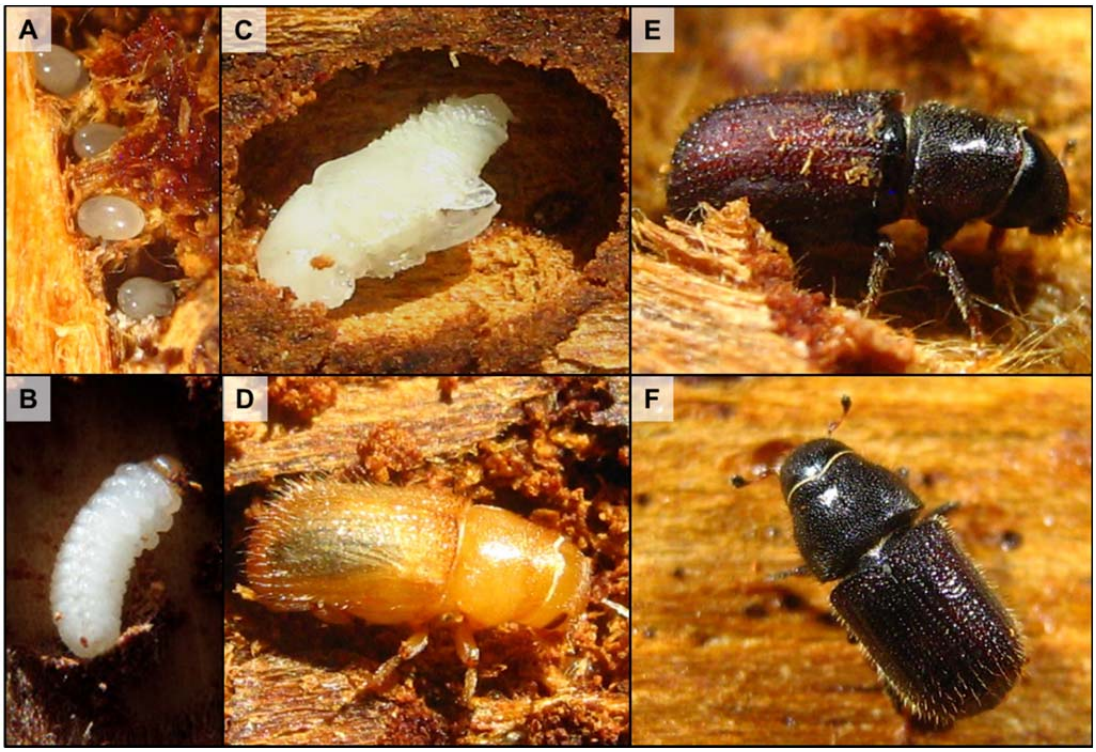

Figure 1.1 (A) Eastern larch beetle eggs packed within frass and located within egg niches, (B) Fourth instar within a feeding chamber at the terminal end of the larval gallery, (C) newly formed pupa within the pupal chamber, (D) callow (teneral) adult beetle removed from the pupal chamber, and (E-F) fully sclerotized adult beetle with black body and maroon elytra. (Photo credits: FRM).

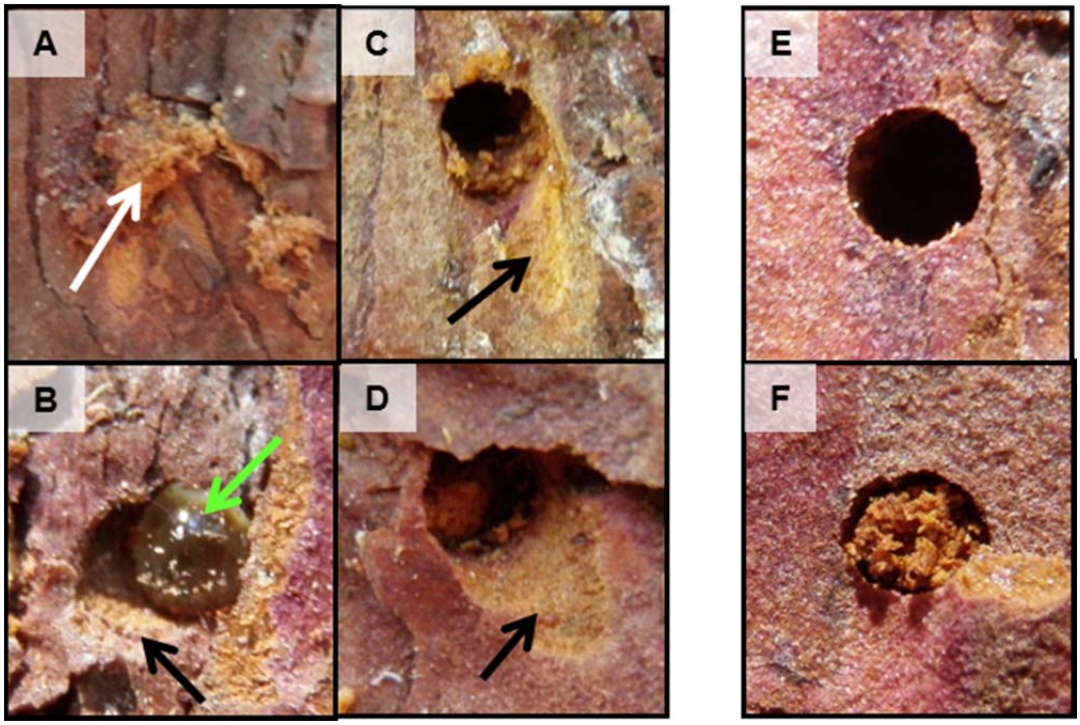

Figure 1.2 (A-D) Entrance holes of attacking eastern larch beetles burrowing into tamarack bark, (E) Exit hole chewed by eastern larch beetles exiting a colonized tamarack, and (F) Ventilation hole chewed by adult beetles in the bark over top of the ovipositional gallery. The white arrow (A) indicates the frass plug that is typically present and that seals the entrance hole. The green arrow (B) points to a resin globule that sealed the entrance hole. The black arrows (B-D) mark the troughs in the bark surface that are characteristic of entrance holes. Note that the bark flaps covering the entrance holes (A-D) were removed to facilitate photography. The frass plug was manually removed from the entrance hole (B-D) to make the trough visible for the photograph. A lack of frass in E is typical of exit holes. The loosely packed frass (F) is characteristic of most ventilation holes. (Photo credits: FRM)

Chapter 2.

Influence of temperature on the reproductive success, brood development, and brood fitness of the eastern larch beetle, *Dendroctonus simplex* LeConte.

2.1 Summary

The eastern larch beetle (*Dendroctonus simplex* LeConte) colonizes the phloem of tamarack (*Larix laricina* (Du Roi) K. Koch), preferring recently dead or moribund trees weakened by insect defoliation or other factors that predispose trees to beetle attack. Outbreaks of eastern larch beetles are typically localized, of short duration, and collapse when the supply of stressed hosts is exhausted. While rare, landscape-level outbreaks of eastern larch beetles can occur if large areas of tamarack become stressed. Since 2000, an ongoing outbreak of eastern larch beetles in the Great Lakes Region of North America has resulted in extensive mortality to more than 85 000 hectares of tamarack forest in Minnesota, USA. This outbreak has no known biotic predisposing factor, such as extensive defoliation. Trends of recent climate warming are suspected to be a contributing factor, however. Current efforts to model the effects of climate on eastern larch beetle population dynamics are hampered by an absence of data relating beetle developmental biology to temperature. In a laboratory study, we studied eastern larch beetle reproductive success, larval development, and offspring fitness at temperatures from 9.9 to 29.4 °C. Offspring production was similar across temperatures. Successful brood development occurred at 9.9 °C, while the minimum and optimal developmental temperatures were calculated to be 7.5 and 27.9 °C, respectively. Offspring size and lipid content were maximized between 20-22 °C. Our results indicate a potential trade-off between temperatures that maximizes eastern larch beetle offspring fitness vs. developmental rate. The implications of such a trade-off are discussed with respect to beetle population dynamics.

2.2 Introduction

Herbivorous insects represent integral components of forest ecosystems, influencing floral and faunal diversity, water quality, stand age, size, and genetic structure, fire regimes, and nutrient cycling (Kurz *et al*. 2008, Raffa *et al*. 2008, Hicke *et al*. 2012). Such landscape-level impacts are frequently tied to dramatic insect population eruptions, which vary in size and extent. Large-scale disturbances from insect outbreaks may be occurring with increasing frequency and severity as thermal constraints that affect the population dynamics of many insects are changing (Bale *et al*. 2002). Indeed, recent changes in climatic patterns have been altering the population dynamics and geographic ranges of several species of forest insects (Bale *et al*. 2002, Carroll *et al*. 2004, Aukema *et al*. 2008, Raffa *et al*. 2008, Waring *et al*. 2009).

More frequent outbreaks of bark beetles (Coleoptera: Curculionidae) such as species within the genus *Dendroctonus*, recognized for their economic impacts on forest resource management (Werner *et al*. 2006, Raffa *et al*. 2008, Bentz *et al*. 2010, Sambaraju *et al*. 2012) have resulted in a widespread increase in forest mortality throughout western North America (Raffa *et al*. 2008, Bentz *et al*. 2010). For example, a hyper-epidemic of the mountain pine beetle (*Dendroctonus ponderosae* Hopkins), in western Canada is orders of magnitude larger than previous epidemics (Aukema *et al*. 2006, Raffa *et al*. 2008, Alfaro *et al*. 2010). Moreover, the spruce beetle (*Dendroctonus rufipennis* (Kirby)), has erupted in areas of western North America (Holsten *et al*. 1999, Jenkins *et al*. 2014) coincident with drought conditions (Chapman *et al*. 2012, Hart *et al*. 2014) and release from thermal constraints (Berg *et al*. 2006, Raffa *et al*. 2008, Bentz *et al*. 2010, Hansen *et al*. 2011).

In the Great Lakes Region of North America, an ongoing outbreak of the eastern larch beetle (*Dendroctonus simplex* LeConte) has been causing extensive mortality of eastern larch (tamarack) (*Larix laricina* (Du Roi) K. Koch) forests in Minnesota, U.S.A., since 2000 (Phillips *et al*. 2012). Concomitantly, activity has been increasing in Wisconsin and Michigan, U.S.A., as well as in Ontario, and Manitoba, Canada, (ONMNR 2012, MIDNR 2013, WIDNR 2013, MBCFB 2014). Moreover, since 2009, the first recorded outbreaks of eastern larch beetles have been occurring in Alberta, Canada (David Langor, Canadian Forest Service, pers. comm.). The distribution of both the eastern larch beetle and tamarack are synonymous across North America

from Alaska in the northwest, eastward throughout the boreal forest of Canada and the northern United States, to the Canadian Maritime provinces, and south to the northeastern United States (Burns and Honkala 1990, Seybold *et al*. 2002). More than 85 000 hectares of tamarack forest has been killed by the beetle in Minnesota since 2000, representing approximately 22% of the tamarack in the state (J. Albers, Minnesota Department of Natural Resources, pers. comm.). This is the third major reported outbreak for this insect, with two large outbreaks occurring concurrently in Alaska (3.3 million ha of tamarack forest affected) and the east coast of North America (> 1.4 million m^3 of tamarack killed) in the late-1970s and early-1980s (Werner 1986, Langor and Raske 1989b, Langor and Raske 1989a). Prior to 1970, no landscape-level outbreaks of eastern larch beetles had been recorded (Langor and Raske 1989b, Langor and Raske 1989a).

Eastern larch beetles typically colonize recently dead tamaracks or those that have been weakened by some stressing agent such as flooding, cold soils, or insect defoliation (Werner 1986). Females are the host-selecting sex, releasing aggregation pheromones to attract conspecifics *en masse* to overcome host defenses and facilitate reproduction (Prendergast 1991). Eggs are laid in the phloem within niches that are cut into the margins of the parental galleries. Enclosed larvae create feeding galleries perpendicular to the parental gallery. After laying the first larval brood in the spring, parent beetles may re-emerge from the colonized tree and establish a second and sometimes a third sibling larval brood in additional trees or host material. Larvae develop to adults by mid-summer or fall. Following pupation, some progeny emerge and drop to the base of the tree where they create overwintering galleries, while others remain in the pupal chamber throughout the winter (Simpson 1929, Wood 1982b, Werner 1986, Langor and Raske 1989b, Langor and Raske 1989a, Seybold *et al*. 2002). The over-wintering life stage is typically the brood adult, although larvae are more cold tolerant than adult life-stages (Venette and Walter 2008). Brood adults purportedly must overwinter to break a reproductive diapause and become reproductively mature the following spring (Langor and Raske 1987b), although laboratory data suggests otherwise (Chapter 3).

When environmental conditions permit, rapid population increases of eastern larch beetles may occur. However, outbreaks are typically ephemeral and confined to small, localized areas of moribund trees. Small, localized infestations of eastern larch beetles have been documented for over 100 years (Hopkins 1909, Wood 1982b). Infestations of healthy trees are typically short-lived and last only a few years, ending when beetles exhaust the proximate, weakened host supply (Langor and Raske 1989b, Langor and Raske 1989a).

Two attributes make the outbreak of eastern larch beetles in Minnesota unique. First, this is the only landscape-scale outbreak of eastern larch beetles ever recorded in central North America. Second, unlike previous eastern larch beetle outbreaks, the outbreak in Minnesota is not associated with any biological disturbance event (*e.g.*, tamarack defoliation) that would predispose the tamaracks to colonization by eastern larch beetles (Albers 2010). While the large outbreaks in Alaska and eastern Canada in the late 1970s and early 1980s were more expansive and pronounced than any previous eastern larch beetle activity, they were associated with prior insect defoliation, localized flooding, and general tamarack decline (Werner 1986, Langor and Raske 1989b, Langor and Raske 1989a). In the absence of a disturbance agent, climatic patterns, such as warming trends in seasonal temperatures, are suspected to be contributing to the beetle outbreak in Minnesota (Venette and Walter 2008).

Efforts to understand how current climate patterns may be affecting the biology of eastern larch beetles, and potential links to outbreak behavior, have been hampered by a lack of data comparing eastern larch beetle reproductive success, larval development, and offspring fitness with environmental temperatures. An understanding of where optimal (or suboptimal) thresholds across these parameters lie in relation to temperature could be used to predict the effects of climate on future population dynamics of eastern larch beetles and the potential for increased forest morality. To that end, a set of laboratory experiments was conducted with the following objectives: i) examine how temperature affects the reproductive success of parent beetles, ii) determine the minimum and optimal temperatures for eastern larch beetle larval development, and iii) relate the developmental temperature to offspring fitness, using size and lipid content as indicators thereof.

2.3 Materials & Methods

2.3.1 Source of experimental tamarack material

Three healthy, non-infested tamaracks with diameter at breast height (DBH; 1.4 m) of 20.5, 18.9, and 18.6 cm growing in the Red Lake Wildlife Management Area, Lake of the Woods Co., MN, U.S.A. (UTM: 15U 0356131 / 5387805) were felled and cut to 2 m lengths on 22 Oct. 2011 and transported to the University of Minnesota, St. Paul, MN. Log ends were sealed with molten paraffin wax to reduce desiccation and stored at 4°C until needed.

2.3.2 Source of parent eastern larch beetles

Four tamaracks containing eastern larch beetle brood adults (first larval brood of 2011) were harvested in the Red Lake Wildlife Management Area (UTM: 15U 0370509 / 5390453) on 29 Oct. 2011 and brought to the University of Minnesota where the log ends were sealed with molten wax. The logs were stored outdoors throughout the winter of 2011-12 before being brought into the laboratory and placed in separate emergence tubes at the first sign of beetle emergence on 19 Apr. 2012. Each emergence tube was fitted with a collecting jar for emergent beetles. The infested logs were held at room temperature (24 ± 0.5°C), ~ 60% RH, with 24 h ambient light. Emergent beetles were collected daily and separated by date, natal host, and sex. The sex of the beetles was determined using the methods of Lyon (1958). Beetles were stored on moist paper towels at 4°C and 60% RH until needed.

2.3.3 Preparation of material for breeding experiments

Logs from each green tamarack were cut into 20 cm long bolts on 23 Apr. 2012, then split lengthwise into two half-logs (hereafter referred to as 'billets') with standardized bark surface areas of 360 cm^2, measuring 18 cm in over-the-bark width and 20 cm in length. Molten paraffin wax was used to seal all wood surfaces and bark-wood interfaces to reduce desiccation. Fifty-

four billets, 18 from each green tamarack, were prepared and stored at 4°C for 24 h. The source tamarack for each billet was recorded.

2.3.4 Colonization of billets with parent eastern larch beetles and collection of brood adults

On 24 Apr. 2012 the billets were removed from cold storage and allowed to warm to room temperature (24°C) for 24 h. Females were introduced to the billets on 25 Apr. 2012 after being removed from cold storage and allowed 2 h to warm to room temperature. Each billet was colonized with one female-male pair. To introduce a female to a billet, a 5 mm diameter hole was drilled through the bark to the surface of the phloem layer. One vigorous female between 3 and 7 d post-emergence, selected at random, was then placed in a 0.5 mL, vented Eppendorf tube open at one end. The open end was inserted into the drilled hole such that the female was free to enter the phloem and commence gallery excavation. All females began excavating ovipositional galleries within 4 h of introduction.

Male beetles were introduced to the billets 24 h after the females in the same manner. Most male beetles entered the egg galleries within 30 s. Males were checked after 2 h. Two males that were present in the Eppendorf tubes at this time were replaced with new males that successfully entered the female ovipositional galleries.

Colonized billets were left at room temperature for 24 h to allow the beetle pairs to mate and begin oviposition. This protocol ensured that oviposition commenced at a similar time in all billets regardless of subsequent rearing temperature treatment. After the 24 h period, each billet was placed in a clear, vented plastic 14 x 10 x 26 cm (W x D x L) rearing container prior to placement in growth chambers with 16:8 L:D photoperiods. Fifty-four pairs of beetles were used (1 pair/billet x 9 billets/rearing temperature x 6 rearing temperatures (see below)).

Colonized billets were checked daily for brood emergence beginning 21 d post-colonization. Because parent beetles can re-emerge, the first brood adult was considered to be either the second male or female beetle to emerge, or the third beetle to emerge (*i.e.*, assuming both parent beetles emerged) as per Smith *et al*. (2009). Upon collection, brood adults were

separated by billet, rearing temperature, and sex. Collection of brood adults continued until 10 d passed with no new emergence. At this time, the bark was removed from the billets and any remaining, live, non-emergent brood adults were collected to account for all progeny, in case some individuals were in a putative diapause state (Chapter 3). Emergent and manually-extracted brood adults were frozen until measured for size and lipid content (see below).

2.3.5 Rearing temperature treatments

Studies of beetle development at six rearing temperatures were planned: 10, 14, 18, 22, 26, and 30°C. During the experiment, repeated malfunctions of the growth chamber for the 14°C treatment dictated removal of those billets from the experiment. HOBO Data-loggers (Onset Computer Corporation, Bourne, MA, U.S.A.) in each growth chamber recorded actual rearing temperatures of 9.9 ± 0.02, 19.2 ± 0.03, 21.6 ± 0.005, 26.1 ± 0.04, and 29.4 ± 0.016°C (mean ± SE) for the intended 10, 18, 22, 26, and 30°C treatments, respectively. Rearing temperatures recorded by the data loggers were used for all analyses.

All billets in the 19.2, 21.6, 26.1, and 29.4°C treatments were held at a constant temperature throughout the experiment. The nine billets in the 9.9°C treatment were exposed to one of three scenarios as follows. One billet was peeled at day 165 to check if successful brood development was occurring. These larvae were not used in any analyses. Four of the remaining eight billets were then retained at a constant 9.9°C for the entire experiment. The remaining four billets were moved from 9.9°C to the 19.2°C growth chamber on day 165 to increase chances of successful pupation, as temperature thresholds for development were unknown prior to this study and the mountain pine beetle requires a minimum temperature of 15°C to successfully pupate, for example (Régnière *et al.* 2012). The developmental rate of beetles at 9.9°C ($RD_{9.9}$) in the temperature-transfer scenario was calculated by solving equation 2.1:

[Equation 2.1]: $1 = (RD_{9.9} \times t_{9.9}) + (RD_{19.2} \times t_{19.2})$

where: 1 = a constant representing an entire progeny adult as a sum of products of developmental rates (expressed as fractional development of an insect per day) multiplied by the number of days to emergence

$RD_{9.9}$ = calculated rate of brood development at 9.9°C (*i.e.*, 1 / time (d) to first emergence)

$t_{9.9}$ = 165 = time (d) spent by brood in a billet at 9.9°C

$RD_{19.2}$ = 0.0195 = mean rate of brood development at 19.2°C (*i.e.*, 1 / time (d) to first emergence)

$t_{19.2}$ = time (d) spent by brood in a billet at 19.2°C

Progeny did complete development at the constant 9.9°C temperature, so we compared observed *vs*. calculated $RD_{9.9}$ of beetles reared in constant 9.9°C and temperature-transfer 9.9°C billets, respectively, to validate the calculated $RD_{9.9}$ values for eastern larch beetles (see Results).

2.3.6 Measuring the effect of temperature on beetle reproduction, brood development, and brood fitness

Brood sex ratio and number of brood per parent female. All brood adults that emerged or were alive under the bark when the billets were debarked were counted and had the sex determined. All offspring in a billet were the progeny of one female, introduced as part of the single female/male pair to each billet.

Beetle development time and development rate. Development time was the number of days from the date of male introduction into a given billet to the date of emergence of the first brood adult from that billet. Beetle development time included the time until the first egg was laid, the time spent as an egg, larva, pupa, and teneral adult, and the period of maturation feeding prior to brood adult emergence. Beetle developmental rate was expressed as the inverse of the number of days needed for development.

Optimal and minimum temperature for brood development. The optimal temperature for beetle development was calculated using beetle development time in days (d). A quadratic equation was fit to the number of days needed for beetle development in each billet *vs*. rearing temperature. The minimum point on the line, solved algebraically, corresponded to the optimal developmental temperature defined as the minimum number of days required to complete beetle development.

The minimum developmental temperature was determined using data for development rate plotted against rearing temperatures below the optimal developmental temperature (*i.e.*, 9.9, 19.2, 21.6, and 26.1°C) where the data formed a linear relationship. After fitting an appropriate statistical model, the equation was solved to determine the temperature where development rate equaled zero.

Degree days required for beetle development. The number of degree days (DD) required for the development of the first brood adult in each billet at each rearing temperature was calculated using equation 2.2:

[Equation 2.2]: $DD = (T_{mean} - DT_{min}) \times t$

Where: T_{mean} = Mean overall temperature (°C) for the billets in each rearing temperature

DT_{min} = 7.5°C = minimum developmental threshold temperature (°C) of eastern larch beetle (calculated above)

t = Time (d) required for the first brood adult from each billet to develop from an egg to emergent adult

Brood adult size and lipid content. Between 27 and 41 female and male brood adults were subsampled randomly from the each of the 9.9, 19.2, 21.6, 26.1, and 29.4°C treatments with the exception of the 9.9°C temperature-transfer treatment. Progeny size to the nearest 0.01 mm was determined by measuring pronotal width at the widest point using a Leica MZ6 microscope with real-time camera and digital micrometer. Once measured, insects were dried for 24 h at 50°C to determine their dry mass (DM). Beetle mass was recorded to the nearest 0.01

mg using a Metler-Toledo AX105 Delta range analytical microbalance. Lipids were extracted using a 500 mL Soxhlet extractor with petroleum ether. Dried beetles were placed in individual, screened, and labeled 0.5 mL Eppendorf tubes. Sixty-four beetles were processed per lipid extraction using 300 mL of warm petroleum ether. Extractions ran for 8 h with one flush of the extractor column per hour. After extraction, beetles were re-dried for 12 h at 50°C and re-weighed to the nearest 0.01 mg to obtain the lean dry mass (LDM). Total lipid content (mg) per beetle was calculated as the difference in dry mass before and after lipid extraction. Percent lipid content for each beetle was calculated as a percent of beetle dry mass prior to lipid extraction (%DM).

2.3.7 Statistical analyses

Analysis of variance (ANOVA) was used to compare observed $RD_{9.9}$ values of brood from billets in the constant 9.9°C treatment to the calculated $RD_{9.9}$ values of brood from billets in the temperature-transfer treatment (9.9°C moved to 19.2°C). The effects of rearing temperature on development time, development rate, insect size, mass, and lipid content were characterized using separate regression analyses. Variables were transformed as necessary (*e.g.*, asin$\sqrt{y}$ for proportional data, log(y+1) or $\sqrt{y}$ for other variables) to fulfill model assumptions of homoscedasticity and normality of errors. Linear and polynomial models were explored during analyses with final models selected based on model fit, (*e.g.*, R^2 values) and simplicity. The number of degree days required for brood development vs. each rearing temperature was analyzed using ANOVA rather than regression since a lack of fit/pure error test indicated treating rearing temperature as a categorical variable yielded significantly more explanatory power using $\alpha = 0.05$. Finally, as a measure of quality control, the effect of host tree was tested for each response variable of interest to detect design artifacts that may have influenced results. The individual tree from which billets originated did not affect any of the variables measured ($P > 0.05$ for all) so are not treated further. Means separation was done using a Tukey's multiple comparisons procedure. All statistical analyses were performed using R (R Development Core Team, 2014).

2.4 Results

2.4.1 Number of brood produced per parent female and brood sex ratio.

Offspring production per female averaged 15.8 ± 5.1 progeny across the billets held at a constant 9.9°C. This production was lower than those billets transferred to a higher temperature (40.8 ± 6.8 brood adults per female) (ANOVA, $F_{1,4}$ = 8.61, P = 0.043) so only the billets held at a constant 9.9°C were used to compare offspring production across rearing temperatures. Rearing temperature did not affect offspring production ($F_{2,36}$ = 1.56, P = 0.23). Parent females produced 44.5 ± 5.5 (mean ± SE) brood adults overall.

Overall, 49.2 ± 2.3 % (mean ± SE) of the brood adults were females. The brood sex ratio did not differ between billets held at a constant 9.9°C and those in the temperature-transfer regime (ANOVA, $F_{1,4}$ = 1.059, P = 0.36). As such, billets for the constant and temperature-transfer 9.9°C treatment were pooled. The sex ratio of the offspring was constant across rearing temperatures ($F_{1,39}$ = 2.61, P = 0.11).

2.4.2 Beetle development time and optimal developmental temperature.

The number of days needed for brood development decreased with increasing rearing temperature in a curvilinear fashion ($F_{2,34}$ = 964.8, P < 0.0001) (Fig. 2.1). The mean (± SE) number of days required for brood development were 235.3 ± 7.9, 51.6 ± 1.4, 43.8 ±2.0, 32.5 ± 0.7, 33.4 ± 0.3 for the constant rearing temperatures of 9.9, 19.2, 21.6, 26.1, and 29.4°C, respectively. The minimum of this line, from which the optimal developmental temperature was determined, was 27.9°C. At the optimal temperature, beetles can complete development in 33.2 days.

2.4.3 Beetle development rate and minimum developmental temperature.

The observed $RD_{9.9}$ for beetles in billets held at a constant 9.9°C were not different than the calculated $RD_{9.9}$ for beetles in the 9.9°C temperature-transfer billets ($F_{1,4}$ = 2.52, P = 0.19) so

the $RD_{9.9}$ data were pooled for further analyses. The mean (± SE) $RD_{9.9}$ values were 0.0043 ± 0.00014 and 0.0037 ± 0.00037 for the 9.9°C constant and temperature-transfer billets, respectively. The rate of beetle development (*i.e.*, (1/development time (d))) increased with increasing rearing temperature ($F_{2,38}$ = 1125.0 , P < 0.0001) (Fig. 2.2; solid line). Mean (± SE) developmental rates were 0.00396 ± 0.000217, 0.0195 ± 0.000501, 0.0232 ± 0.000838, 0.0309 ± 0.000660, 0.0299 ± 0.000274 for rearing temperatures of 9.9, 19.2, 21.6, 26.1, and 29.4°C, respectively. Data from the linear portion of the developmental rate curve (*i.e.*, 9.9, 19.2, 21.6, and 26.1°C) were used to calculate the minimum developmental threshold of 7.5°C (Fig. 2.2; dashed line).

2.4.4 The effect of rearing temperature on offspring size and dry mass.

The pronotal widths of female and male brood adults were 1.82 ± 0.0059 and 1.81 ± 0.0060 mm, respectively. Because this difference was not statistically significant (ANOVA, $F_{1,371}$ = 0.65, P = 0.42), and no interaction existed between beetle sex and rearing temperature on beetle size (ANOVA, $F_{1,371}$ = 0.25, P = 0.62), data for female and male beetles were pooled. The largest brood adults occurred at 21.1°C, with progeny becoming slightly smaller at both cooler and warmer temperatures ($F_{2,372}$ = 44.3, P < 0.0001) (Fig. 2.3A). Even though males and females were similar in size, the females exhibited greater mean (± SE) dry mass than male brood adults (3.67 ± 0.056 *vs*. 3.49 ± 0.052 mg, respectively; ANOVA, $F_{1,373}$ = 5.65, P = 0.018). There was an interaction between beetle sex and rearing temperature on beetle dry mass (ANOVA, $F_{1,371}$ = 11.78, P < 0.001). The heaviest female and male brood adults were produced at 20.8 and 20.4 °C respectively, with lighter beetles developing both above and below these temperatures (Fig. 2.3B).

2.4.5 The effect of beetle dry mass on total and percent lipid content.

Beetle total lipid content (mg) was positively correlated with beetle dry mass (mg) (R^2 = 0.790) (ANOVA, $F_{1,373}$ = 1410.7, P < 0.0001) (Fig. 2.4A). This relationship was consistent between males and females (ANOVA, $F_{1, 372}$ = 0.46, P = 0.50) so beetles were pooled. Beetle

percent lipid content (%DM) had a positive curvilinear relationship with beetle dry mass (mg) (R^2 = 0.531) (ANOVA, $F_{2,372}$ = 212.5, P < 0.0001) (Fig. 2.4B). Percent lipid content was not effected by beetle sex (ANOVA, $F_{1,370}$ = 0.23, P = 0.63) or by an interaction between beetle sex and dry mass (ANOVA, $F_{1,370}$ = 0.28, P = 0.60) so data for both sexes were pooled.

2.4.6 The effect of rearing temperature on the total and percent lipid content of offspring.

The total lipid (mg) content of female brood adults was significantly greater than that of males, averaging 0.92 ± 0.032 and 0.81 ± 0.030 mg, respectively (ANOVA, $F_{1,373}$ = 5.89, P = 0.016). Total lipid content had a concave parabolic relationship with rearing temperature ($F_{2,371}$ = 272.9, P < 0.0001) that was influenced by beetle sex ($F_{1,371}$ = 10.0, P < 0.0001). Progeny had the greatest total lipid content at temperatures of 20.2°C for females and 19.7°C for males (Fig. 2.5A).

Percent lipid content (%DM) of brood adults averaged 23.9 ± 0.6 and 22.3 ± 0.5% overall for female and male beetles, respectively, and did not differ significantly (ANOVA, $F_{1,373}$ = 3.27, P = 0.07). Beetles were pooled by sex for further analyses. The optimum rearing temperature for percent lipid content of brood adults was 20.2°C, where insects exhibited 27.9% lipid content on average. There was substantial variability in this relationship, however, with only 38.7% of the variation in the data explained by the regression line ($F_{2,372}$ = 119.0, P < 0.0001) (Fig. 2.5B).

2.4.7 Number of degree days needed for eastern larch beetle development.

The mean ± SE number of degree days ≥ 7.5°C required for a beetle to develop from an egg to an emergent brood adult did not differ among rearing temperatures below the optimal developmental temperature of 27.9°C, but was significantly greater for the 29.4°C rearing temperature (ANOVA, $F_{4,32}$ = 10.1, P < 0.001) (Fig. 2.6). The mean (± SE) degree day requirements for brood development from egg to progeny emergence are 604.1 ± 9.1 when pooled for rearing temperatures below 27.9°C. At 29.4°C, 732.3 ± 6.7 degree days were required for development.

2.5 Discussion

Successful progeny development and emergence at a constant 9.9°C demonstrates that the minimum developmental thresholds of all life-stages of the eastern larch beetle are ≤ 9.9°C or that the sub adult life-stages lack individualized minimum developmental thresholds. The spruce beetle, like the eastern larch beetle, can also complete development at temperatures below 10°C but is subject to a facultative larval diapause that results in a semi- *vs.* uni-voltine lifecycle (Hansen *et al*. 2001a, Hansen *et al*. 2011). Inclusion of a 14°C treatment may have allowed us to detect a similar larval or prepupal diapause, although reports of a uni-voltine lifecycle for eastern larch beetles from cool, northern latitudes (Werner 1986) suggest that this insect lacks any sub adult diapause.

Other than diapause, prevention of development to cold-sensitive life-stages such as pupae and adults can also be achieved through higher developmental temperature thresholds of larval life stages. In mountain pine beetle, for example, developmental thresholds of late instars and pupae of 16.2°C and 15°C, respectively, slow development as fall temperatures decline so pupae and adults are not typically subjected to lethal winter temperatures (Bentz *et al*. 1991, Régnière *et al*. 2012). The selective pressure to evolve high developmental thresholds at sub adult life stages may be reduced for eastern larch beetles, however, since adults are quite cold hardy (Venette and Walter 2008). Indeed, beetles colonize hosts early in the spring such that larval development to the cold-hardy adult life-stages is largely complete prior to onset of freezing winter temperatures. Moreover, the behavior of many brood adults to migrate from pupal chambers to the tree base to over-winter beneath the snow line also reduces winter mortality (Hopkins 1909, Simpson 1929, Werner 1986, Langor and Raske 1987a). Developmental thresholds, such as those for 4th instar development and for pupation in the mountain pine beetle (Régnière *et al*. 2012) also act to synchronize beetle emergence and host procurement activities to the summer months when water deficit conditions enhance tree vulnerability to beetle attack (Logan and Bentz 1999, Powell and Logan 2005, Safranyik and Carroll 2006, Régnière *et al*. 2012). In contrast, eastern larch beetle adults attack host trees early in the spring when

translocation of oleoresin may be reduced by frozen root systems for tamaracks growing in cold climates or in areas with saturated soils (Werner 1986, FRM pers. obs.). Developmental thresholds pose major constraints to bi-voltine development in mountain pine beetles (Bentz *et al*. 2014). However, reduced physiological limitations to development at low temperatures (*i.e.*, ≤ 9.9°C) due to low developmental thresholds for the life stages of eastern larch beetles may allow this insect to shift voltinism in response to climate warming quite readily.

Although previous studies indicate a diapause for eastern larch beetle adults (Swaine 1911, Simpson 1929, Langor and Raske 1987b), recent laboratory (Chapter 3) and field studies (Chapters 4 & 5) suggest that an adult diapause is facultative. Moreover, the high optimal developmental temperature of 27.9°C for eastern larch beetles suggests that eastern larch beetles could take advantage of additional heat units due to climate change without developmental complications. Indeed, the maximum developmental rate, averaged across all life stages, occurs at a higher temperature for eastern larch beetles than for the mountain pine beetle (25°C) and the southern pine beetle (*D. frontalis* Zimmermann) (27°C) (Stephen 2011, Régnière *et al*. 2012). Some bark beetle species with large geographic ranges exhibit regional adaptations to prevailing climatic conditions that alter the effect of temperature on beetle development, such that populations from northern latitudes develop faster at a given constant temperature (Bentz *et al*. 2001, Bentz *et al*. 2011, Bracewell *et al*. 2013). Similar relationships likely exist in eastern larch beetles as well, as populations from higher latitudes in the Canadian Maritimes take 80, 42, and 40 d to develop at 12, 18, and 24°C, respectively (Langor and Raske 1987b) while the data in this study indicate developmental times of 154, 61, and 37 d for the same temperatures for populations representing the near-southern extent of the eastern larch beetle distribution (Fig. 2.1; Seybold *et al.,* (2002) If eastern larch beetles from higher latitudes possess the ability to develop faster than the beetles observed in this study when exposed to similar environmental temperatures, this system may be highly sensitive to climate warming on a broad scale. As is the case with the mountain pine beetle (Bentz *et al*. 2001, Bentz *et al*. 2011), adaption of eastern larch beetle populations to local climate is likely to synchronize beetle activity at the landscape scale. Under climate warming scenarios, the sensitivity of eastern larch beetle development to

temperature may become manifested in an increase in the number of larval broods that are established and that successfully develop to adults each year. An increase in the number of eastern larch beetle larval broods produced each year may result in increased frequency and severity of beetle outbreaks and tamarack mortality.

Our method of using infested billets (*e.g.*, Smith *et al*. 2009) rather than phloem sandwiches (*e.g.*, Hansen *et al*. 2011) yields a developmental rate averaged across all life-stages, rather than rates specific to each life-stage, which vary among the eastern larch beetle (Langor and Raske 1987b) and several other species of bark beetles (Vité and Rudinsky 1957, Bentz *et al*. 1991, Wermelinger and Seifert 1998, Hansen *et al*. 2001a). Here, we are most interested in minimum and optimal thresholds for complete development, and do not capture variability across all progeny. Later-emerging progeny at a given temperature, for example, could reflect a later date of oviposition or a reduced development rate, or both. We also note that linear extrapolation of development rate data to estimate the minimum temperature for development may over-estimate the lower developmental threshold (Beck 1983, Wermelinger and Seifert 1998, Briere *et al*. 1999), although the difference of 20.4°C between our estimates of optimal (27.9°C) and minimum (7.5°C) developmental temperatures falls within the related 95% confidence interval of 19.1 – 20.5°C reported by Dixon *et al.,* (2009) for most temperate insects. The lower developmental threshold temperature for eastern larch beetles calculated in this study is quite similar to that reported for its closest relative, the Douglas-fir beetle *D. pseudotsugae* Hopkins of approximately 8°C (Vité and Rudinsky 1957).

Bias in the sex ratios of eastern larch beetles are reported for field populations (Werner 1986, Langor and Raske 1987b) but was not present in the beetles of our laboratory study, suggesting that rearing temperatures between 10 and 30°C were not sufficient to induce unequal survivorship between sexes. Brood sex ratios in bark beetles can become skewed to favor the fittest sex in the presence of some stressing agent (Amman and Pace 1976), such as competition, predation and/or parasitism, host defenses, dessication, or lethal overwintering temperatures affecting juveile life stages (Cole 1973a, Amman 1984, Rankin and Borden 1991). In some bark beetles, sex ratios favor the host selecting sex (Bentz *et al*. 2011, Lachowsky and

Reid 2014), although this is inconsistent (Safranyik and Whitney 1985, Wermelinger and Seifert 1999, Bentz *et al*. 2014). Although little is known regarding beetle mortality between host emergence and procurement (Raffa 2001), host-seeking behavior in this system may skew sex ratios if females with greater lipid content than males are better conditioned dispersers (Evenden *et al*. 2014).

Although the host selecting sex in scolytid beetles is usually larger (Wood 1982b), we did not find that to be true in our study, mirroring inconsistencies among field-captured populations of eastern larch beetles (Werner 1986, Langor and Raske 1987b). Moreover, we did not find that cooler temperatures resulted in larger offspring as has been reported in other bark beetles (Atkins 1967, Safranyik and Whitney 1985, Bentz *et al*. 2001) and insects in general (Roff 1980, Nylin and Gotthard 1998, Kingsolver and Huey 2008). In this study, the largest offspring were produced between 21 and 22°C (Fig. 2.3A&B). A similar relationship has also been observed in the pine weevil *Hylobius abietis* (Inward *et al*. 2012). Smaller progeny at the lower and upper rearing temperatures may be due to temperature stress (Kingsolver and Huey 2008). Larger body sizes are often associated with greater survival, fecundity, mating success, and dispersal potential (McGhehey 1971, Roff 1980, Anderbrant 1988, Honěk 1993, Kingsolver and Huey 2008, Williams and Robertson 2008, Evenden *et al*. 2014). Similar to body size-temperature relationships, the peak in lipid content for beetles that developed at temperatures of approximately 20°C suggests that these beetles possess a fitness advantage relative to beetles that develop at other temperatures since increased lipid content, like larger body size, has also been shown to increase dispersal, survival, host attack, and fecundity in bark beetles (Atkins 1966, Thompson and Bennett 1971, Anderbrant 1988, Jactel 1993, Elkin and Reid 2005, Williams and Robertson 2008, Evenden *et al*. 2014). The optimal temperature for developmental rate, however, occurred at a much higher temperature of 27.9°C. This incongruence of optimal temperatures for size, lipid content, and development rate may represent confounding developmental effects of associated symbionts, such as fungi (Jacobs *et al*. 1997), which provide nourishment to developing beetles (Bentz and Six 2006, Bleiker and Six 2007).

This potential tradeoff between developmental rate, beetle size, and beetle lipid content may help to maximize beetle survival and reproductive potential over a wide range of temperatures and environmental conditions. For example, warmer temperatures may foster higher rates of insect development and increased potential for population growth, but also result in smaller, less lipid-rich offspring (Nylin and Gotthard 1998). Bark beetles must attack host trees in sufficient numbers to kill part, or all of, the host tree in order to overcome host defenses and reproduce successfully (Raffa and Berryman 1987, Raffa *et al*. 2005). Thus, when conditions are sub-optimal for development (*i.e*., cool temperatures) and beetle population growth potential is low, larger and more lipid-rich individuals may increase individual survivorship when fewer beetles are available to participate in host attack and the risk of mortality for each individual is greater (Raffa and Berryman 1983, Raffa and Berryman 1987). Conversely, warm environmental conditions that promote rapid beetle development may also result in smaller, less lipid-rich individuals that experience reduced survivorship when attacking host trees individually. However, the advantage offered by a larger population of beetles to cooperatively attack a host tree, helps to ensure successful host tree colonization and beetle reproduction, and allows the beetle population to increase.

Several aspects of the biology of the eastern larch beetle related to temperature, such as absence of an obligate diapause, a high optimal developmental temperature, and a minimal developmental temperature for all life-stages below 9.9°C suggest that this insect has the potential to become problematic under climate change scenarios and cause more forest mortality than previously observed. This would be especially true if this insect could become bi-voltine, as patterns of voltinism in forest insects have enormous ramifications for the potential of an insect to undergo population outbreaks and cause significant forest mortality. We are currently examining temperature signals at landscape levels to determine whether implications for population increase from this laboratory study are realized by changes in forest mortality in the field.

2.6 Acknowledgements

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2.7 Figures

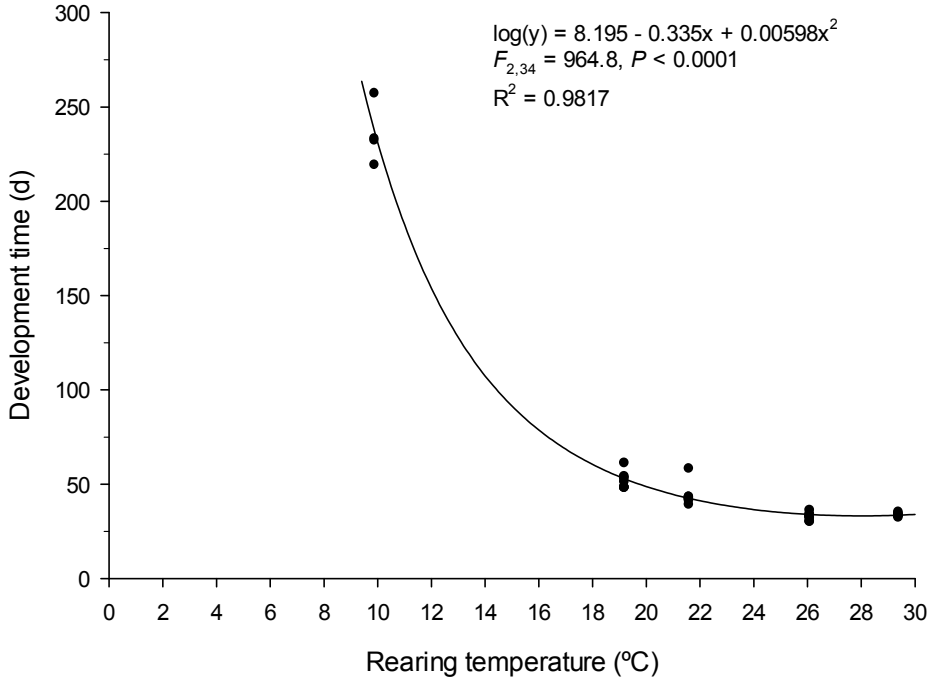

Figure. 2.1 Number of days needed for eastern larch beetle development from egg to emergent adult at five rearing temperatures. Each point represents the number of days until the emergence of the first brood adult from each billet exposed to a constant rearing temperature. Number of billets = 4, 9, 8, 8, and 8 for 9.9, 19.2, 21.6, 26.1 and 29.4°C temperatures, respectively.

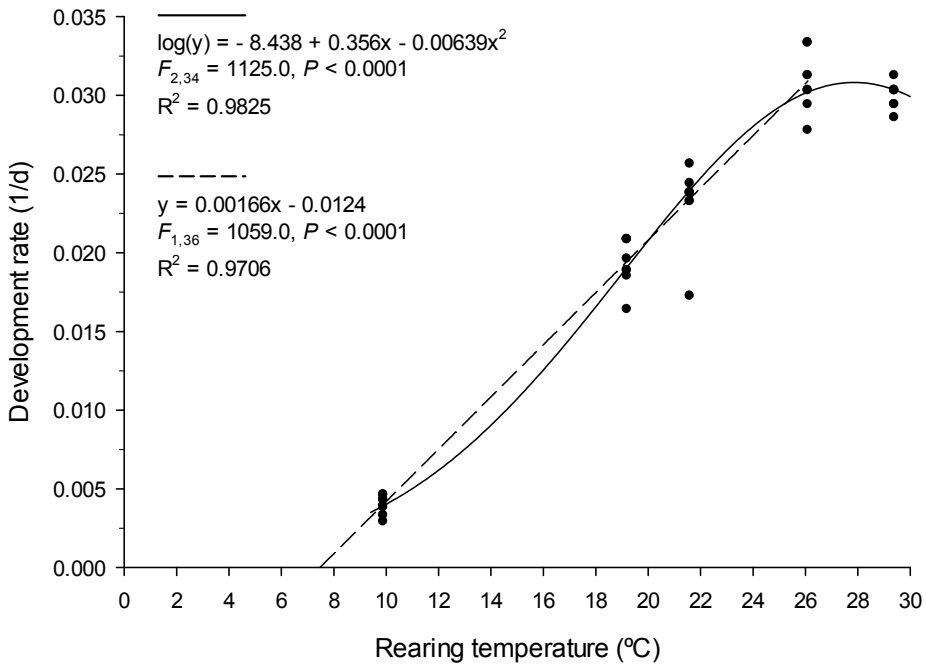

Figure 2.2 Developmental rates of eastern larch beetles at five rearing temperatures. Each point represents the developmental rate of the first brood adult to emerge from each billet. Number of billets = 8, 9, 8, 8, and 8 for 9.9, 19.2, 21.6, 26.1 and 29.4°C temperatures, respectively.

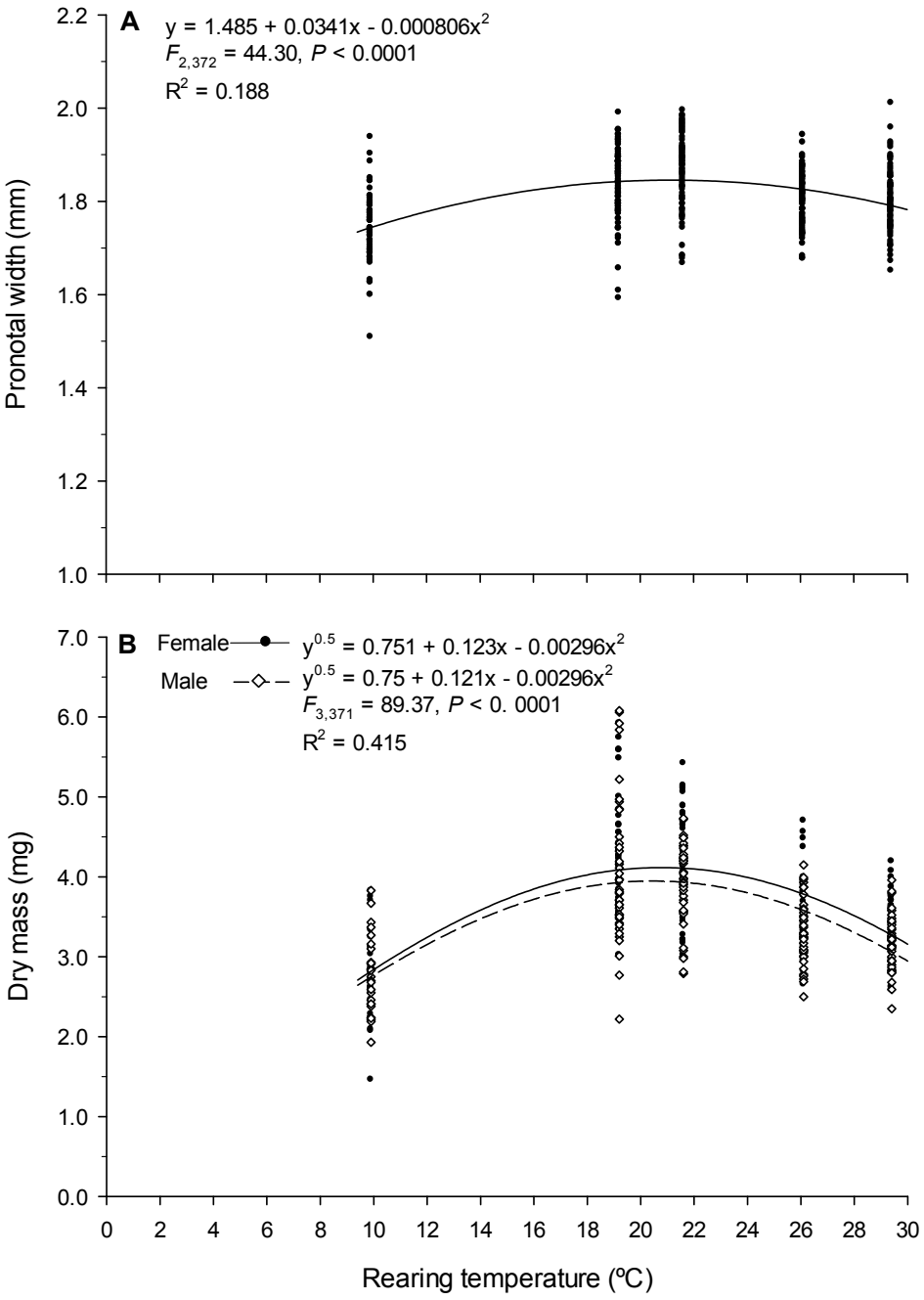

Figure 2.3 (A) Pronotal width (mm) of eastern larch beetle brood adults at five rearing temperatures. (B) Dry mass (mg) of eastern larch beetle brood adults at five rearing temperatures. For each graph, each point represents one beetle (*n* = 186 females, 189 males). Male and female beetles were pooled when considered appropriate by statistical analyses.

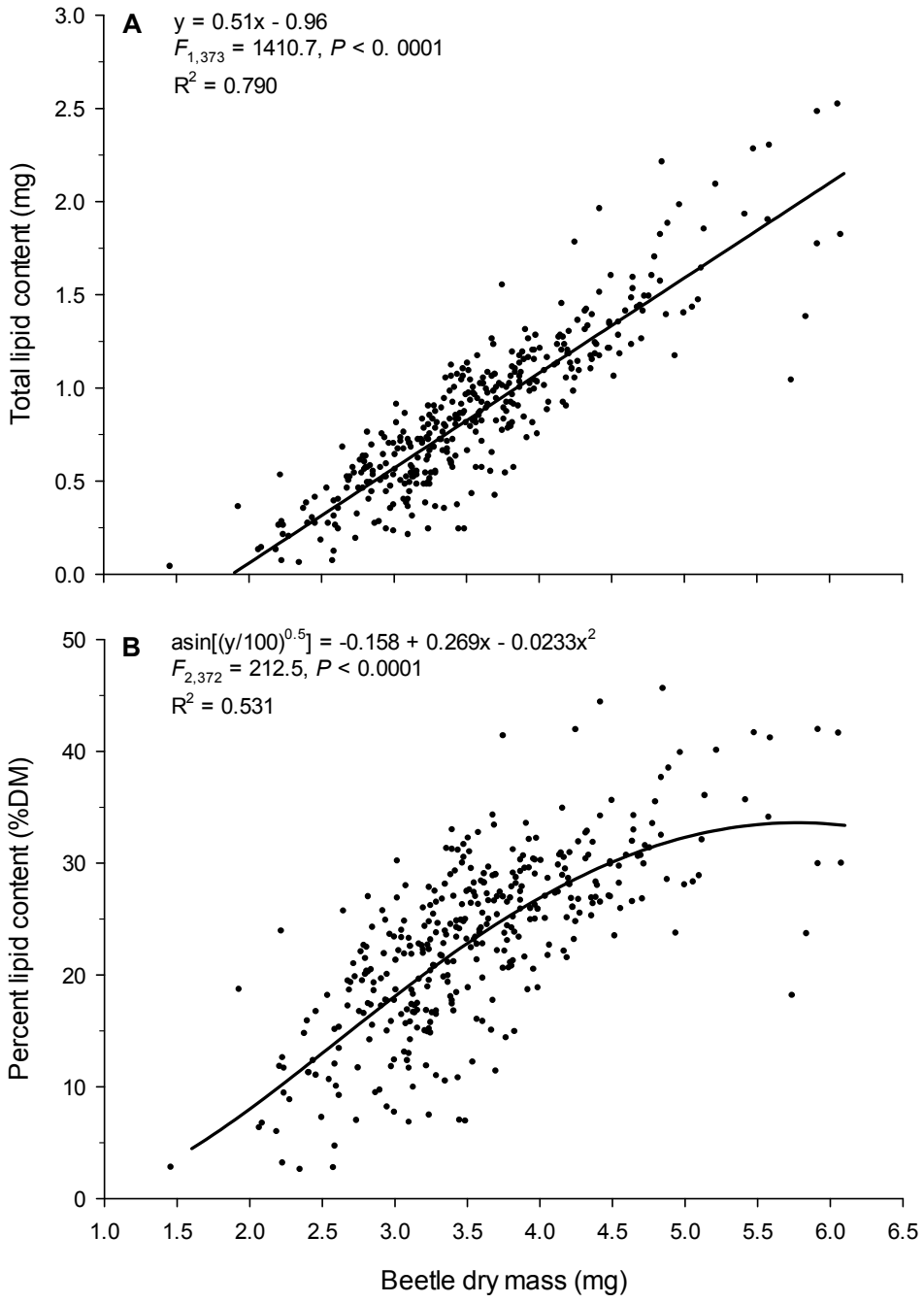

Figure 2.4 The effect of beetle dry mass (DM) (mg) on (A) total lipid content (mg) and (B) percentage lipid content (%DM) of eastern larch beetle brood adults. Beetles from all rearing temperatures were pooled. Each point represents one beetle (*n* = 186 females, 189 males).

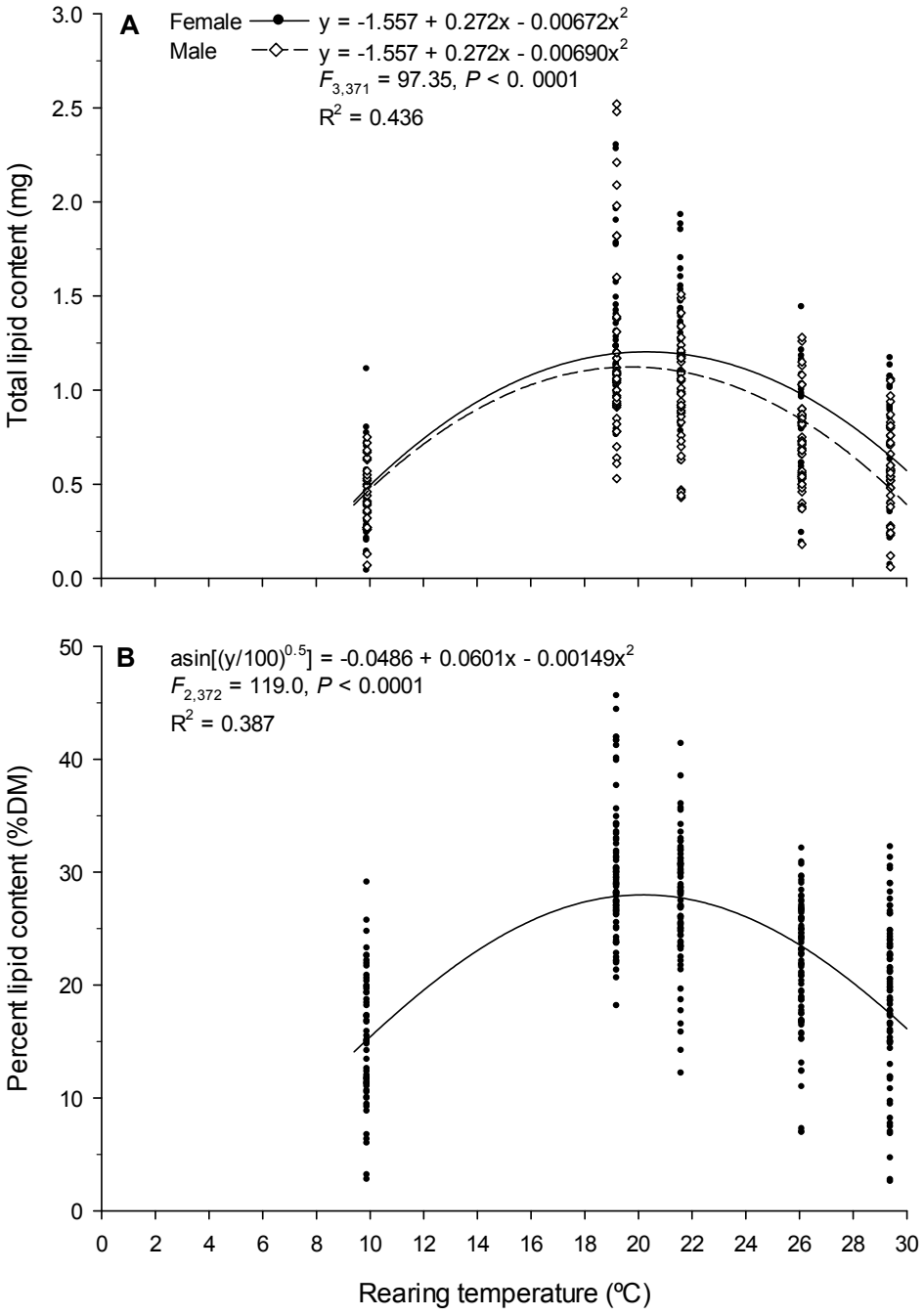

Figure 2.5 (A) Total lipid content (mg) and (B) percentage lipid content (%DM) of eastern larch beetle brood adults at five rearing temperatures. Each point represents one beetle (*n* = 186 females, 189 males). Male and female beetles were pooled as deemed appropriate by statistical analyses.

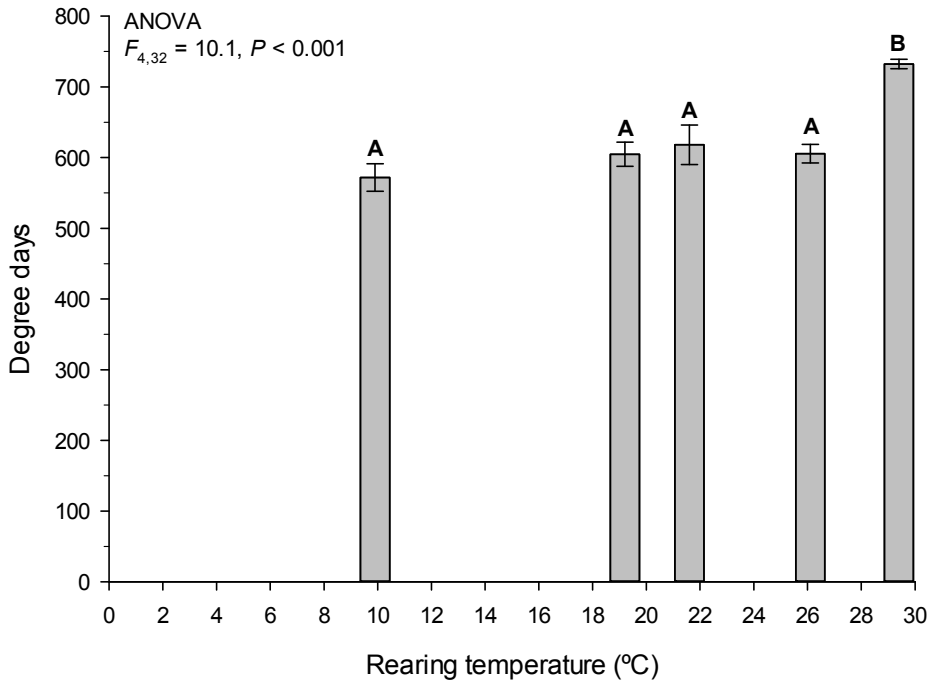

Figure 2.6 Number of degree days ≥ 7.5°C needed for eastern larch beetles to develop from eggs to emergent brood adults at five rearing temperatures of 9.9, 19.2, 21.6, 26.1 and 29.4°C. Degree days were recorded for the first brood adult to emerge from each infested tamarack billet at each rearing temperature. Number of billets = 4, 9, 8, 8, and 8 for 9.9, 19.2, 21.6, 26.1 and 29.4°C temperatures, respectively

Chapter 3.

Successful reproduction by the eastern larch beetle in the absence of an overwintering period.

3.1 Summary

Eastern larch beetles, (*Dendroctonus simplex* LeConte) (Coleoptera: Scolytinae), are monophagous, phloem-feeding herbivores of eastern larch (tamarack), (*Larix laricina* (Du Roi) K. Koch) (Pinaceae). Recently dead or moribund trees are preferentially colonized. Outbreaks of eastern larch beetles are generally localized and short-lived, although a large outbreak has been occurring in the Great Lakes region of North America since 2000. The beetle is reported as univoltine, with a single, spring-emergent, reproductive parent generation establishing one to three sibling broods per year. Some progeny emerging during summer or fall re-enter the tree bole close to ground level to overwinter, while remaining brood adults overwinter within pupal chambers *in situ*. Due to these behaviors, eastern larch beetles have been suggested to possess an obligate overwintering reproductive diapause. However, studies have not confirmed this hypothesis. We tested the reproductive viability of non-overwintered progeny in three laboratory experiments. Non-overwintered progeny were reproductively viable, suggesting that a portion of the population may exhibit a facultative adult overwintering diapause. Progeny that emerged naturally from the host (*i.e.*, putative fall-emergers) demonstrated reproductive rates almost 6-fold those of manually extracted insects demonstrating a propensity to remain *in situ* (*i.e.*, putative spring-emergers). These results shed new light onto the reproductive behavior of eastern larch beetles, and suggest that future population dynamics may be influenced by a warming climate.

3.2 Introduction

The eastern larch beetle, (*Dendroctonus simplex* LeConte), (Coleoptera: Curculionidae: Scolytinae) is a monophagous bark beetle that attacks and colonizes the phloem of its host tree,

eastern larch (tamarack) (*Larix laricina* (Du Roi) K. Koch) (Wood 1982b). The range of the eastern larch beetle is extensive and closely matches that of tamarack, found throughout the Canadian boreal forest as well as the northeastern and northcentral United States and Alaska (Seybold *et al*. 2002) Historically, the eastern larch beetle has been considered a non-aggressive bark beetle and thus the subject of fewer studies than other tree-killing *Dendroctonus* species (Hopkins 1909, Langor and Raske 1989b, Langor and Raske 1989a). Unthrifty tamaracks stressed from flooding, wind-throw, defoliation, drought, pathogen infection, or other predisposing agents are often colonized (Hopkins 1909). Under favorable conditions, the insect will attack and kill relatively healthy trees, with such infestations typically being of short duration (*e.g.*, 2-3 years) (Langor and Raske 1988a). In recent decades, however, the eastern larch beetle has exerted increasing landscape-scale mortality, affecting millions of hectares of tamarack forest in separate outbreaks in Alaska and the Canadian Maritimes region (Werner 1986, Langor and Raske 1989b).

Reproductively mature adult beetles typically emerge in early spring from tamarack material colonized the previous year, disperse, and locate new host trees. Pheromone-mediated mass-aggregation (Prendergast 1991) enables beetles to overwhelm host defenses, killing the tree and facilitating successful brood production. Eggs of the first larval brood are laid shortly thereafter. Parent beetles often re-emerge from colonized tamaracks and establish a second, and sometimes a third, sibling brood in separate host material. Larvae develop rapidly, and by early summer the adult beetles of the first brood begin to emerge from pupal chambers. Depending on the year and sibling cohort, 20 – 80% of brood adults will exit the host throughout the summer and fall, drop to the lower bole of the natal tree, re-enter the bark, and overwinter in non-reproductive galleries constructed in the base of the tree (Simpson 1929, Werner 1986). Remaining brood adults overwinter in the pupal chambers (Werner 1986, Langor and Raske 1987b, Seybold *et al*. 2002). The brood adult is the predominant overwintering life-stage (Langor and Raske 1987a), although both adults and larvae are extremely cold hardy, surviving temperatures as low as – 42 and – 49°C, respectively (Venette and Walter 2008).

Previous studies report an absence of reproductive efforts by emerging brood adults in the summer and fall such that new host trees are not attacked even though sufficient time often remains in a season for the development of a new generation of beetles (Swaine 1911, Langor and Raske 1987b). Because only one reproductive generation of eastern larch beetles per year has ever been observed historically (Hopkins 1909, Prebble 1933, Dodge 1938, Werner 1986, Langor and Raske 1987b), evidence suggests that the insect possesses an obligate reproductive diapause (Langor and Raske 1987b). An obligate overwintering reproductive diapause reportedly occurs in the Douglas-fir beetle (*D. pseudotsugae* Hopkins) (Furniss 1976), the closest relative of the eastern larch beetle (Wood 1982b) as well as in other species of bark beetles such as the spruce beetle (*D. rufipennis* (Kirby)) (Hansen *et al*. 2011). However, no published studies have tested whether the eastern larch beetle can reproduce without an overwintering period (*i.e*., lack of reproductive diapause).

Since 2000, an ongoing outbreak of eastern larch beetles in Minnesota, U.S.A., has killed over 85,000 ha of tamarack in the state (J. Albers, Minnesota Department of Natural Resources, pers. comm., 2014). Eastern larch beetle activity is also increasing throughout other areas of the Great Lakes region including Wisconsin and Michigan, U.S.A., as well as in Manitoba and Ontario, Canada (ONMNR 2012, MIDNR 2013, WIDNR 2013, MBCFB 2014). In Minnesota, the cause of the outbreak is not known, as beetle activity is not associated with any known predisposing conditions (Albers 2010). As part of a larger study to elucidate the insect's seasonal phenology and reproductive capacity, a series of three experiments were undertaken with the following objectives: i) to determine if eastern larch beetle progeny (F_1) from the field could reproduce successfully without an overwintering period, ii) to determine whether their offspring (F_2) were reproductively viable without a subsequent overwintering period, and iii) to explore potential differences in the reproductive abilities of brood adults (F_1) that emerge from the host prior to winter *vs*. those brood adults that remain in the pupal chambers to overwinter. These studies seek to determine whether a second generation of eastern larch beetles in field settings is hypothetically possible in a single year in the absence of an overwintering period and if such reproduction could be a factor contributing to the current beetle outbreak.

3.3 Methods

3.3.1 Experiment 1: Are F_1 eastern larch beetle progeny that do not emerge from natal hosts in the fall capable of reproduction without an overwintering period?

To obtain beetles, three infested tamaracks containing the first spring brood of 2011 were harvested on 19 June 2011 from the Red Lake Wildlife Management Area (RLWMA), Lake of the Woods, Minnesota, U.S.A. (UTM: 15U 0349837 / 5382193). Parent beetles (F_0) were re-emerging from the infested trees at the time of cutting and developing offspring were in the egg, first, and second instar life-stages. The infested logs were brought to the University of Minnesota, St. Paul, MN. The cut ends of the logs were sealed with molten paraffin wax prior to placement in rearing tubes. Brood (F_1) development continued at 23°C and a 24:0 L:D photoperiod. Collecting jars were attached to the rearing tubes to capture emerging progeny.

Because we were interested only in the reproductive ability of beetle progeny (F_1) that did not emerge from the infested material, all re-emergent parent (F_0) eastern larch beetles (Simpson 1929, Langor and Raske 1987a) and emergent brood (F_1) adults were discarded. The infested logs were peeled on 20 August 2011 after a period of 10 d without beetle emergence. At that time, fully sclerotized (*i.e.*, black and maroon) or dark brown brood adults were extracted directly from pupal chambers. Henceforth, such beetles are referred to as 'manually-extracted' while those that emerged into the collecting jars are deemed 'naturally-emergent.' These progeny were separated by sex and natal host, and stored at 4°C on moist paper towel for 48 h until use.

To obtain breeding material for these progeny, three green tamaracks were harvested on 19 August 2011 from the RLWMA (UTM: 15U 0356109 / 5387806). The diameter at breast height (DBH: 1.4 m) of the trees were 24.1, 22.4, and 22.7 cm. Eighteen bolts, six from each green tamarack, each 50 cm in length were prepared 20 August 2011. Cut ends of the bolts were sealed with wax.

The female progeny (F_1) were removed from 4°C storage and allowed 2 h to warm to room temperature (~23°C) on 22 August 2011. Ninety vigorous, 2 d-old females, 30 from each

infested tamarack, were randomly selected and pooled. Five females were introduced to each bolt using starter holes 5 mm wide, drilled into the phloem, and spaced evenly around the bolt circumference, 5 cm from one end. One female was sealed in each hole using aluminum screening. Females were checked after 2 h and four females that were not boring into the bolts (*i.e.*, female still present in starter hole and boring frass absent) were replaced. Bolts were placed horizontally in rearing tubes to allow females to excavate the egg gallery.

The next day, the male progeny (F_1) were removed from 4°C storage and likewise warmed for 2 h at room temperature. One hundred and eight vigorous, 3 d-old males, 36 from each infested tree, were randomly selected and pooled. The aluminum screening was removed from the female entrance holes. Six males were placed on the bark of each bolt and allowed to locate the female egg galleries. Males were checked 6, 12, and 24 h after introduction. Males that had fallen from the bolts were placed back on to the bark surface. No beetles were found at the 24 h check indicating that all had joined the female colonizers in the log. Bolts were stored horizontally at 23°C.

The bolts were monitored for emerging offspring (F_2) every 2 d, beginning 21 d after male introduction. The first emergent offspring was observed on 19 October 2011. Emergent offspring were counted and separated by date, and bolt. The bolts were peeled 4 November 2011 to collect adult offspring (F_2) from the pupal chambers. Offspring were counted and separated by bolt and sex and stored at 4°C for use in a subsequent breeding experiment (see below). Egg galleries of eastern larch beetles tend to meander and quickly intersect one another to form egg gallery networks (Langor and Raske 1987b) making it difficult to attribute specific reproductive success to each female when multiple females are present. Thus, the reproductive success of introduced progeny was expressed as the number of offspring per female by dividing the total number of offspring produced in a bolt by the number of introduced females. This method was also used for Experiments 2 and 3 (see below).

3.3.2 Experiment 2: Is the F_2 generation of non-wintered eastern larch beetle progeny reproductively viable?

Non-emergent F_2 progeny of the non-emergent F_1 beetles (see Experiment 1) were used to determine if the F_2 brood were also reproductively viable without an over-wintering period. Three healthy tamaracks (DBHs: 20.5, 18.9, and 18.6 cm) harvested from the RLWMA on 8 October 2011, and stored as previous, were sectioned into five bolts, each 50 cm in length, on 5 November 2011. Two bolts were cut from each of two tamaracks, and a single bolt was sourced from the third tamarack. The midsection diameters of the bolts were 15.1, 13.9, 14.1, 13.8, and 16.1 cm.

These bolts were infested with F_2 female beetles on 7 November 2011. Females were removed from cold storage and warmed for 2 h at 23°C. Fifty vigorous, 3 d-old females, five from each of 10 natal bolts of Experiment 1, were selected randomly and pooled. Ten females were introduced to each of the new bolts by placing the females on the bark and allowing them to select their own entry points. On 9 November 2011, 60 vigorous, 5 d-old F_2 males, six from each of the 10 natal bolts of Experiment 1, were randomly selected and pooled. Twelve males were placed on the bark of each breeding bolt and allowed to locate the female entrance sites. All beetles were checked at 6, 12, and 24 h post-introduction, again with fallen beetles being placed back on the bark. No beetles were found at the 24 h check, indicating that all beetles had entered the bolts. Bolts were kept at 23°C.

The bolts were checked for offspring (F_3) emergence every 2 d beginning 21 d after male beetle introduction. Emergent offspring were first recorded 27 December 2011. Emergent offspring were collected and separated by emergence date, and bolt. Bolts were peeled on 21 February 2012 to collect offspring from pupal chambers under the bark. Reproductive capacity was expressed as the number of offspring produced per parent female.

3.3.3 Experiment 3: Does the reproductive ability of non-wintered F_1 beetles that emerge naturally vs. reside in pupal chambers differ?

As stated, and experienced in Experiments 1 and 2, a proportion of insects in the field and laboratory choose to reside in place in their pupal chambers putatively for overwintering rather than emerging from under the bark (Werner 1986, Langor and Raske 1987a, Seybold *et al*.

2002). We examined whether the reproductive capabilities of the naturally emergent and manually extracted groups of F_1 beetles differed prior to going through the winter. To obtain non-overwintered eastern larch beetle brood adults (F_1), two tamaracks infested with the first spring brood of 2012 were cut from the RLWMA (UTM: 15U 035603 / 5387792) on 28 July 2012. Phenology data of eastern larch beetle activities collected for a separate study (Chapter 4) indicated that parent beetles (F_0) had completed re-emergence from spring brood trees by 6 July 2012, and brood adults had been emerging since 13 July 2012. The infested logs were brought to the University of Minnesota, and were prepared and placed in emergence tubes as previously described. Brood adults (F_1) continued to emerge and were collected every 1 – 2 days, separated by date, natal host, and sex, and stored at 4°C on moist paper towel. After 7 d without emergence, the infested logs were peeled and remaining brood adults were extracted by hand from pupal chambers on 15 August 2012.

Three healthy tamaracks (DBHs: 18.1, 18.0, and 18.8 cm) were cut from the RLWMA (UTM: 15U 0356113/5387823) on 1 July 2012. Again, ends were sealed with paraffin wax, and the logs were stored at 4°C. On 6 August 2012, eight bolts, 30 cm in length, were cut from the green logs. Four bolts were prepared for the naturally emerging insects, and four for those insects that were manually-extracted from logs upon peeling. For each beetle group, two bolts were made from one tamarack and single bolts were made from each of the other two tamaracks. The ends of the bolts were sealed with paraffin wax prior to storage at 4°C until needed. The diameters of the four bolts were 12.8, 13.3, 13.2, and 14.6 cm and 13.3, 12.5, 14.4cm, and 14.5 cm for the naturally-emergent and manually-extracted brood adults, respectively.

On 6 August 2012, naturally-emergent female beetles (F_1) were removed from cold storage and warmed for 2 h at 23°C. Forty vigorous females, 20 from each of the two infested tamaracks were randomly selected and pooled. Ten females were placed on the bark of each bolt. Again, the females selected their own entry sites. On 8 August 2012, naturally-emergent male beetles (F_1) were removed from cold storage and warmed for 2 h at 23°C. Forty-eight vigorous males, 24 from each of the two infested tamaracks were randomly selected and pooled. Twelve male beetles were placed on the bark of each bolt, and allowed to locate female entry

sites. All beetles were ≤ 6 d old. The methods for introducing manually-extracted beetles to the parallel set of bolts was identical to the naturally-emergent beetles, and occurred on 19 August (females) and 21 August 2012 (males).

All bolts were monitored for emergent offspring (F_2) every 2 d beginning 21 d after beetle introduction. Emergent offspring were first observed 23 September 2012 and 8 October 2012 from bolts infested with naturally-emergent and manually-extracted beetles, respectively. Emergent offspring were separated by emergence date, and bolt. Bolts were peeled 30 d after the initial emergence of offspring, when the emergence had dropped to near zero, to collect and count any remaining live offspring.

Lengths of parental galleries were recorded for each bolt, as well as the types of galleries. Different types of gallery architecture have been noted in other studies of eastern larch beetles (Simpson 1929, Werner 1986), but their functions remain unclear. For our purpose, we characterized the galleries into three types (Table 3.1).

3.3.4 Statistical Analyses

Analysis of variance (ANOVA) was used for five analyses: i) comparing offspring production per female between the F_1 and F_2 generations of non-wintered brood adults (Expt. 1 *vs*. Expt. 2), ii) comparing offspring production per female between the naturally-emergent and manually-extracted (F_1) brood adults (Expt. 3), iii) comparing offspring production per centimeter of egg gallery between the naturally-emergent and manually-extracted brood adults (Expt. 3), iv) comparing mean total length per bolt of each gallery type for naturally-emergent brood adults (Expt. 3), and v) comparing mean total length per bolt of each gallery type for manually-extracted brood adults (Expt. 3). Per capita reproduction was calculated by dividing the number of introduced females into the number of offspring produced per bolt. The bolt was the unit of replication for analyses of offspring production per female and total length of each gallery type per bolt. All analyses were performed using R v2.14.1 (R Development Core Team, 2010). Variables were transformed as necessary (*e.g.*, $\sqrt{y}$) to fulfill model assumptions of homoscedasticity and normality of errors.

3.4 Results

In both Experiments 1 and 2, we observed successful reproduction by the F_1 and F_2 generations of manually-extracted eastern larch beetle progeny that had not been exposed to an overwintering period. Non-wintered F_1 brood from the first spring brood of 2011 (Expt. 1) produced 6.1 ± 2.6 (mean ± SE) offspring per female, and non-wintered F_2 brood produced 6.7 ± 3.6 offspring per female (Expt. 2) (Fig. 3.1). These reproductive outputs were not significantly different (ANOVA, $F_{1,21} = 0.17$, $P = 0.68$). Thus, it appears that some eastern larch beetles are able to reproduce with some success without an obligate overwintering reproductive diapause. In Experiment 2, in which all source beetles were raised in a uniform laboratory thermal environment, we found that 94.9% of the insects emerged naturally. The remaining 5.1% required extraction from their pupal chambers by hand.

When we compared insects that emerged naturally *vs*. those extracted manually from pupal chambers in Expt. 3, we found successful reproduction by both F_1 groups. Naturally-emerged beetles produced 43.0 ± 2.7 (mean ± SE) offspring per female, whereas the manually-extracted beetles produced significantly fewer offspring at 7.4 ± 3.0 per female (ANOVA, $F_{1,6} = 24.7$, $P = 0.0025$) (Fig. 3.2A). Differences in bolt diameter were not responsible for this trend, as the mean ± SE bolt diameters of 13.5 ± 0.4 and 13.7 ± 0.5 cm for the naturally-emergent and manually-extracted beetles, respectively, were not significantly different (ANOVA, $F_{1,6} = 0.11$, $P = 0.77$).

Despite the pronounced difference in total reproduction per female, offspring production per cm of egg gallery for naturally-emergent and manually-extracted F_1 brood adults was similar (ANOVA, $F_{1,5} = 0.55$, $P = 0.49$). The number of offspring per cm of egg gallery averaged 1.6 ± 0.1 SE and 1.3 ± 0.3 for naturally-emerged and manually-extracted beetles, respectively. The difference in reproductive success was due to longer egg galleries excavated in the bolts infested with naturally-emerged F_1 beetle parents (Fig. 2.2B; ANOVA, $F_{1,6} = 18.43$, $P = 0.0051$). The total length of egg gallery in these bolts was more than 5-fold those of the bolts infested with F_1 beetle

parents had been manually extracted directly from pupal chambers prior to introduction to the bolts. Naturally-emergent beetles excavated 282.0 ± 33.3 cm (mean ± SE) of egg gallery per bolt while the manually-extracted beetles only excavated 56.0 ± 18.9 cm of egg gallery per bolt.

The types of galleries excavated differed between the naturally-emergent and manually-extracted F_1 beetles. Naturally-emergent beetles excavated only egg galleries (Fig. 3.3). In contrast, manually-extracted beetles excavated egg, pseudo-egg, and hibernal galleries in equal proportions (Fig. 3.3) as the total length of each gallery type per bolt did not vary (ANOVA, $F_{2,9}$ = 0.95, P = 0.42). The mean length of individual egg galleries of naturally-emergent beetles was 94.1 ± 18.0 cm (n = 12). In contrast, the egg galleries of the manually-extracted beetles averaged less than half of that amount, only 37.4 ± 11.2 cm in length (n = 6). Pseudo-egg galleries (n = 6) and hibernal galleries (n = 14) averaged 12.7 ± 2.6 and 5.3 ± 1.0 cm in length, respectively.

3.5 Discussion

While previous studies are consistent with the understanding that a univoltine life cycle for eastern larch beetles is governed by an obligate adult diapause (Hopkins 1909, Swaine 1911, Simpson 1929, Dodge 1938, Werner 1986, Langor and Raske 1987b). similar to Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopkins) (Coleoptera: Curculionidae), the present work suggests that there may be genetic variability in adult diapause capacity within a population. In Experiment 2, for example, where all parent beetles were subjected to the same laboratory thermal regime, 95% of the brood were naturally emergent whereas 5% appeared to be in a diapause state. The number of beetles in a putative diapause state may have been higher if the naturally emerging beeltes were actually emerging to seek overwintering sites at the base of a tree (Langor and Raske 1987a). Flight musculature appeared robust, however, suggesting they were reproductively mature and ready to seek new hosts (see below). If diapause capacity is a plastic trait, this minority proportion of brood may have entered diapause with a lower accumulation of thermal units. Such genetic variation in diapuase capacity has also recently

been described within the pine weevil (*Hylobius abietis* L.) (Coleoptera: Curculionidae). Other than a facultative prepupal diapause, it had been thought that pine weevils needed to overwinter prior to becoming reproductively viable (Clark 1975). In field settings, however, a proporation of adults can mature eggs without overwintering (Tan *et al*. 2010, Wainhouse *et al*. 2014).

Propensity to enter a diapause state is likely triggered by an environmental cue such as temperature during a critically sensitive life stage (Tauber *et al*. 1986). The spruce beetle, (*Dendroctonus rufipennis* (Kirby)) (Coleoptera: Curculionidae), for example, exhibits a facultative prepupal diapause and what is thought to be an obligate adult diapause (Safranyik *et al*. 1990). When spruce beetle pre-pupae experience threshold cool temperatures of appropriate duration, a facultative diapause arrests development until the following season. If favorably warm environmental temperatures prevail, then spruce beetles proceed to the adult overwintering diapause stage uninterrupted (Hansen *et al*. 2001a, Hansen *et al*. 2011). Differences in environmental conditions (*i.e.*, climate) may partially explain why eastern larch beetle brood adults from previous studies did not attempt immediate reproduction upon emergence (Hopkins 1909, Swaine 1911, Werner 1986, Langor and Raske 1987b), whereas a portion of the brood adults in this study were reproductively viable.

We note two lines of evidence for adult facultative *vs*. obligate diapause states. First, one study reports the existence of eastern larch beetle progeny emerging from host trees in the field in summer with underdeveloped flight muscles (Langor and Raske 1987a), a characteristic of adult bark beetles and weevils in a state of diapause (Danks 1987, Nordenhem 1989, Tan *et al*. 2010, Ryan *et al*. 2015). However, emergent progeny collected from rearing jars in the present experiments would often fly rapidly towards ceiling lights, indicating the functionality of the flight muscles. Second, differences in gallery construction behavior could reflect differences in reproductive maturity. Bark beetles in a state of diapause exhibit barren ovarioles or underdeveloped seminal vesicles (Ryan 1959, Langor and Raske 1987a). In the present work, naturally-emergent beetles only excavated egg (*i.e.*, reproductive) galleries, while manually-extracted beetles placed equal effort into excavating egg, as well as non-reproductive pseudo-egg, and hibernal galleries (Fig. 3.3). The construction of feeding and hibernal galleries by non-

reproductive brood adults has also been noted in other studies (Simpson 1929, Werner 1986). Over-wintering galleries constructed by brood adults in tamaracks in the field are similar to the so-called hibernal galleries observed in this study (FRM, pers. obs.), and may reflect adults in a state of diapause.

This study suggests that eastern larch beetles may be capable of bivoltinism under appropriate conditions that facilitate reproductive maturity in brood adults. Both spring-emergent and summer-emergent reproductive generations of beetles may be possible in a single season when sufficient heat units are available to allow the insects to complete development (Chapter 2). This may be particularly true if climate warming creates a longer window suitable for beetle development due to a warmer and earlier spring as well as warmer and later fall season. A second generation of eastern larch beetles in one summer could contribute substantially to beetle population growth, increase the frequency and severity of beetle outbreaks, and resultant tamarack mortality. The mean number of progeny produced per F_1 brood adult female that had emerged naturally from the bolts in the present study (Fig. 3.2A; 43 ± 2.7 SE insects) is similar to that of spring-emergent beetles in three separate, and independent, laboratory experiments (44.5 ± 5.5 (Chapter 2), 40.8 ± 4.9, and 58 ± 4.8; McKee and Aukema, unpubl.).

The information found in this study challenges traditional assumptions regarding the potential for eastern larch beetles to become a significant forest pest and disturbance agent if it is considered as a strictly univoltine insect colonizing moribund trees. Detailed studies to elucidate the mechanisms that govern variation within the reproductive maturation and reproductive capacity of eastern larch beetles are warranted, such as work examining respiratory or developmental rates to quantify potential diapause events in immature stages (*e.g.*, Hansen *et al*. 2011, Chapter 2). Such studies will be particularly important to predict future population dynamics of this insect and its ability to inflict increased mortality to tamarack forests under scenarios of a changing climate.

3.6 Acknowledgments

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3.7 Tables

Table 3.1 Characteristics of the galleries excavated by eastern larch beetle brood adults when introduced to tamarack bolts.

Gallery type[1]	Gallery characteristics
Egg	Sinuous. Egg niches, larval mines, and pupal chambers present. Beetle reproduction evident.
Pseudo-egg	Sinuous. Egg niches present, but larval mines and pupal chambers absent. Beetle reproduction not evident.
Hibernal[2]	Straight. Egg niches, larval mines, and pupal chambers all absent. Beetle reproduction not evident.

[1] Names given for gallery types are unofficial terms used by the authors for convenience.

[2] Name based on field observations of similar galleries excavated in the basal bark of natal trees by emergent brood adults and in which beetles overwinter.

3.8 Figures

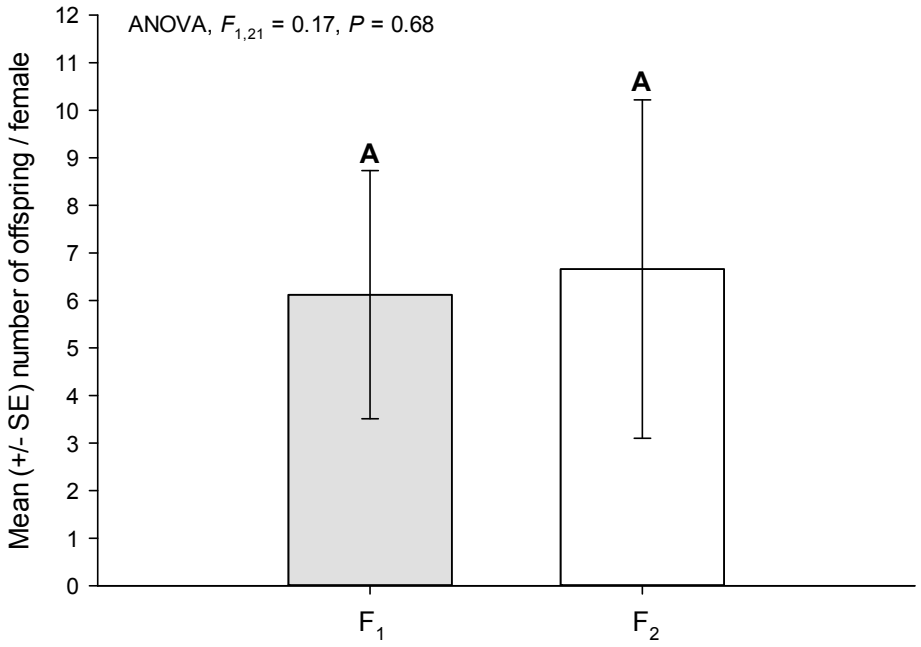

Figure 3.1 Number of offspring (mean ± SE) produced per non-wintered, manually extracted F_1 female (Experiment 1) and F_2 female (Experiment 2). Parent beetles used in Experiment 1 were progeny from the first spring larval brood of 2011. Parent beetles used in Experiment 2 were offspring from Experiment 1.

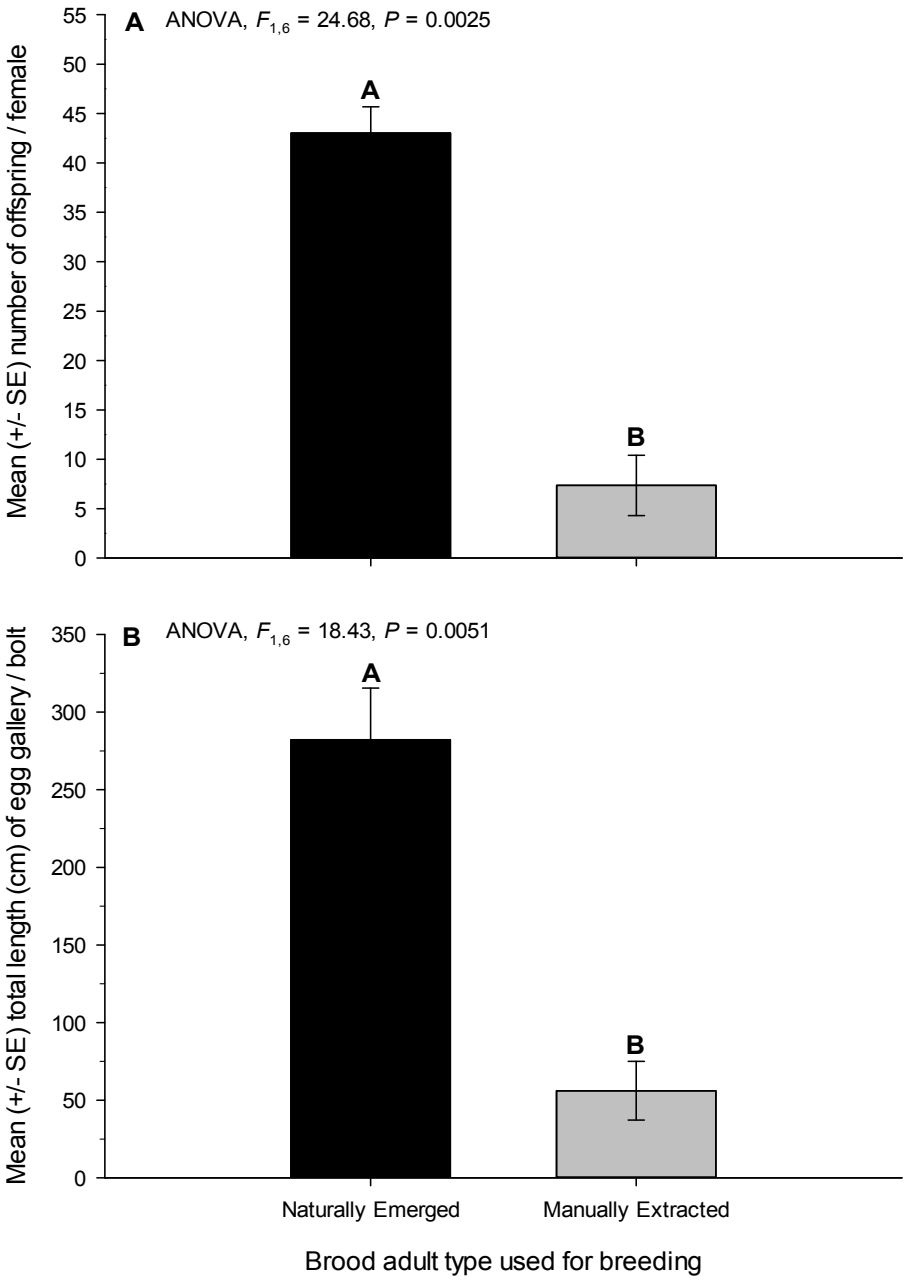

Figure 3.2 (A) Number of offspring (mean ± SE) produced per female for naturally emerged and manually extracted beetles in Experiment 3. (B) Total length (cm) of egg gallery (mean ± SE) excavated per bolt by naturally emergent and manually extracted parent beetles. Beetles from both groups had not experienced an overwintering period before the reproductive trials. n = 10 females per bolt, n = 4 bolts for each of the naturally emergent and manually extracted beetle groups.

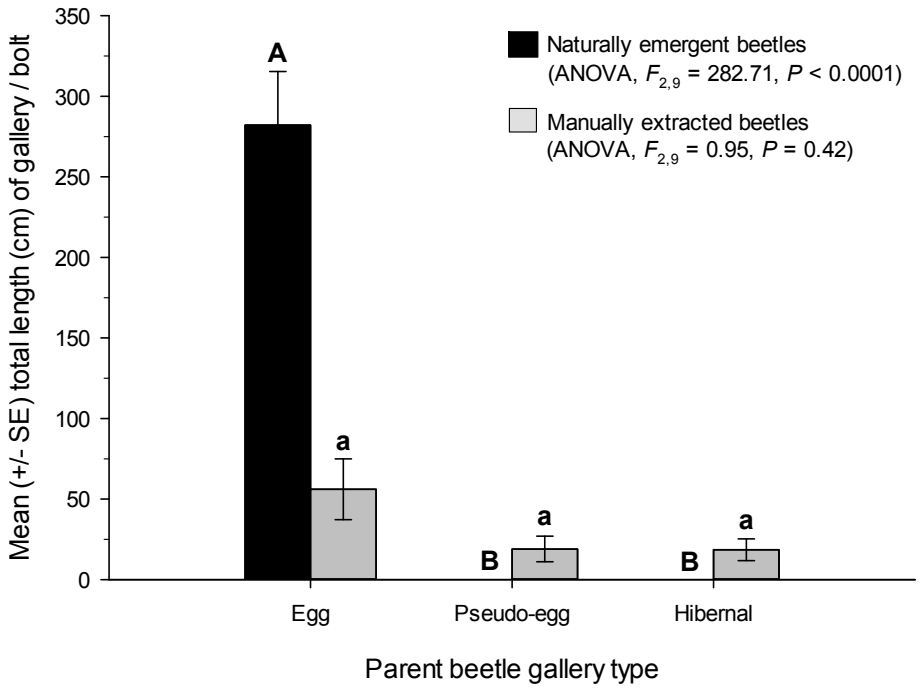

Figure 3.3 Mean total length of gallery type per bolt for naturally emergent and manually extracted beetles. Different letters indicate significant differences in total gallery length per bolt for naturally emergent beetles (upper case letters) and manually extracted beetles (lower case letters).

Chapter 4.

Seasonal phenology and life-history of the eastern larch beetle, *Dendroctonus simplex* LeConte in the Great Lakes region of North America.

4.1 Summary

The eastern larch beetle, (*Dendroctonus simplex* LeConte) (Coleoptera: Scolytinae) is distributed throughout the North American boreal forest wherever its primary host, the eastern larch (tamarack), (*Larix laricina* (Du Roi) K. Koch) is found. Small, localized infestations on stressed or recently dead tamaracks are typical of this insect. Studies on the biology of eastern larch beetles are limited, and derived from research within Alaska and the Canadian Maritime provinces following rare beetle outbreaks in those regions. Since 2000, outbreaks of eastern larch beetles have killed over 100,000 ha of tamarack in Minnesota and neighboring states and Canadian provinces in the Great Lakes region of North America. The causes of the outbreak have not been readily apparent, and research efforts have suffered from gaps in knowledge on the biology of this insect. We present field data from 2011 – 2014 in areas of high beetle activity in northern Minnesota. We describe degree day markers associated with beetle flight periods, emergence of overwintering adults each spring, timing of attacks on tamaracks throughout the flight season, re-emergence behavior of parent beetles from colonized tamaracks, and development of each larval brood, including pre-winter emergence behavior by adult progeny. Re-emergence by parent beetles from the first set of colonized hosts occurred rapidly, allowing a second brood to be established by early summer. In 2012, a third brood was established. The first brood developed to adults well before winter, with many beetles emerging to re-locate to the base of the host tree. The second brood often reached adulthood and began emergence prior to winter. The third brood overwintered as adults, pupae, and late-instars, and resumed development the following spring. Broods established by re-emergent beetles may contribute appreciably to the following year's adult beetle population. Moreover, adult beetles of different broods displayed marked emergence synchrony the following spring, allowing beetles from all

broods to cooperatively mass-attack new host trees. Knowledge of the biology of eastern larch beetles along the southern margin of its range in the Great Lakes region will aid in understanding how population dynamics of this insect may change with a changing climate, and lend insights into management strategies to reduce forest mortality in the future.

4.2 Introduction

The eastern larch beetle, (*Dendroctonus simplex* LeConte), (Coleoptera: Scolytinae) is a phloem-feeding herbivore that colonizes the main bole as well as larger limbs and exposed roots of the eastern larch (tamarack), (*Larix laricina* (Du Roi) K. Koch) (Wood 1982b). Tamarack is the primary host of the eastern larch beetle; however, exotic *Larix* spp. planted in sympatry with tamarack, and allopatric North American *Larix* spp. are also suitable host species (Furniss 1976, Seybold *et al*. 2002). The large geographic distribution of the eastern larch beetle in North America overlays the distribution of tamarack, and is found in Alaska, across the boreal forest of Canada and the north-central United States, eastward to the Canadian Maritime provinces, and south into the northeastern United States (Burns and Honkala 1990, Seybold *et al*. 2002).

Historically, the eastern larch beetle has been considered a non-aggressive bark beetle, colonizing tamaracks stressed from flooding, wind-throw, defoliation, drought, pathogen infection, or other agents that weaken and predispose tamaracks to beetle colonization (Hopkins 1909, Werner 1986, Langor and Raske 1989a, Langor and Raske 1989b). However, under certain conditions, relatively healthy tamaracks can be attacked and killed. Such infestations are typically of short duration; *i.e.*, 2 – 3 years (Langor and Raske 1988a). While not generally considered a significant agent of landscape-level forest mortality, the potential for the eastern larch beetle to become problematic has been recognized for over 100 years (Hopkins 1909). In the late-1970s and early-1980s, for example, millions of hectares of mature tamaracks were killed during concurrent outbreaks in Alaska and on the east coast of Canada and the United States (Werner 1986, Langor and Raske 1989a, Langor and Raske 1989b). These outbreaks were

associated with flooding, defoliation, and general decline of tamarack over large areas that predisposed the trees to bark beetle attack.

In the past 15 years, activity of eastern larch beetles has been increasing in many areas with tamarack across the Great Lakes region including Wisconsin and Michigan, U.S.A., as well as in Ontario, and Manitoba, Canada (ONMNR 2012, MIDNR 2013, WIDNR 2013, MBCFB 2014). In Minnesota, for example, a current outbreak of eastern larch beetles has caused severe tamarack mortality to more than 85,000 ha of tamarack forest since 2000. To date, approximately 22% of the tamarack forest type in Minnesota has been killed (J. Albers, Minnesota Department of Natural Resources, pers. comm.). Unlike previous landscape-level outbreaks of eastern larch beetles, no obvious predisposing agents have been noted (Albers 2010), thus putting enhanced scrutiny on the insect, its development, and behavior.

Much of what is known of the biology and ecology of eastern larch beetle comes from studies in its western and eastern range margins of Alaska and the Canadian Maritimes (Werner 1986, Langor and Raske 1987a, b). In general, adult beetles emerge in early spring from tamaracks colonized the previous year, disperse, and locate new host trees or tamarack material. Similar to most bark beetles, pheromone-mediated mass-attack facilitates the colonization of host trees and increases the reproductive opportunities of the beetle (Prendergast 1991). Following oviposition, female beetles may re-emerge from colonized tamaracks to establish a second, and occasionally, a third sibling brood within separate host material (Simpson 1929, Langer and Raske 1987a). Development is temperature-dependent, and larvae may complete development to adults prior to the onset of winter (Langor and Raske 1987b). Some adult progeny overwinter in place in pupal chambers (Werner 1986, Langor and Raske 1987b). Others emerge prior to winter, drop to the lower bole of the natal tree, and cut non-reproductive, hibernal galleries under the bark and overwinter there (Simpson 1929, Werner 1986).

Current patterns of mortality in the Great Lakes region along the southern range margin of tamarack suggest that subtle changes in climate may be exacerbating insect activity. Timing of life-history events of eastern larch beetles vary between eastern and western range margins (Werner 1986, Langor and Raske 1987a, b), although to date nothing is known concerning the

phenology of the eastern larch beetle in central North America along the southern portion of its range. Existing reports are brief descriptions that rely on data gathered in other areas of the beetle's range (*e.g.*, Dodge 1938). To that end, as a component of a larger research project examining eastern larch beetle population dynamics and the potential causes of the current outbreak in the Great Lakes region, the current study aimed to describe in detail the ecology of eastern larch beetle in that region. Sampling tamarack-dominated northern conifer stands in northern Minnesota from 2011 – 2014, we characterized patterns of flight from spring to fall within stands. Examining individual trees within these stands, we also investigated in detail the 1) emergence of overwintering adults each spring; 2) timing of attacks on tamaracks throughout the flight season; 3) re-emergence of parental beetles from colonized tamaracks, a phenomenon of which we know little in *Dendroctonus* spp.; and 4) the weekly development of each brood, including 5) pre-winter emergence behavior by adult progeny.

4.3 Methods

4.3.1 Study site location

Four study sites with localized, tree-killing populations of eastern larch beetle were selected in the Red Lake Wildlife Management Area, near Lake of the Woods, MN, U.S.A. on 10 June 2011 (Supplemental Methods S1). At time of study initiation, tamarack mortality was confined to small epicenters or the outside edges of each stand, and each site was surrounded by healthy, green tamaracks. Study seasons occurred from 10 June – 22 October 2011, 24 March – 2 November 2012, 23 April – 28 October 2013, and from 28 April – 23 June 2014.

4.3.2 Characterization of flight periods

Twelve 16-unit Lindgren funnel traps (Lindgren 1983) were distributed among the four study sites, with two to four traps per site. Traps with cups suspended 1m above the ground (Werner 1986) were placed in small forest openings, 200 - 600 m from areas where studies of insects in individual trees were occurring to minimize any effects of pheromone baits on beetle

behavior within those sites. Traps were baited with a 50/50 blend of +/- seudenol in a bubble-cap dispenser (release rate = 2.3 mg per day at 20°C) and a 5.0 mL Eppendorf tube containing *α*-pinene (release rate = 2.3 mg per day at 20°C) (Contech, Victoria, BC, Canada) (Werner 1986, Prendergast 1991). Each funnel trap was fitted with dry collection cup containing a 3 x 3 cm piece of Ortho® Home Defense ® Max^{TM} No-Pest ® Strip (19.2% Dichlorvos, active ingredient). Seudenol and *α*-pinene dispensers were replaced as necessary, approximately every three weeks. Collections of trap contents occurred weekly (Supplemental Methods S1). Captured beetles were stored on ice in the field and transferred to a – 25°C freezer until processing. Funnel trap data is presented as the mean (± SE) number of beetles captured per funnel trap per week.

4.3.3 Spring emergence of adult beetles

To study beetle colonization of, and development within, individual trees, each year, spring-emergent, overwintered adult beetles were captured from tamaracks killed the previous year. Beetles were captured in 16.5 x 30 cm (width x height) screened cages affixed to the bark and fitted with a 150 mL collection cup. Four cages were attached to each infested tamarack. Two cages were attached on both the north and south bole aspects centered at 0.4 and 1.8 m above ground level. The cages at 1.8 m were placed over areas caged the previous year to sample re-emergence of parent beetles and emergence of brood progeny. These cages captured spring-emergent brood adults that overwintered in the pupal chamber *in situ* (see below). The cages at 0.4 m were placed over areas of bark not sampled the previous year. These cages captured spring-emergent brood adults that overwintered in pupal chambers *in situ*, as well as brood adults that emerged from pupal chambers the previous fall, descended the host tree, and overwintered in the bark at the base of the host tree. Spring emergence of adult beetles was recorded in 2012, 2013, and 2014.

In total, 55 tamaracks were caged. Cages were emptied twice weekly, and collected beetles were catalogued by emergence date, tree, trap aspect, trap height, and sex. Degree day accumulations associated with 50% spring emergence (*i.e.*, E50) were calculated for each of the

trees (Supplemental Methods S1). ANOVAs were used to analyze whether i) the degree days needed for E50 varied between different broods within a year, and ii) the degree days needed for the E50 varied for analogous broods between years. Where significant differences existed (α = 0.05), a Tukey's HSD means comparison procedure was used to identify significant differences in degree day accumulations.

4.3.4 Adult beetle attack on healthy tamaracks

On 10 June 2011, 103 green, apparently healthy, non-attacked tamaracks with a minimum diameter at breast height (dbh; 1.4m) of 10 cm were selected at random across three of the sites for weekly assessments of beetle colonization. An additional 100 trees were added for monitoring in 2013 due to unexpectedly high rates of tree mortality from the beetles (Supplemental Methods S1). Assessments of each tree for beetle attack began 17 June 2011, 31 March 2012, and 30 April 2013 and continued every 7 d until the cessation of beetle activity each fall. The lower 2.5 m of bole was visually assessed for bright orange frass produced by beetles boring into tamarack bark.

Upon locating a beetle attack, an observation "window" was installed, consisting of four push pins bordered with string on the south face of the bole 1.6 m above ground-level. In 2011, observation windows measured 20 x 20 cm (H x W) but were enlarged to 40 x 25 cm (H x W) in 2012 and 2013 to more effectively include attacks by beetles during the early stages of host colonization when the density of attacks was low. Each attack was marked with a pin to prevent double counting in successive weeks. For purposes of this work, we classify new attacks as those deemed successful (*i.e.*, beetles active within the phloem) and unsuccessful (*i.e.*, beetles "pitched out" after entering the phloem). Attack data is presented as the total number of new attacks summed from all study tamaracks per week.

4.3.5 Adult beetle re-emergence from colonized tamaracks

Once no new attacks were observed between two successive sample dates, colonization of a tree was deemed to be complete, emergence cages identical to those used to capture spring

emerging adults were installed. Up to seventeen trees attacked per brood group were sampled (Supplemental Methods S1). Re-emerging parent beetles were collected twice per week in the same manner as the spring-emergent beetles. Captured beetles were catalogued by emergence date, specific host tree, trap aspect, trap height and beetle sex. Data are presented as the mean (± SE) number of re-emergent parent beetles captured per cage per week.

4.3.6 Recording larval development

Weekly sampling of between three and six trees per brood group (*i.e.*, the first, second, or third brood) for development of progeny began when it was apparent that 1) beetle attack was likely to continue, and 2) tree death was highly probable. With the exception of two trees (out of 92 attacked), both criteria occurred when beetle attack density was ≥ 10 attacks per 1000 cm^2. Two 10 x 10 cm bark samples on opposite aspects of the tree were removed weekly with a utility knife and chisel, beginning at 0.5 m and continuing vertically to approximately 2.5 m (Supplemental Methods S1). Using a cloth apron to prevent loss of immature life stages dropping from the inner bark, samples were placed in paper bags and transported to the laboratory on ice, where all eggs and larvae were placed in 95% ethanol the same day. Larval samples were divided into instar classes based on head capsule measurements (Supplemental Methods S1). Each brood group continued to be sampled until all individuals consisted of adult life-stages for two consecutive sampling periods. When a brood was not able to complete development to the adult life-stage due to the onset of cold weather, sampling was continued in the spring of the following year. Data of life-stage development for each brood group is present as the mean (± SE) proportion of each life-stage present per 100 cm^2 bark sample per week.

4.3.7 Pre-winter emergence of beetle progeny

Cages used to capture re-emerging parents post initial-colonization were maintained in place to capture emerging brood progeny throughout the summer and fall months. Progeny were collected twice per week, and catalogued by emergence date, tree, trap aspect, trap height, and beetle sex (Supplemental Methods S1). A logistic regression was used to determine the

probability of pre- *vs.* post winter emergence by adult progeny from each brood of each study year. The model utilized a term for site incorporated as a random effect.

4.3.8 Calculations of degree day accumulations

Daily air temperature data from the spring of 2011 to the spring of 2014 was recorded from weather stations located at the Baudette International Airport, Baudette, MN and at the Norris Camp Field Office, Minnesota Department of Natural Resources, Red Lake Wildlife Management Area, MN, U.S.A. The maximum straight-line aerial distance from a study site to one of these locations was 35.4 km. The data for both locations was obtained from the NOAA National Climatic Data Center (National Oceanic and Atmospheric Administration, 2014). Daily mean air temperatures for all study sites were calculated from daily minimum and maximum air temperatures using data from both weather station locations.

The number of degree days (DD) above 5°C that had accumulated at the time of each sampling period was determined using air temperature data from the two weather stations. A base threshold of 5°C was used because 5°C is the minimum temperature required for many adult beetle activities such as spring emergence, host attack, and re-emergence (Langor & Raske, 1987), and thus allows comparisons in phenology between larval development, emergence of adult progeny, and the previously-mentioned adult beetle activities (Supplemental Methods S1). A threshold temperature of 7.5°C is the estimated minimum temperature for development of eastern larch beetles (Chapter 2). As such, this base temperature was used in two instances: 1) calculating the average degree days required for an entire brood group to completely develop from eggs to the adult life-stage (n = 29 tamaracks), and 2) calculating the average degree days that elapse between the establishment of a brood group as eggs to the onset of pre-winter emergence by adult progeny (n = 55 tamaracks).

4.4 Results and Discussion

4.4.1 Annual temperature profiles

The profile of daily maximum and minimum temperature (°C) for the study area varied considerably between years (Fig. 4.1A-D). As such, annual degree day accumulations (5°C base) differed between study years with 2011, 2012, 2013, and 2014 totaling 1832, 1895, 1771, and 1690 DD, respectively. In 2012, temperatures were warmer from Julian day (JD) 0 – 65 than in all other years. Furthermore, 2012 was characterized by an early spring with rapid warming suitable for eastern larch beetle spring emergence beginning around JD 65 – 70 (early-March, Fig. 4.1B; see section on spring emergence below). In contrast, 2013 had, in particular, a cool, wet spring with daily mean temperatures above 5°C not occurring until almost 110 JD, resulting in a much later onset of beetle spring emergence. Moreover, a cool period occurred mid-summer (JD ~200 – 225) (Fig. 4.1C). The year 2011 (Fig. 4.1A) was intermediate relative to 2012 and 2013, while 2014 was the coolest overall (Fig. 4.1D). The maximum temperatures for 2011, 2012, 2013, and 2014 were 33.0, 32.8, 32.5, and 31.7°C, respectively, while the minimum temperatures were -38.3, -31.4, -35.8, and -38.0°C. Degree days based on mean daily temperature accumulated over a 227 d period in 2012 – the longest of any year in this study (Fig. 4.1 B). The shortest period of degree day accumulation was just 172 d in 2013 (Fig. 4.1C). The yeas 2011 and 2014 were between these extremes (Fig. 4.1A&D).

4.4.2 Characterization of flight periods

Beetles were captured almost immediately when traps were deployed in June of 2011 (Fig. 4.2A). However, the captures likely did not represent the initial spring flight, based on how early flying beetles were captured in 2012 and 2013 (13 Apr. and 20 May in Figs. 4.3A, 4.4A, respectively). As such, it appears that beetles begin to be captured in the spring when the cumulative degree days reach 90. In 2012, this threshold was breached after temperatures on four days of the week preceding 13 Apr. exceeded the flight threshold of 5°C (Langor and Raske 1987a) at 5.6 ± 0.3°C, even though mean daily temperatures for the entire week were only 2.6 ± 1.5°C. Beetles began to fly in greater numbers (13.5 ± 4.2 beetles per trap) by 106 DD when daily air temperatures averaged 6.2 ± 1.2°C (mean ± SE) during the previous week. Similarly, a rapid and large initial onset of beetle flight in 2013 (608.0 ± 228.2 beetles per trap) occurred at

111 DD following a rapid increase in temperature, with mean daily temperature in week of initial flight of 13.7 ± 0.8°C. The peak of beetle flight in 2013 occurred at 191 DD and lasted until 356 DD.

Smaller flight peaks occurred after the initial spring emergence comprising second and third flight periods (Langor and Raske 1987a). In 2011, the flight event captured from 877 to 1574 DD was likely the second flight period. In 2012, the second beetle flight was initiated much earlier at 451 DD and lasted until 835 DD. The second beetle flight of 2013 was similar to 2011, beginning at 809 DD and lasting until 1702 DD. In 2012, a third flight event occurred at 954 DD and continued until 1888 DD, although the majority of the flight was complete by 1455 DD. The first, second, and third flights of 2012 accounted for 61.6, 18.8, and 19.6% of beetles captured (*n* = 265,349 beetles), respectively, while the first and second flight periods of 2013 accounted for 85.8, and 14.2% of beetles (*n* = 104,789) captured, respectively.

Captures of bark beetles in funnel traps do not always adequately characterize emergence from hosts or attack on new hosts (Bentz 2006). Highly attractive lures deployed during periods of low beetle colonization may capture a disproportionately large numbers of beetles, while capturing fewer beetles during times of high beetle activity when natural aggregation pheromone plumes inundate an area (Aukema *et al*. 2000, Bentz 2006, McMahon *et al*. 2010). Among mountain pine beetles, re-emerging parents in the spring can be mistaken as early-emerging brood adults (DeLeon *et al*. 1934, Bentz 2006). This mischaracterization is less likely in this system, because spring-emergent eastern larch beetle parents typically perish within the second or third set of host trees (Simpson 1929) leaving few, if any, re-emergent adult beetles to over-winter and fly early the following spring.

4.4.3 Spring emergence of adult beetles from trees

More than 4,200 beetles were captured emerging from trees. Subsequent sections describe the ecology of different broods established the prior year, but we describe their emergence behaviors as adults in the spring following establishment in this section. In the spring of 2012, a total of 1,162 (590 females, 572 males) and 674 adult beetles (359 females, 315

males) were captured in cages on the first and second sets of brood trees of 2011, respectively. In 2013, 516 (280 females, 236 males), 117 (59 females, 58 males), and 486 adult beetles (242 females, 244 males) were captured from the first, second, and third sets of 2012 brood trees, respectively. Finally, in 2014, 995 (486 females, 509 males) and 305 adult beetles (152 females, 153 males) were captured emerging from the first and second sets of brood trees of 2013.

Overwintered adult progeny from all broods that developed the previous year demonstrated a high degree of spring emergence synchronicity the following year. Spring emergence of adult beetles in 2012 (Fig. 4.3B) from tamaracks colonized for the first and second broods of 2011 began at degree day accumulations of 93 and 90 DD, respectively. In 2013 (Fig. 4.4B), the spring emergence of adult beetles from the first, second, and third broods of 2012 began simultaneously at 72 DD, slightly earlier than the previous year. In the spring of 2014, overwintered progeny from the first and second broods of 2013 were first observed in emergence cages at 68 and 129 DD, respectively (Fig. 4.5); the slight delay may have been due to prolonged, wet, and cool weather as beetles typically disperse during clear, sunny periods (Langor and Raske 1987a).

The ecology of “sister” or “sibling” broods among bark beetles has not been well studied, but in general the young adults of inaugural broods emerge prior to subsequent broods (Walters 1956, Wermelinger and Seifert 1999, Humphreys 2000, Dworschak *et al*. 2014a, Öhrn *et al*. 2014). Eastern larch beetles appear to be atypical in this regard with highly synchronous emergence between brood groups of the previous year. In the only other published study recording three broods, concurrent spring emergence occurred by adult beetles from the first and second broods, while adults of the third brood emerged two months later (Simpson 1929). In the present study, the vast majority of beetles from the third brood of 2012 emerged during the main spring emergence period in 2013, with only 1.2% of the beetles emerging in early July. The synchronicity of emergence enhances likelihood of host procurement with higher numbers of conspecifics to engage in cooperative mass attack (Raffa *et al*. 2015).

Averaged across all trees, the mean onset of spring emergence across the three years occurred at 85.1 ± 8.2 DD, almost identical to the 85 DD (base = 5°C) required by beetles in

Alaska (Werner 1986). Degree day accumulation has been shown to be the most reliable predictor of spring emergence and flight activities of another northerly-distributed bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytinae) (Öhrn *et al*. 2014). Maximum daily temperature superseding a flight threshold appears to be a more accurate predictor of spring emergence of many southerly-distributed bark beetles, including several *Dendroctonus* spp. (Gaylord *et al*. 2008). The 5°C flight threshold of eastern larch beetles (Langor and Raske 1987a) may not be a reliable indicator of spring emergence, since maximum mean air temperature in the present study superseded 5°C for 18, 25, and 29 d during the 30 d period leading up to the onset of beetle emergence in 2012, 2013 and 2014, respectively. The overall mean (± SE) maximum air temperature for the days above 5°C was 12.5 ± 0.5°C (range: 5.3 – 21.6°C) but did not stimulate beetle emergence.

Although emergence between brood groups began synchronously in the spring, we found that inaugural brood groups generally emerged at a higher rate. For example, in the spring of 2012, the E50 for adult progeny of the first and second broods of 2011 occurred at mean (± SE) degree day accumulations of 186.4 ± 4.0 and 205.4 ± 6.6 DD, respectively (ANOVA, $F_{1,11}$ = 6.49, P = 0.027). In 2013, the degree days required by adults of the first, second, and third brood groups of 2012 to reach the E50 averaged (± SE) 140.5 ± 7.9, 168.4 ± 24.4, and 195.6 ± 26.3 DD, respectively (ANOVA, $F_{2,20}$ = 3.43, P = 0.053). For the spring of 2014, the E50 of adults from the first and second broods of 2013 occurred at mean degree days of 115.0 ± 4.3 and 158.4 ± 11.1 DD, respectively (ANOVA, $F_{1,11}$ = 19.18, P = 0.0011). Faster spring emergence by the inaugural *vs.* subsequent broods from the previous year likely reflects greater physiological maturity among individuals from the inaugural brood. This is discussed further in the section on brood development.

Across years, declining degree days needed for adults of analogous brood groups (*i.e.*, first brood of 2011 *vs.* 2012) to reach the E50 of spring emergence may involve differences between air and bark temperatures as noted in other bark beetle studies (Beal 1934, Bartos and Amman 1989, Wainhouse *et al*. 2014). As the beetle infestations progressed throughout the duration of the study period, cumulative tamarack mortality resulting from eastern larch beetle

activity opened the forest canopy and reduced sub-canopy shading. Reduced shading likely resulted in more sunlight directly striking the boles of infested tamaracks and caused the bark temperature (experienced by beetles) to become increasingly warmer than the air temperature (used to calculate DD sums). As such, measurements of degree day accumulations based on air temperature may have lagged behind the actual degree day accumulations experienced by beetles in the bark of the infested trees. Therefore, the reduction in the number of degree days required for analogous brood groups of subsequent years to reach the spring E50 may be due to an increased discrepancy between air and bark temperatures during the later years of the study.

In general, funnel trap captures of eastern larch beetles in 2012 and 2013 mirrored the periods of beetle emergence quite well (Figs. 4.3A&B, 4.4A&B). The completion of beetle emergence was also captured fairly well by funnel traps, although early re-emergent parent beetles (discussed below) captured in the funnel traps sometimes created a tail on the first flight period data. Thus, it appears that funnel trap sampling provides a relatively efficient and manageable option for forest health managers to generally monitor eastern larch beetle spring emergence for the inaugural brood, but should not be used to infer specific details on the biology of this insect.

4.4.4. Adult beetle colonization of tamaracks

In each of the three years of our study, the season was marked by two (2011, 2013) or three (2012) distinct episodes of tree-killing activity. Each set of attacked tamaracks resulted in the successful development of a separate brood.

First set of brood trees ("first brood"). The initiation of the spring attack period on tamaracks for the first brood lagged behind the start of beetle emergence by 60 – 100 DD, which corresponded to 12-13 d, depending on the year. However, because the assessment for beetle attacks occurred once per week in our study, attacks may have begun up to 6 d earlier post-emergence. A lag between beetle emergence and host attack has been observed in bark beetles previously (Langor and Raske 1987a, Öhrn *et al*. 2014), and may reflect a requirement to burn lipids through flight, becoming physiologically primed to initiate attacks on host trees (*e.g.*, Atkins

1966, Wood 1982a, Bentz 2006). Beetle attack in 2012 and 2013 began at similar degree day accumulations of 151 (Fig. 4.3C) and 173 DD (Fig. 4.4C), respectively, but was sustained for almost 300 DD longer in 2013. In 2012, the timing of beetle attack closely matched the spring emergence of adult beetles from trees (Fig. 4.3B&C), but these events did not align well in 2013 (Figs. 4.4B&C). However, the first beetle flight period in the stand and the attack period on the first set of brood trees did align closely for both years (Figs. 4.3A&C, 4.4A&C). Sixty-seven tamaracks were killed by adult beetles establishing the first brood of 2012. For the first brood of 2013, forty-four tamaracks were killed comprising six of the seventeen tamaracks remaining alive from the group selected in 2011, as well as thirty-eight tamaracks from the group selected in 2013.

Second set of brood trees ("second brood"). Attacks on tamaracks for the second brood occurred concurrently with beetle re-emergence from tamaracks recently colonized for the first brood group. No delay between re-emergence and attack would be expected if these re-emergent parents were already physiologically engaged in oviposition (see subsequent section). Beetle attacks on the second set of tamaracks in 2011 were underway at 523 DD (Fig. 4.2C) but were scattered, light (≤ 0.4 ± 0.4 attacks per 400 cm^2), and appeared unsuccessful (*i.e.*, many pitch-filled attack points). This period of attack was coincident with high rates of parent beetle re-emergence from the first set of brood trees (see next section; Fig. 4.2B). Attack increased considerably (1.0 ± 0.4 attacks per 400 cm^2) by 972 DD and peaked at 1160 DD (Fig. 4.2C), coincident with the onset of pre-winter emergence by adult progeny of the first brood of 2011 (Fig. 4.6H). Beetle attack on the second set of brood trees of 2012 and 2013 both began at similar degree days of 451 and 488 DD, respectively (Figs. 4.3C & 4.4C), slightly earlier than in 2011. The peak of attack on the second set of brood trees of 2012 closely matched the re-emergence pattern of parent beetles from the first set of brood trees of that year (Fig. 4.3C&D). In 2013, the second set of brood trees experienced two peaks of beetle attack (Fig. 4.4C). The first peak in attack occurred at 488 DD when the re-emergence of parent beetles from the first set of brood trees was greatest (Fig. 4.4C&D). The second peak occurred at 711 DD, coincident with the delayed emergence of a small group of beetles from two tamaracks that were colonized late the

previous year and that were a part of the third set of brood trees of 2012 (Fig. 4.4B&C). Attack on the second set of brood trees of 2012 and 2013 ended at 715 and 918 DD, respectively, which was much earlier than in 2011 (*i.e.*, 1577 DD). In total, seven, three, and four tamaracks were killed for the second brood of 2011, 2012, and 2013, respectively.

Third set of brood trees (2012 only; "third brood"). In 2012, a third period of attack on healthy tamaracks started at 954 DD (17.0 ± 0.0 attacks per 1000 cm^2) (Fig. 4.3C). Re-emergence of parent beetles from the second set of brood trees of 2012 was largely complete (*i.e.*, < 1 beetle per cage per week) when the third period of attack began (Fig. 4.3C&D). The onset of beetle attack on the third set of trees in 2012 was associated with the beginning of pre-winter emergence by brood adults of the first brood of 2012 (Figs. 4.3C & 4.6H). This pattern was similar to observations of increased attack on the second set of brood trees of 2011 and also occurred at similar degree day accumulations (972 and 954 DD for 2011 and 2012, respectively). Seven tamaracks were killed by beetles establishing the third brood of 2012.

4.4.5 Adult beetle re-emergence from colonized tamaracks

After colonization of each set of tamaracks during the springs and summers of 2011, 2012, and 2013, adult beetles were observed re-emerging from all sets of brood trees colonized. Re-emergence of parents to putatively commence other broods is a common phenomenon in a number of bark beetle species (Wood 1982b, Byers 1989).

First set of brood trees. Re-emergence of parent beetles was similar across study years. In 2011 (Fig. 4.2B), 2012 (Fig. 4.3D), and 2013 (Fig. 4.4D) the first captures of re-emergent adult beetles were recorded at 390, 451, and 369 DD, respectively, and was complete after similar degree day accumulations of 972, 954, and 909 DD. Records of adult beetle density per 100 cm^2 taken while sampling larval development (*i.e.*, before brood trees were fully colonized) indicated a decline in parent beetle density in brood one trees occurring by 317 DD in 2012 (Fig. 4.3E) and 285 DD in 2013 (Fig. 4.4E). Thus adult beetles began re-emerging before host trees were fully colonized, and continued until living beetles were no longer present within the host tree (*e.g.*, Fig. 4.3D&E). This phenomenon is a new finding for eastern larch beetle, as past studies have

reported re-emergence occurring approximately 20 – 30 d after host trees or material becomes fully colonized (Swaine 1911, Simpson 1929, Langor and Raske 1987a).

Residence time of parental bark beetles within colonized host trees prior to re-emergence has been shown to decline with increased attack density (McMullen and Atkins 1961, Wagner *et al*. 1981, Anderbrant 1986, 1989, Byers 1989). Rapid re-emergence from the first set of brood trees by beetles in our study is reported during an epidemic population phase where attack densities on tamaracks averaged 2.2 ± 0.7 (SD) per 100 cm^2 (Appendix 1) whereas previous reports were taken during low-density endemic conditions (*e.g.*, Swaine 1911, Simpson 1929). However, Langor and Raske (1987a) also report delayed re-emergence by eastern larch beetles during the late stages of an outbreak despite similar attack densities (*i.e.*, 2.4 ± 1.2 SD per 100 cm^2). Re-emergence rates are also correlated with ambient air temperatures (Wagner *et al*. 1981, Anderbrant 1986, 1989), and degree day accumulation (Anderbrant 1986, Öhrn *et al*. 2014). The ambient air temperature reported by Langor and Raske (1987a) during the attack and host colonization phases of eastern larch beetles was approximately 10 – 11°C, whereas average ambient temperatures during these same phases in our study were 13.1, 15.1, and 13.6°C, for 2012, 2013, and 2014, respectively. The warmer temperatures in our study potentially resulted in more rapid re-emergence activity.

Early re-emergence and rapid colonization of subsequent tamaracks for a second brood cohort may have several advantages for bark beetles relative to delayed re-emergence. First, an early exit from colonized host trees reduces intraspecific competition and can increase the reproductive success of the parent beetles (McMullen and Atkins 1961, Byers 1989, Zhang *et al*. 1992) as well as generate more fit offspring (Anderbrant 1988). Second, earlier re-emergence in bark beetles can be associated with greater lipid reserves in the parental beetles (Anderbrant 1988), which in turn is associated with increased fecundity, dispersal, pheromone production, mate procurement, and survival (Atkins 1966, Thompson and Bennett 1971, Anderbrant *et al*. 1985, Anderbrant 1988, Jactel 1993, Elkin and Reid 2005, Williams and Robertson 2008, Evenden *et al*. 2014). Enhanced fitness may contribute to the ability to successfully attack and colonize standing, live, apparently healthy trees for the establishment of a second brood cohort

as was observed in each year of this study. Previous, reports list suitable host material for re-emergent beetles as being logging slash or other downed, low-vigor material located near colonized tamaracks (Hopkins 1909, Swaine 1911, Simpson 1929, Langor and Raske 1987b). Large diameter, healthy tamaracks that are able to be successfully attacked and killed by eastern larch beetles allow parent beetles to produce abundant progeny and may also provide abundant phloem resources that reduce inter- and intraspecific competition among developing larvae (Appendix 1.). Such relationships have been observed in other bark beetle – conifer systems as well (Atkins and McMullen 1960, Cole and Amman 1969, Cole *et al*. 1976, Fargo *et al*. 1979, Haack *et al*. 1984, Raffa 2001). Third, earlier establishment of the second cohort increases the likelihood that the cohort will develop to the adult life-stage prior to winter (see section on Brood Development).

Second set of brood trees. In 2011, re-emergence began relatively late in the year, after 1371 DD (Fig. 4.2B). In 2012 and 2013, however, re-emergence occurred much earlier, and at similar degree days of 715 and 809 DD, respectively (Figs. 4.3D, 4.4D). Similarly, re-emergence in 2011 finished at 1828 DD but was complete by 1288 and 1212 DD in 2012 and 2013, respectively.

In both 2011 and 2013, beetle re-emergence from the second set of brood trees did not accompany an additional period of beetle attack on green tamaracks. In 2012, the year that a third set of brood trees was colonized, beetle re-emergence from the second set of brood trees generally did not coincide with beetle attack on the third set of brood trees (Fig. 4.3C&D). It seems likely, therefore, that re-emergent beetles did not participate in a meaningful way to attacking the third set of tamaracks in 2012.

Third set of brood trees (2012 only). Re-emergence by adult beetles was observed from the third set of brood trees of 2012, occurring from 1288 – 1894 DD (Fig. 4.3D). Unlike the density of adult beetles in the first and second sets of brood trees of each study year, the density of adult beetles in the third set of brood trees did not reach zero before the onset of winter, ending the year with a density of 0.4 ± 0.1 beetles per 100 cm^2 (mean ± SE) (Fig. 4.3E).

4.4.6 Brood development

The mean (± SE) number of degree days required for a complete brood cohort to develop from eggs to adults was 694.6 ± 22.9 DD. This value was calculated as the degree day accumulation (base = 7.5°C, Chapter 2) between the date of initial tamarack colonization and the date when all progeny reached the adult life-stage (*n* = 25 trees sampled for brood development).

First brood. The pattern of larval development of the first brood was similar across study years. At the initial sample in 2011 (402 DD), eggs, first, second, and third larval instars accounted for 52 ± 6, 25 ± 3, 20 ± 4, and 3 ± 2 % (mean ± SE) of observations, respectively (Fig. 4.6A-D). These life-stages were present in similar proportions at 400 DD in 2012 (Fig. 4.7A-D) and 2013 (Fig. 4.8A-D). By 365 DD in 2012, eggs accounted for 98 ± 2% of observations, with first-instars accounting for just 2 ± 2% of observations, indicating that egg hatch likely began at approximately 350 DD. A similar result was observed in 2013 at 379 DD, when eggs, first- and second-instars accounted for 91 ± 4, 7 ± 3, and 2 ± 1 % of the sampled brood, respectively. It appears that egg-hatch begins approximately 200 DD after the initial attack on brood trees begins since 214 and 206 DD elapsed between the first observations of beetle attack and the early stages of egg hatch in 2012 and 2013, respectively.

In general, the majority of the brood cohort developed to a subsequent life-stage each week of the sampling period since successive, sub-adult life-stages peaked in abundance at each weekly sampling episode (Figs. 4.6A-G, 4.7A-G, & 4.8A-G). In 2011, the first, second, third, fourth-instar, and pupae were most abundant at 402, 462, 542, 651, and 745 DD, respectively (Fig. 4.6A-G). This was very similar to 2012 and 2013. In 2012, only eggs were observed until 321 DD. Thereafter, brood development occurred rapidly. Peaks in the abundance of first, second, third, fourth-instar, and pupae occurred at 466, 466, 546, 629, and 731 DD, respectively (Fig. 4.7A-G). In 2013, eggs were observed until 296 DD, after which first, second, third, fourth-instars, and pupae were most abundant at 488, 488, 594, 711, and 828 DD, respectively (Fig. 4.8A-G). In 2013, the peak abundance of the first and second-instars occurred at degree days similar to 2011 and 2012 but development of the subsequent life-stages lagged slightly behind the previous years.

In each year, brood adults first occurred at relatively similar degree day accumulations of 745, 851, and 828 DD for 2011, 2012, and 2013, respectively (Figs. 4.6G, 4.7G, 4.8G). Brood adults accounted for all offspring observations by 1277 and 1190 DD for 2011 and 2012, respectively. However, brood adults of 2013 accounted for all offspring observations by 990 DD, earlier than previous years.

Second brood. The timing of the establishment and subsequent development of the second brood of 2011 differed markedly from that of the second broods of either 2012 or 2013. Indeed, the development of the second brood of 2011 (Fig. 4.6) was most similar to the third brood of 2012 (see section below; Fig. 4.7). In 2011, trees containing the second brood were first sampled for development at 1109 DD, and eggs were the only life-stage observed. However, by 1174 DD brood development was occurring with eggs, and first- and second-instars accounting for 84 ± 9, 12 ± 8, and 4 ± 4 % of the observations, respectively. In contrast, eggs of the second larval brood of 2012 (Fig. 4.7) were present until 629 DD, which was nearly 500 DD earlier than in 2011. By 731 DD in 2012, eggs were only 40 ± 18% of observations while first and second-instars each accounted for approximately one-third each of the observations. Similarly, eggs of the second brood of 2013 (Fig. 4.8) were the only life-stage present in bark samples at 594 DD. By 711 DD, the relative proportions of eggs, first, second, and third-instars advanced to 65 ± 15, 17 ± 8, 11 ± 8, and 7 ± 6 %, respectively.

Unlike the first brood groups, the majority of larval development of the second broods often took longer than one week to reach a subsequent life-stage. In 2011, first, second, third, fourth-instars and pupae were most abundant on 1371, 1371, 1470, 1574, and 1698 DD, respectively (Fig. 4.6), which was much later than in 2012 or 2013. In contrast, peaks in abundance for first, second, third, fourth-instars, and pupae of the second brood of 2012 occurred at 731, 731, 851, 972, and 1083 DD, respectively (Fig. 4.7) while peaks in abundance for the same life-stages in 2013 occurred just slightly later at 828, 918, 1130, 1065, and 1233 DD, respectively (Fig. 4.8).

Brood adults were first observed at 1612, 1083, and 1233 DD in 2011, 2012, and 2013, further marking the disparity between the development of the second brood of 2011 to that of

2012 and 2013. In 2011, the second brood did not completely develop to the brood adult life-stage by 1827 DD, the amount of degree days accumulated prior to the onset of cold temperatures that prevented further brood development. As such, this brood entered the winter of 2011-12 as fourth instars, pupae, and brood adults (Fig. 4.6). Development resumed in the spring of 2012. Fourth instars, pupae, and brood adults accounted for 11 ± 6, 0 ± 0, and 89 ± 6 % of individuals, respectively at 106 DD, the initial sample in 2012. All individuals reached the adult life-stage by 191 DD in 2012. Again in contrast to 2011, the second brood of 2012 and 2013 completed development to the adult life stage within the same year as being laid as eggs. Brood adults comprised all observations by 1559 DD in 2012 and by 1771 DD in 2013.

Third brood (2012 only). At the time of the first larval development sample on 1083 DD, eggs accounted for 85 ± 8% of observations while first and second-instars represented 13 ± 7 and 2 ± 1 % of observations, respectively (Fig. 4.7A-C). Thus, development had occurred since 954 DD, when attacks on the third set of brood trees were first observed. The third brood was characterized by a lack of developmental synchronicity; *i.e.*, most immature life-stages were present concurrently throughout most of the sampling period. This brood continued to develop until 1894 DD were accumulated, but, like the second brood of 2011, not all individuals were able to complete development to adults. This brood entered the winter of 2012-13 as fourth-instars, pupae, and brood adults in proportions of 7 ± 3, 19 ± 9, and 72 ± 12 % of the population, respectively (Fig. 4.7E-G). Development resumed in the spring of 2013. All individuals reached the adult stage by 472 DD. However, 472 DD may over-estimate heat accumulation since 2 weeks separated the second-to-last and final sample periods in the spring of 2013. It is therefore likely that the brood had completely reached the brood adult stage at least a week earlier, at approximately 369 DD.

Larval development was most synchronous for the inaugural brood cohorts of each year with a majority of individuals present in a similar life-stage. The decline in developmental synchronicity in the subsequent brood cohorts may be due to a prolonged and less concentrated (*i.e.*, peak-less) attack and egg laying period by re-emergent adult beetles. Eastern larch beetles exhibit life-stage specific development rates (Langor and Raske 1987b) with the minimum

threshold temperature for development below 9.9°C for all life-stages (Chapter 2). Differences in the life-stage specific developmental thresholds for eastern larch beetles may not be as pronounced as in other *Dendroctonus* spp. such as mountain pine beetle (*D. ponderosae*) (Bentz *et al*. 1991, Régnière *et al*. 2012), making it potentially difficult for the larvae of inaugural and subsequent broods to become synchronized developmentally via ambient temperature alone. However, adult eastern larch beetle progeny from inaugural and subsequent broods that successfully develop to adulthood prior to winter are able to emerge during a common period the following spring.

Development to the adult life stage prior to winter provides several advantages for eastern larch beetles. First, overwintering as adult progeny may reduce overwintering mortality by allowing beetles to overwinter beneath the snow (Langor and Raske 1987a). Larval and adult eastern larch beetles are very cold hardy, however, and can survive temperatures of –49 and –42°C, respectively, however (Venette and Walter 2008), and we did not observe late fall temperatures sufficient to cause near-complete mortality of adult progeny from second brood cohorts (Langor and Raske 1987a). We do note, however, that brood groups entering the winter as a mixture of fourth-instars, pupae, and adult progeny experienced a decline in the proportions of fourth-instars and pupae relative to adult progeny between the late fall and early spring, suggesting that some winter mortality occurred to the sub-adult life-stages (Figs. 4.6E-G, 4.7E-G). Furthermore, the sex ratios of spring-emergent beetles from these brood cohorts was skewed at times to favor females on the northern – and presumably coldest – bole aspects of colonized tamaracks (Supplemental Results R1), suggesting sex-related differences in overwintering survivorship (Lachowsky and Reid 2014).

Enhanced synchronicity between the inaugural and subsequent broods that emerge the following spring constitutes a second advantage to completing development to adults prior to winter. Increased numbers of beetles emerging concurrently facilitates host procurement through cooperative mass attack (Raffa *et al*. 2015) and increases the likelihood of reproductive success of the latter brood groups. Finally, overwintering as adults may increase fitness relative to those insects that complete development in the spring. Recent studies of *I. typographus* , for example,

have demonstrated that spring-emergent adult beetles that over-wintered as larvae had lower survivorship, lipid content, and dispersal capacities as adults relative to beetles that had overwintered as adults (Dworschak *et al*. 2014a).

4.4.7 Pre-winter emergence of progeny

Pre-winter emergence by adult progeny of eastern larch beetle is a common occurrence for this insect (Swaine 1911, Simpson 1929, Langor and Raske 1987a). Because temperatures below the snowline are significantly warmer than above (Werner 1978), earlier pre-winter emergence of adult eastern larch beetle progeny from pupal chambers to overwintering galleries beneath the snowline likely serves to increase overwintering survival (Werner 1986). Based on pre-winter emergence data of adult progeny, we found that the probability of pre-winter emergence in this study was between 13 – 85% depending on brood group and study year. Overall these rates are similar to the rates of pre-winter emergence by adult progeny in the spruce beetle (*D. rufipennis* (Kirby)) (Schmid and Frye 1977).

The mean (± SE) degree day accumulation (7.5°C base) between the date when beetle attack on a tamarack was first observed and the date of initial pre-winter emergence of brood adults was 655.9 ± 22.8 DD, calculated from all caged tamaracks from all brood cohorts from 2011 – 2013 (n = 43, not including the first set of brood trees of 2011). This degree day value is slightly higher than laboratory studies showing developmental time from eggs to emergent brood adults of 604.1 ± 9.1 DD (Chapter 3), and could reflect weekly sample intervals in the field.

Emergence from the first set of brood trees. The onset of brood adult emergence from the first set of brood trees began at similar degree day accumulations for each year, beginning at 972, 954, and 976 DD for 2011, 2012, and 2013, respectively (Figs. 4.6H, 4.7H, & 4.8H). Emergence continued in all years at variable levels until the onset of cold daily temperatures (≤ 5°C) corresponding to a maximum degree day accumulation of 1828, 1894, 1771 DD in 2011, 2012, and 2013, respectively. An increase in the number of beetles emerging after 1600 DD in each year suggest that cool temperatures interspersed with days of relatively warm day-time temperatures (≥ 10 – 12°C) stimulate an increase in the rate of brood adult emergence that

continues until average daily temperatures fall below 5°C. The probability of pre-winter emergence by brood adults from the first set of brood trees was 55.5, 84.5, and 44.6% in 2011, 2012, and 2013, respectively.

Emergence from the second set of brood trees. Emergence of brood adults from the second set of brood trees of began at 1612, 1288, and 1582 DD for 2011, 2012, and 2013, respectively (Figs. 4.6H, 4.7H, & 4.8H). As with brood adults from the first broods of each year, emergence of brood adults from the second sets of brood continued for as long as degree days above 5°C continued to accumulate and was punctuated by an increase in emergence late in the year. Emergence terminated at 1828, 1894, and 1771 DD in 2011, 2012, and 2013, respectively. In 2011, 2012, and 2013 the probability of beetle emergence from the second sets of brood trees prior to winter was 13.3, 58.2, and 34.6%, respectively.

Emergence from the third set of brood trees (2012 only). Adults of the third brood started to emerge on 1785 DD and increased the rate of emergence until 1873 DD (Fig. 4.7H). A significant drop in mean air temperature from 10.6 ± 2.0°C to 1.3 ± 0.5°C severely reduced brood adult emergence late in the season. However, a return of mean air temperatures to 6.8 ± 0.9°C resulted in a peak of emergence at 1888 DD which then declined but continued until the degree day accumulation for the year reached the maximum of 1894 DD. The probability of pre-winter emergence for this brood was 36.2%.

4.5 Conclusions

This is the first study to comprehensively record the biology of the eastern larch beetle in the Great Lakes region of North America. We have highlighted several new discoveries, such as the number of degree days required to produce a generation in the field (655 DD, using base 7.5°C), and the successful development to adult life-stages of three separate brood cohorts in one year. While the results of some studies indicate a questionable contribution of subsequent brood cohorts to the overall population dynamics of eastern larch beetles (Langor and Raske 1987a), other data indicate that such brood cohorts likely contribute substantially to the number of

reproductive adult beetles available the following spring (Swaine 1911, Simpson 1929 McKee & Aukema, manuscript in prep.).

There exist several future areas of research for this insect that continues to decimate the tamarack resource along the southwestern margin of its range. Little remains known about the insect's chemical ecology (*e.g.*, Baker *et al*. 1977, Werner *et al*. 1981, Prendergast 1991, Werner 1995), interactions between eastern larch beetle and its natural enemies (Langor and Raske 1988b, Langor 1991), and with its symbiotic microorganisms (*e.g.*, Jacobs *et al*. 1997). Continued research on the eastern larch beetle is warranted given the tamarack mortality that the beetle has already caused in the Great Lakes region and that beetle populations and associated tamarack mortality are increasing in other areas of the beetle's range in North America.

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4.7 Figures

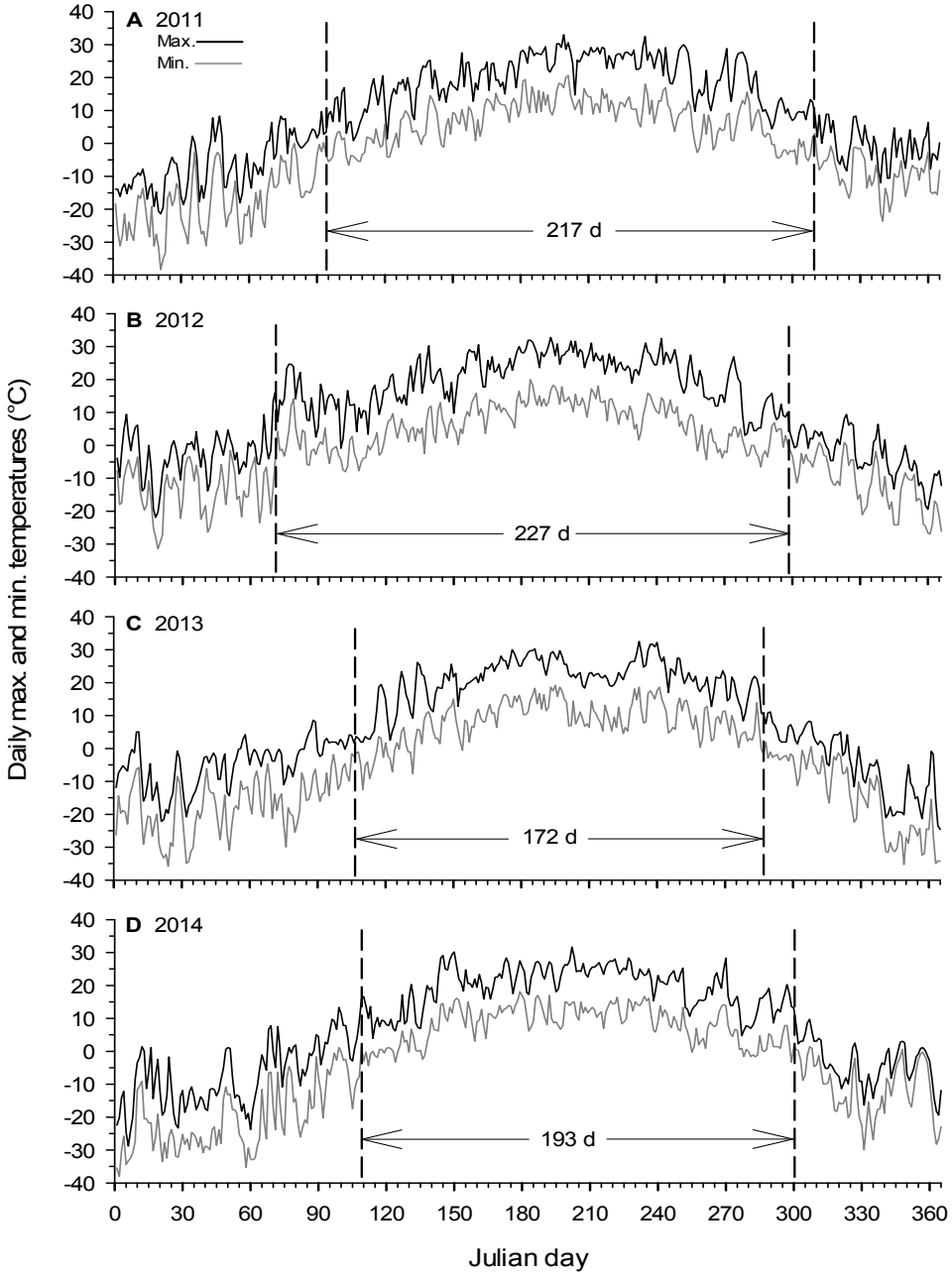

Figure 4.1 Daily maximum and minimum air temperatures for the eastern larch beetle phenology study area, Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A., Accumulated degree days (5°C base) were 1832, 1895, 1771, and 1690 DD for 2011, 2012, 2013, and 2014, respectively. The vertical lines represent the period of degree day accumulation where daily mean temperature exceeded 5°C based on the average of the daily maximum and minimum temperature.

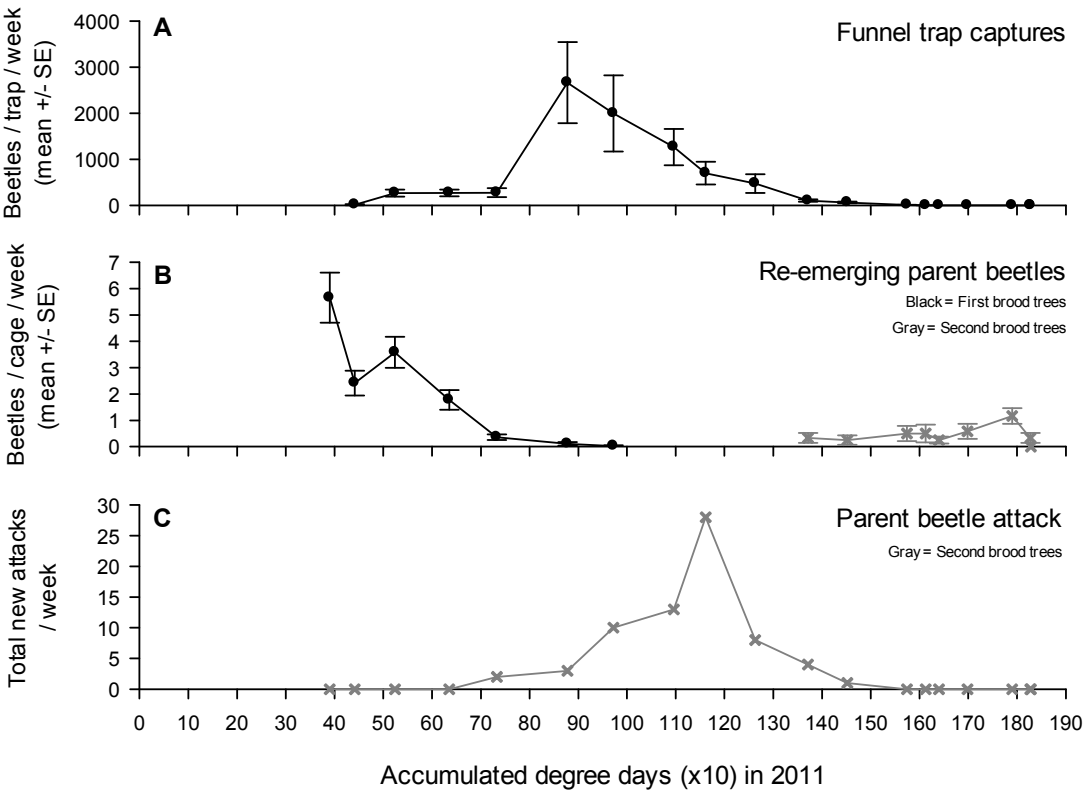

Figure 4.2 (A) Captures of parent eastern larch beetles in funnel traps, (B) Re-emergence of parent beetles from tamaracks colonized for each brood, and (C) Attacks by parent beetles on tamaracks for the second brood, relative to the accumulated degree days (5°C base) from 17 June to 22 Oct., 2011 (first and last data point, respectively). This study was conducted in the Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A.

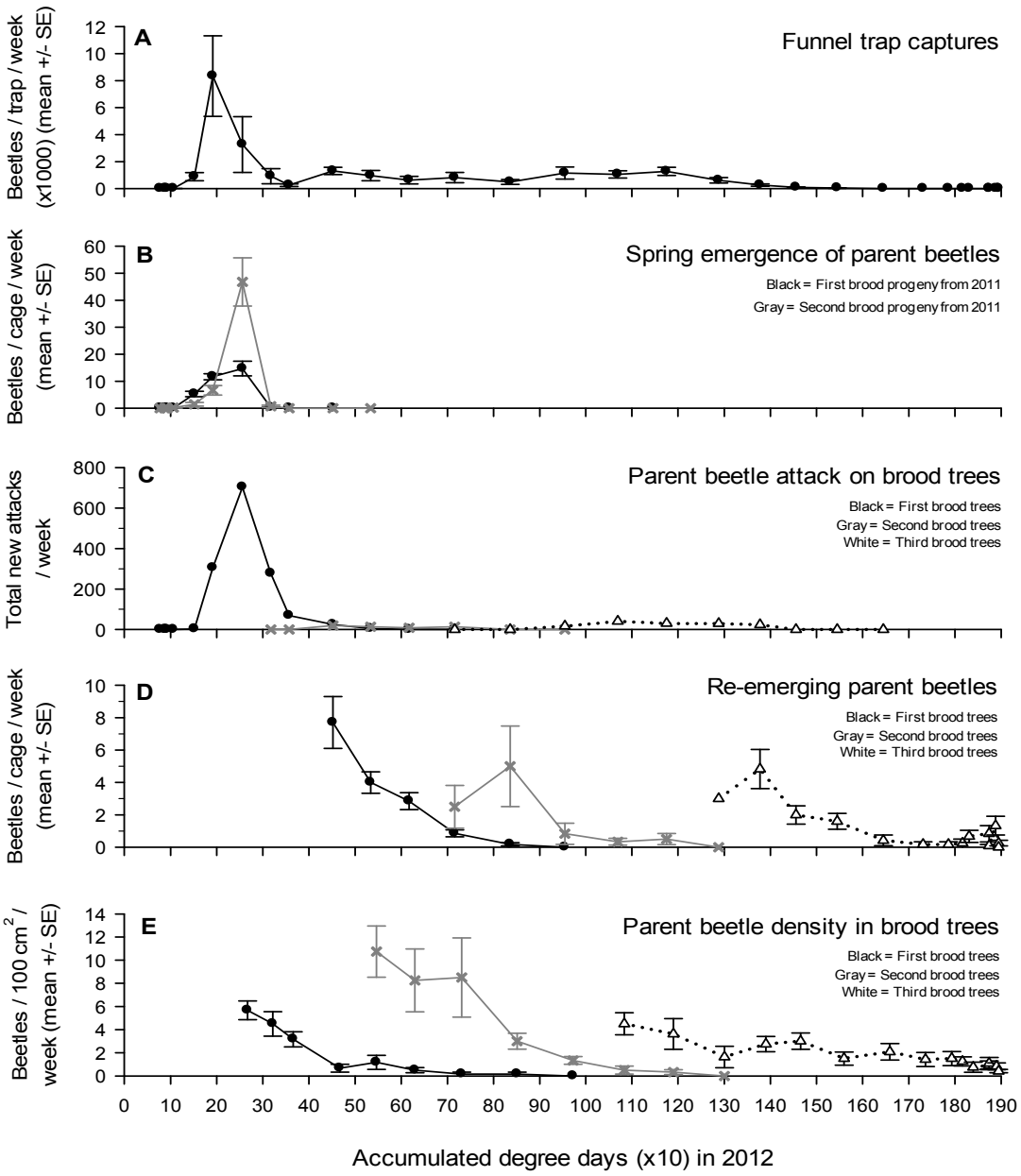

Figure 4.3 (A) Captures of parent eastern larch beetles in funnel traps, (B) Spring emergence of parent beetles from tamaracks killed the previous year, (C) Attacks by parent beetles on tamaracks for each brood, (D) Re-emergence of parent beetles from attacked tamaracks, and (E) Parent beetle density in tamaracks attacked for each brood, all relative to accumulated degree days (5°C base) from 30 Mar. to 2 Nov., 2012 (first and last data point, respectively). This study was conducted in the Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A.

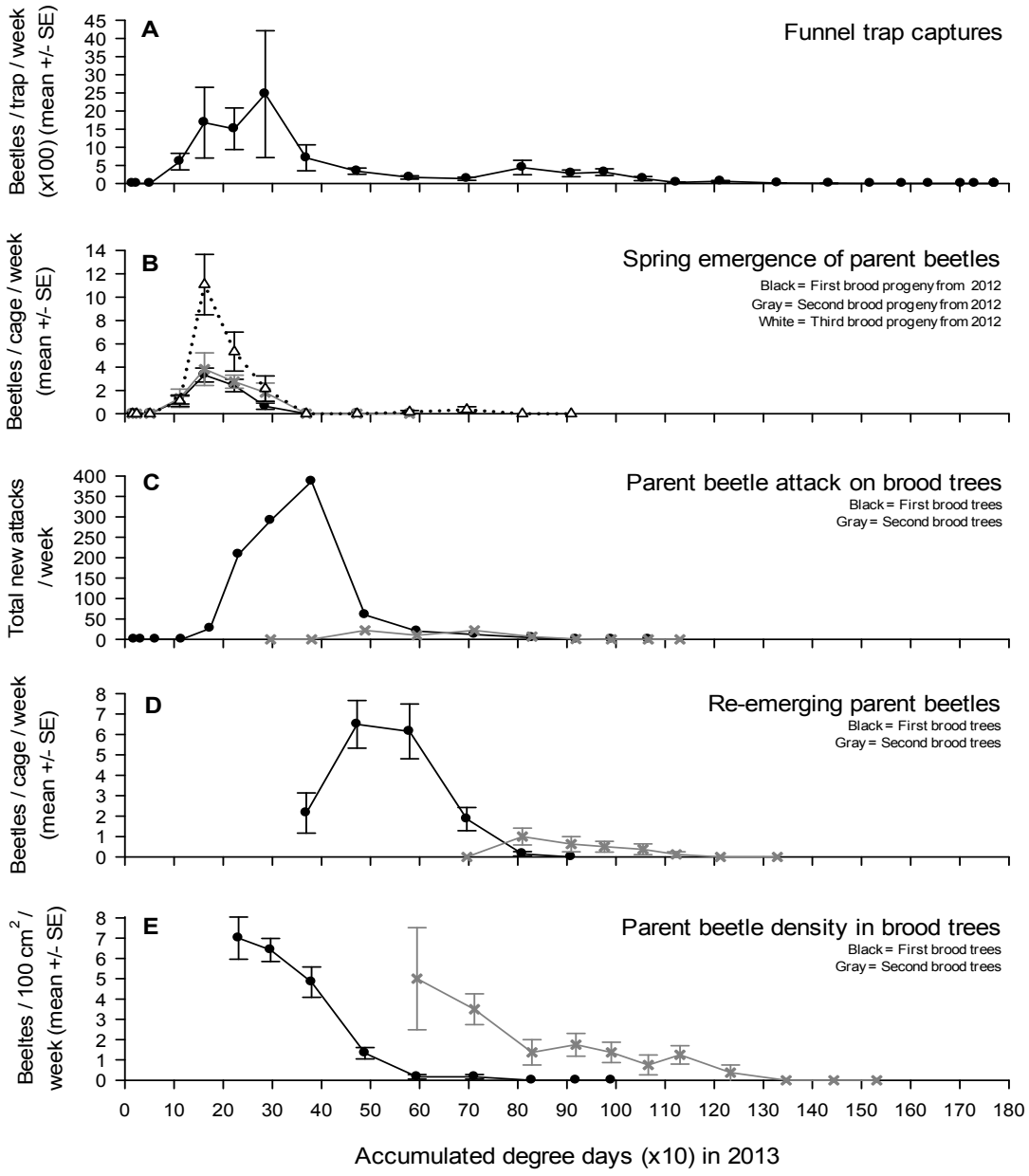

Figure 4.4 (A) Captures of parent eastern larch beetles in funnel traps, (B) Spring emergence of parent beetles from tamaracks killed the previous year, (C) New attacks by parent beetles on tamaracks for each brood, (D) Re-emergence of parent beetles from attacked tamaracks, and (E) Parent beetle density in tamaracks attacked for each brood, all relative to accumulated degree days (5°C base) from 29 Apr. to 28 Oct., 2013 (first and last data point, respectively). This study was conducted in the Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A.

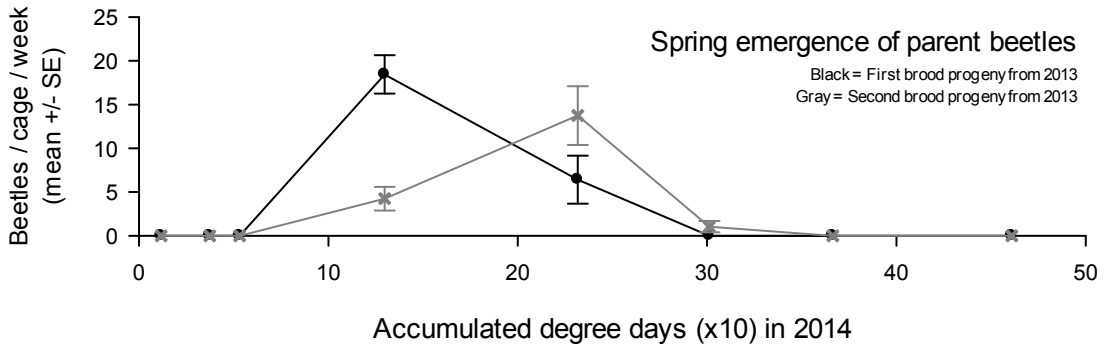

Figure 4.5 Spring emergence of parent beetles from tamaracks attacked the previous year relative to the accumulated degree days (5°C base) from 5 May to 23 June, 2014 (first and last data point, respectively). This study was conducted in the Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A.

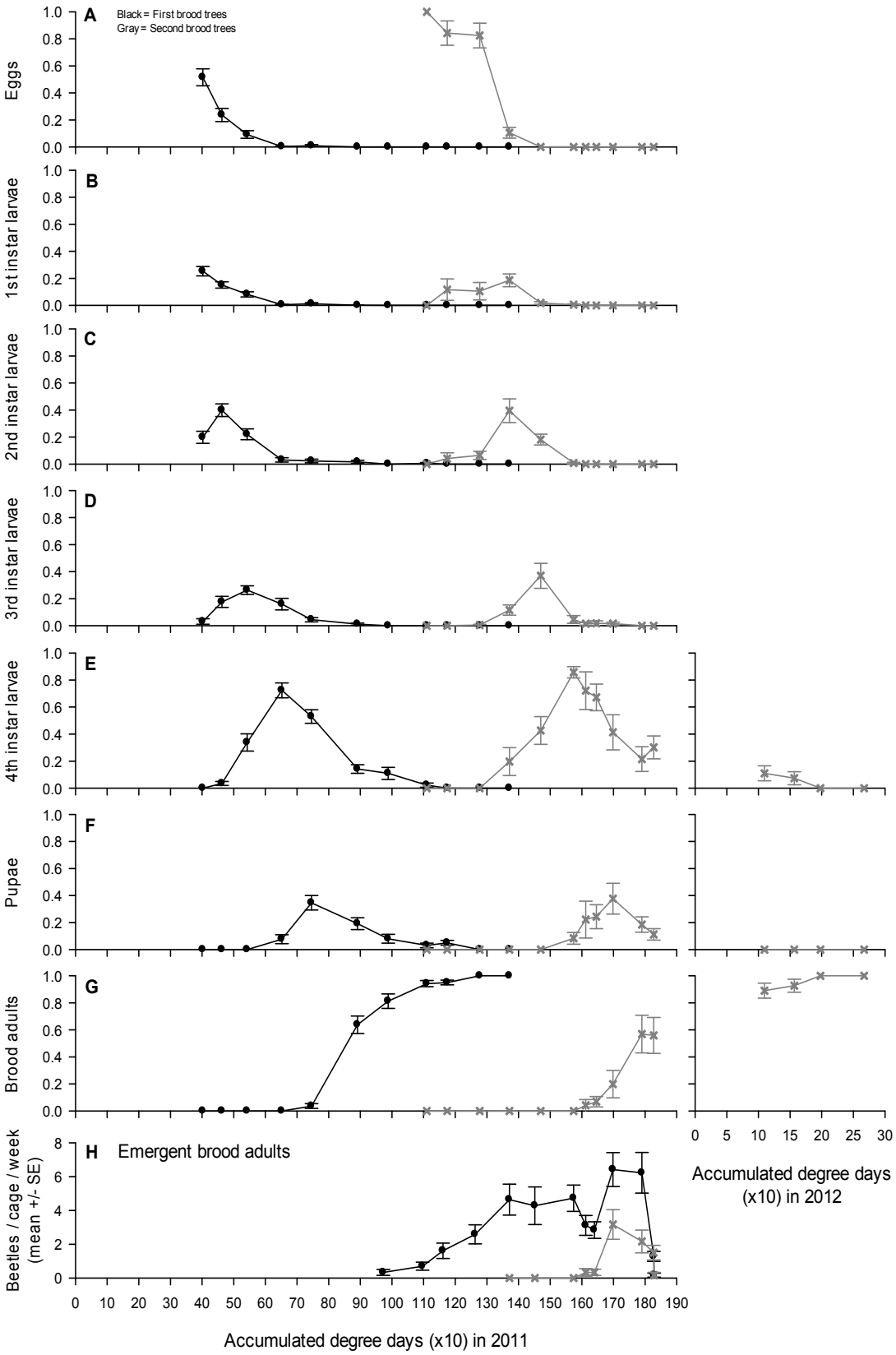
A
Black = First brood trees
Gray = Second brood trees
Eggs
B
1st instar larvae
C
2nd instar larvae
D
3rd instar larvae
E
4th instar larvae
F
Pupae
G
Brood adults
H
Emergent brood adults
Beetles / cage / week
(mean +/- SE)
Accumulated degree days (x10) in 2011
Accumulated degree days
(x10) in 2012

Figure 4.6 (*Continued from previous page*) (A – G) Proportion (mean ± SE) of eastern larch beetle life-stages per sample per week within each tamarack colonized for each brood, and (H) Number (mean ± SE) of emergent brood adults per cage per week on each tamarack colonized for each brood, relative to accumulated degree days (5°C base) from 18 June to 22 Oct., 2011 and from 28 Apr. to 25 May, 2012 (where applicable). This study was conducted in the Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A.

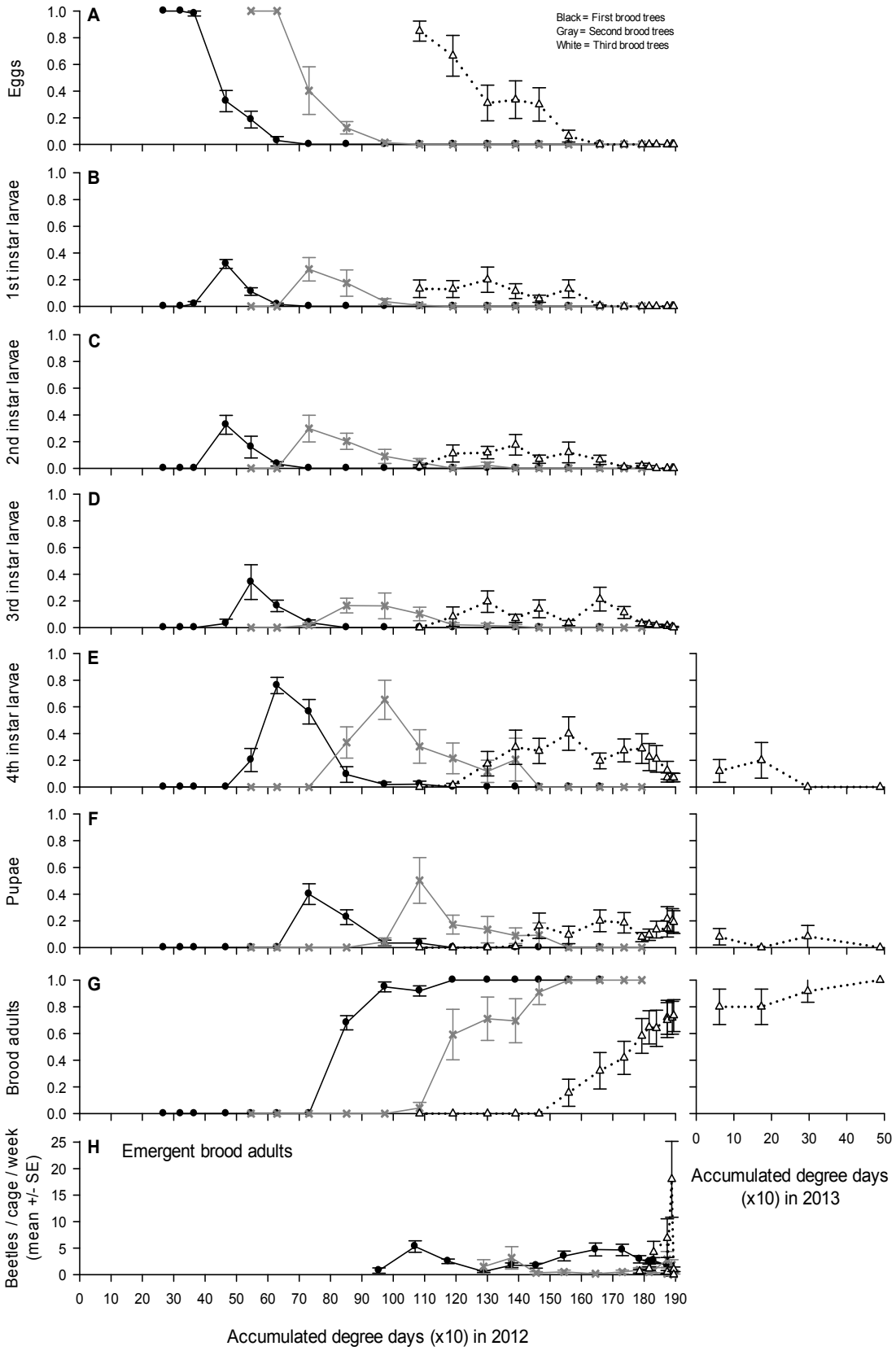
A
Eggs
Black = First brood trees
Gray = Second brood trees
White = Third brood trees
B
1st instar larvae
C
2nd instar larvae
D
3rd instar larvae
E
4th instar larvae
F
Pupae
G
Brood adults
H
Emergent brood adults
Beetles / cage / week (mean +/- SE)
Accumulated degree days (x10) in 2012
Accumulated degree days (x10) in 2013

Figure 4.7 (*Continued from previous page*) (A – G) Proportion (mean ± SE) of eastern larch beetle life-stages per sample per week within each tamarack colonized for each brood, and (H) Number (mean ± SE) of emergent brood adults per cage per week on each tamarack colonized for each brood, relative to accumulated degree days (5°C base) from 19 May to 2 Nov., 2012 and from 14 May to 25 June, 2013 (where applicable). This study was conducted in the Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A.

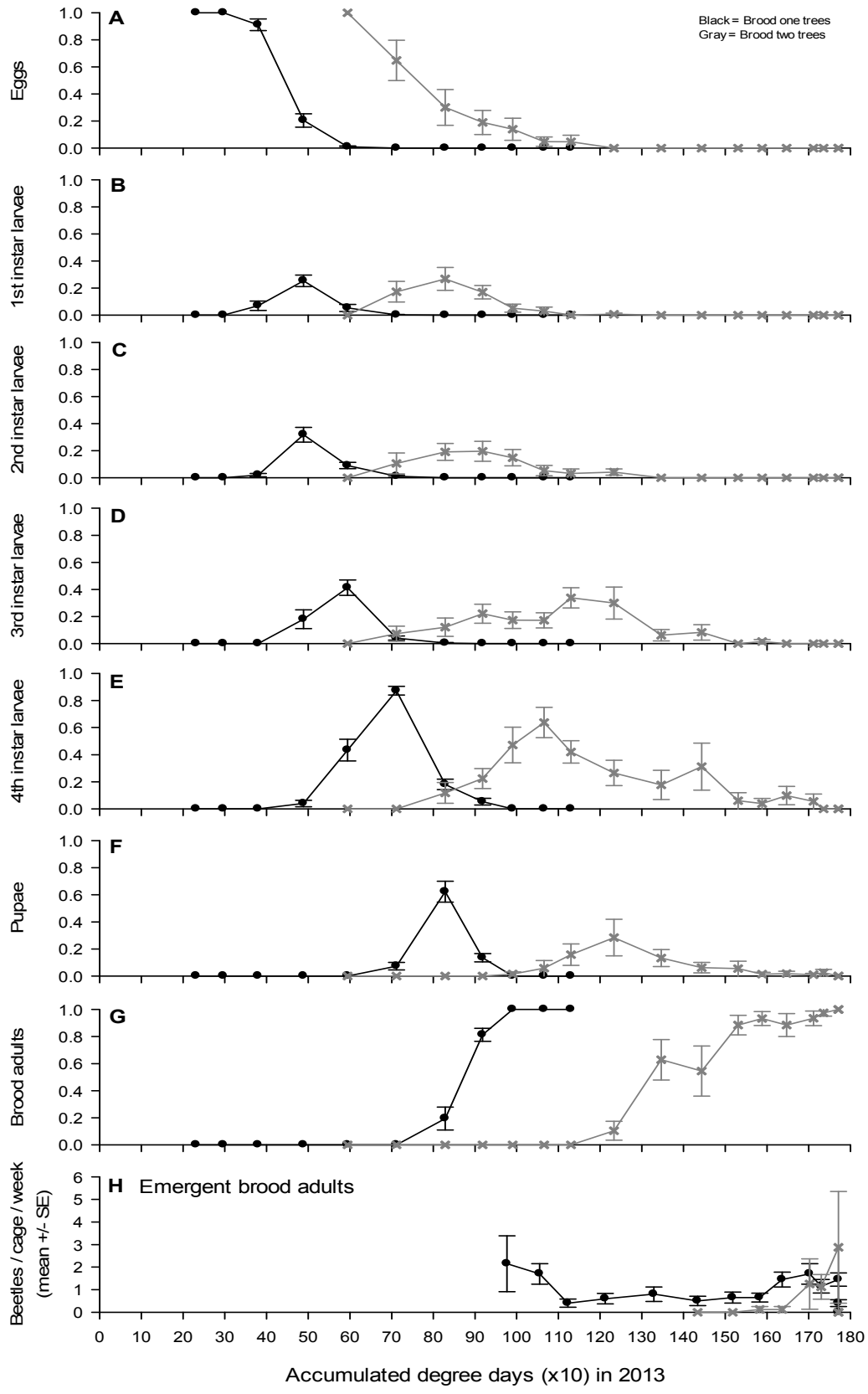
A
Black = Brood one trees
Gray = Brood two trees
Eggs
B
1st instar larvae
C
2nd instar larvae
D
3rd instar larvae
E
4th instar larvae
F
Pupae
G
Brood adults
H Emergent brood adults
Beetles / cage / week (mean +/- SE)
Accumulated degree days (x10) in 2013

Figure 4.8 (*Continued from previous page*) (A – G) Proportion (mean ± SE) of eastern larch beetle life-stages per sample per week within each tamarack colonized for each brood, and (H) Number (mean ± SE) of emergent brood adults per cage per week on each tamarack colonized for each brood, relative to accumulated degree days (5°C base) from 4 June to 28 Oct., 2013. This study was conducted in the Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A.

4.8 Supplemental Methods

This supplement contains greater detail about the methods employed to study seasonal phenology of eastern larch beetle, (*Dendroctonus. simplex*), from 2011 – 2014. The subheadings match the subheading sections of the manuscript proper.

4.8.1 Study site location

Three sites, *PS1*, *PS2*, and *PS3*, were located on the Pitt Grade Forest Road in the Beltrami Island State Forest (UTMs: 15U 0370411 / 5384452, 15U 0370374 / 5382114, and 15U 0370509 / 5390453, respectively). A fourth site, *HS1*, was located on the Hogsback-O'Brien Forest Road (UTM: 15U 0349789 / 5382180). All sites were used to study flight patterns of insects (see below) but only *PS1*, *PS2*, and *PS3* were used to study insect development in trees.

At the time of project initiation and site selection in spring of 2011, adult eastern larch beetles had recently completed spring emergence. Ten, eight, and ten tamaracks at *PS1*, *PS2*, and *PS3*, respectively, had been recently attacked by spring emergent adult beetles and that contained the first larval brood of 2011. The presence of eggs and adult beetles within the galleries suggested that beetle attack on the trees had begun approximately 14 – 18 d previously. The mean ± SE [range] diameter at breast height (dbh = 1.4 m) of the infested trees were 21.1 ± 0.7, [18.7 – 25.9]; 20.7 ± 1.3, [15.4 – 25.9]; and 21.0 ± 0.5, [19.3 – 23.8] cm at *PS1*, *PS2*, and *PS3*, respectively.

4.8.2 Characterization of flight periods

Funnel traps were placed 200 – 600 m from areas where studies of insects in individual trees were occurring. Funnel traps were placed in the field on 15 June 2011, 24 Mar. 2012, and 23 Apr. 2013. Collections occurred every seven days throughout the study seasons, with slight variations due to site inaccessibility from flooding in 2012.

4.8.3 Spring emergence of adult beetles

On 24 Mar. 2012, tamaracks containing the first and second brood groups of 2011 (*n* = nine and six trees, respectively) were caged to record the spring emergence of adult beetles. Similarly, on 22 Apr. 2013, tamaracks containing the first, second, and third brood groups of 2012 (*n* = 17, three, and six trees, respectively) were caged to record the spring emergence of adult beetles. Finally, on 28 Apr. 2014, tamaracks containing the first and second brood groups of 2013 (*n* = 10 and four trees, respectively) were caged to record adult beetle spring emergence. To avoid temporal bias in the spring emergence data, caged tamaracks were selected from among the trees colonized during each week of the eastern larch beetle mass-attack periods pertaining to each brood within each study year.

The cages placed at 0.4 and 1.8 m on tamaracks in the spring of 2012 were re-installed over areas caged in 2011 (*i.e.*, the year of attack and tree death) to record the re-emergence of adult beetles (see below section) and pre-winter emergence of the adult progeny. In the springs of 2013 and 2014, four cages were again installed on tamaracks killed the previous year, as per above, however only the cages at 1.8 m had previously sampled re-emergent adult beetles and pre-winter emergent adult progeny because analysis of these beetle groups did not indicate a sampling advantage of using cages at 0.4 and 1.8 m *vs.* cages only at 1.8 m. Thus, traps were not installed at 0.4 m on newly attacked tamaracks during the 2012 and 2013 seasons to sample these latter two groups of beetles (Fig. S4.1) (see Supplemental Results).

To calculate the degree days associated with the spring emergence E50 for the adult beetles from each caged tamarack of each brood for each study year, the number of emergent beetles captured in all cages was pooled for each collection date for each tamarack. Using pooled emergence data from both collections per week, a dataset of cumulative beetle emergence was generated for each caged tamarack. A generalized linear model was used analyze the number emerged beetles *vs.* the number of beetles left to emerge from each tamarack as a function of degree day accumulation. The logit link function (e^y / (1 + e^y)) for the model was solved for the value of y (*i.e.*, 0) that reduced the logit link to the fraction 1/2 (*i.e.*, 50%). By substituting $y = 0$ into the linear equation $y = \beta_0 + \beta_1 x$, substituting the coefficients for

the model intercept and degree day terms, and solving for x, we were able to calculate the degree day accumulation associated with 50% beetle emergence for each of the 55 caged tamaracks.

4.8.4 Adult beetle attack on healthy tamaracks

On 10 June 2011, 28, 33, and 42 (*n* = 103 total) green, apparently healthy, non-attacked tamaracks were randomly selected in site *PS1*, *PS2*, and *PS3*, respectively, from the area surrounding the infested tamaracks at each location. The stem diameter of each green tamarack measured at 1.4 m *(i.e.*, diameter at breast height (dbh)) was ≥ 10 cm. The mean ± SE [range] dbh of the green tamaracks at site *PS1*, *PS2*, and *PS3* were 16.5 ± 0.3 [13.1 – 20.6], 17.9 ± 0.5 [11.6 – 24.6], and 16.8 ± 0.7 [10.6 – 29.7] cm, respectively.

During the spring, the tamaracks were monitored for attack by spring-emergent adult beetles. Monitoring of the green tamaracks continued throughout the summer to record attacks made by re-emergent adult beetles exiting fully colonized host trees (*e.g.*, trees colonized for the first brood) and attempting to establish subsequent broods (see below). In 2011, it was not possible to record beetle attack on the first set of brood trees resulting from spring-emergent adult beetles since spring emergence was complete when the study began. However, such attack was recorded in 2012 and 2013. Monitoring the green tamaracks for attack by re-emergent adult beetles occurred in all years.

Eastern larch beetle attack dynamics were recorded on tamaracks attacked for the second brood in 2011 (*n* = 15 trees; 8 killed), as well as on tamaracks attacked for the first, second, and third broods in 2012 (*n* = 68, three, and seven trees, respectively; all killed). Due to the high rate of beetle-caused mortality to the original group of 103 green tamaracks selected for monitoring, only 17 small-diameter (dbh = 13.4 ± 0.5 cm) tamaracks remained alive at the end of the 2012 season. Concerns were raised that during the upcoming 2013 season eastern larch beetles may either avoid attacking the remaining small-diameter tamaracks, or, that the beetles may exhaust the remaining supply of green tamaracks prior to the end of beetle attack activity, resulting in lost or incomplete data of the beetle attack periods during the entire 2013 season. Therefore, a second group of healthy tamaracks was selected in the fall of 2012 after beetle

activity ceased in which to also observe the beetle attack periods of 2013. In total, 100 additional tamaracks (dbh ≥ 10 cm) were tagged from areas immediately adjacent to sites *PS2* and *PS3* (*n* = 50 from each location). Additional tamaracks could not be selected at site *PS1* due to extreme tamarack mortality in that area. In 2013, eastern larch beetle attacks were recorded on 44 tamaracks for the first brood (including six of the 17 tamaracks remaining from the group of 103 selected in 2011) as well as five tamaracks attacked for the second brood (all tamaracks from the group of 100 selected in 2012). For purposes of this work, we do not include abandoned attacks (*i.e.*, beetles boring through the outer bark to the outermost layer of phloem before abandonment).

4.8.5 Preventing wood-pecker damage to study trees during the over-wintering period

All tamaracks that were selected to study specific aspects of eastern larch beetle biology (*i.e.*, adult beetle re-emergence, pre-winter emergence of beetle progeny, spring emergence of adult beetles, and larval development – see below) were screened with poultry wire at the end of each field season. This prevented extensive bark damage to the study trees by foraging black-backed *Picoides arcticus*, three-toed *Picoides dorsalis*, and hairy *Picoides villosus* woodpeckers during the winter months. The poultry wire was formed into a cylinder that extended 10 cm beyond the bole surface, preventing the woodpeckers from reaching the bark. The wire cylinders protected the study trees from ground level to a height of 3 m. Protection of study trees from woodpeckers during the field season was not required since the woodpeckers avoided the lower bole of the study trees, possibly due to the presence of the beetle emergence cages affixed to the bole, or, because of the extensive removal of bark by the investigators during larval sampling. The poultry wire was removed from the study trees in the spring prior to beetle emergence so that cages could be re-attached.

4.8.6 Adult beetle re-emergence from colonized tamaracks

Successfully colonized tamaracks were caged to record adult beetle re-emergence from fully colonized host trees of each brood group. Again, to avoid temporal bias in the re-emergence

data, caged tamaracks were selected from among trees colonized during each week of the mass-attack period specific to each brood group. The cages used to capture re-emergent adult beetles were identical to those described previously for capturing spring-emergent adult beetles. In 2011, tamaracks containing either the first and second broods were caged (*n* = nine and six trees, respectively). In 2012, cages were placed on tamaracks containing the first, second, and third brood groups (*n* = 17, three, and six trees, respectively). In 2013, tamaracks were caged that contained the first and second broods (*n* = 10 and four trees, respectively).

4.8.7 Recording larval development

Larval development was recorded from tamaracks colonized for the first and second broods of 2011 (*n* = six and three trees, respectively), the first, second, and third broods of 2012 (*n* = three, three, and six trees, respectively), and the first and second broods of 2013 (*n* = six and four trees, respectively). Tamaracks used for larval sampling were selected from among trees that were colonized during each week of the mass-attack period for each larval brood to avoid temporal bias in the development data.

To record larval development, two 10 x 10 cm bark samples (with phloem attached) were removed from selected tamaracks using a utility knife and chisel. Sampling occurred between 0.5 and 2.5 m on the tree bole. In 2011, bark samples were removed from the north and south aspects of the tree bole but sampling was changed to the east and west bole aspects in 2012 and 2013 to minimize interference with the cages used to capture re-emergent adult beetles and emergent beetle progeny. During the initial stages of beetle colonization when beetle attack densities are relative low, bark samples were removed from overtop of beetle entry points to increase the probability of sampling an active parental gallery rather than non-colonized phloem. A cloth apron pinned to the bark beneath the sample area and to the sampler caught any developing offspring that fell from the bark samples as they were removed from a tree.

In the field, eggs clinging to the sapwood were counted and all other visible and easily accessible life-stages were removed from each sample and placed in a vial with 95% ethanol. The same day, a Leica MZ6 dissection microscope was used to examine the frass-packed

parental galleries and surrounding phloem in each bark sample for eggs (*i.e.*, the frass was teased apart and removed with a blunt probe). Any larvae in the sample not observed in the field were collected at this time as well and added to the appropriate sample vial. Only life-stages that were alive on the day of sampling were included in the larval collection.

Late in 2012 before the poultry fencing was installed, two of the six tamaracks being sampled for the development of the third brood sustained woodpecker damage that reduced the amount of bark area available for sampling. Thus, the sample period of this brood cohort in the spring of 2013 had to be extended to once every 14 d.

4.8.8 Designating sampled larvae to an instar class

Independent datasets of larval head capsule widths were generated for each brood group using randomly sub-sampled larvae from each of the two broods of 2011 (n = 654 and 500 larvae, respectively), and the first, second, and third broods of 2012 (n = 314, 319, and 693 larvae, respectively). We opted not to use the published reports of larval sizes (*i.e.*, head capsule widths) for eastern larch beetles because the published reports disagree somewhat regarding the size of late-instars, and do not account for potential differences in larval size due to brood group and study year (Prebble 1933, Langor and Raske 1987b).

First, the head capsule widths of all larvae subsampled from each brood were measured to the nearest 0.001 mm using a Leica MZ6 microscope with real-time camera and digital micrometer. Then, the raw head capsule width data for each brood cohort was analyzed separately using mixture distribution modeling in R. This procedure separated the head capsule width data into four statistically-probable size classes – one class for each instar. Using the statistical output specific to each brood group in each year, the sub-sampled larvae from each brood were assigned to an instar class. Because brood group and study year did not influence larval size, the data for the larvae from all brood groups (n = 2480 larvae) were pooled and re-analyzed to provide an overall estimate of larval head capsule widths (Table S4.1). All larvae collected in 2013 were assigned to an instar class using Table S4.1.

4.8.9 Pre-winter emergence of beetle progeny

A generalized linear mixed effects model was used to determine if cage placement influenced the sex of emergent adult progeny of each brood cohort and study year. A term for site was incorporated as a random effect.

4.8.10 Calculations of degree day accumulation

In calculating the average degree days required for an entire brood group to completely develop from eggs to the pre-emergent adult life-stage (*i.e.*, still within the bark), each attacked tamarack from all broods that was sampled for larval development was included (n = 29 tamaracks). In calculating, the average degree days that elapse between the establishment of a brood group as eggs to the onset of pre-winter emergence by adult progeny, each tamarack that was attacked by eastern larch beetles and subsequently caged to record progeny emergence was included (n = 55 tamaracks).

4.9 Supplemental Results

4.9.1 The influence of cage placement on the number and sex of beetles captured from colonized tamaracks

There was no effect of cage aspect, height (when applicable), or an aspect*height interaction on the sex of spring-emergent adult beetles from any brood group (*i.e.*, first, second, or third, as applicable) in the spring of 2012, 2013, or 2014 (P > 0.05, in all cases, results not shown). Similarly, no effect of cage aspect, height (when applicable), or an aspect*height interaction (when applicable) existed on the sex of adult beetles that re-emerged from tamaracks colonized for any brood group (*i.e.*, first, second, or third, as applicable) in 2011, 2012, or 2013 (P > 0.05, in all cases, results not shown). With two exceptions, there was no effect of cage aspect, height (when applicable), or an aspect*height interaction (when applicable) on the sex of pre-winter emergence of adult progeny of any brood group (*i.e.*, first, second, or third, as applicable) in 2011, 2012, or 2013 (P > 0.05, in all but two cases, insignificant results not shown, significant

results presented below). Adult progeny from the second brood of 2011 had a significantly greater probability of female beetle emergence on the north *vs.* south bole aspect of host trees (59.2 and 37.5%, respectively) (GLMEM, Z = -2.12, P = 0.034). Similarly, the third brood of 2012 had a 58.4 *vs.* 48.3% probability of female beetle emergence on the north *vs.* south bole aspects of host trees (GLMEM, Z = -2.01, P = 0.044).

4.10 Supplemental Tables

Table S4.1. Head capsule widths of the four instars of eastern larch beetle. Larvae were sampled from six and three tamaracks containing the first and second broods of 2011, respectively, and from three, three, and six tamaracks containing the first, second, and third broods of 2012, respectively. Infested tamaracks were located in the Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A.

Instar	Larval head capsule width (mm)		*n*
	Mean ± SE	Range	
First	0.47 ± 0.0019	0.41 – 0.52	386
Second	0.60 ± 0.0026	0.53 – 0.68	429
Third	0.79 ± 0.0035	0.69 – 0.89	403
Fourth	1.02 ± 0.0021	0.90 – 1.15	1262
			2480

4.11 Supplemental Figures

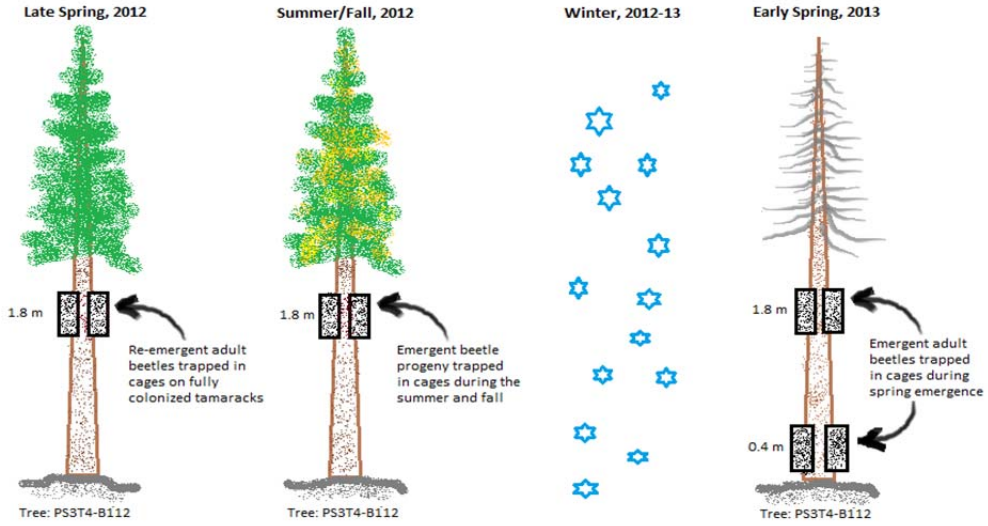

Figure S4.1. An illustration demonstrating the methods for cage placement, 2011 – 2013 on the north and south bole aspects of tamaracks colonized by the eastern larch beetle during this study (note that a tamarack from the 2012 study year is shown). In 2011, emergence cages were also installed at the base of the trees (0.4 m) throughout the season. The cages were used to capture and record (from left to right) the re-emergence of adult beetles from fully colonized host trees, pre-winter emergence of adult progeny, and the spring emergence of adult beetles. Cages were removed from tamaracks during the winter months and reinstalled early the following spring. Boles were protected with chicken wire during the winter.

Chapter 5.

Evidence for a shift in voltinism by the eastern larch beetle, *Dendroctonus simplex* LeConte, (Coleoptera: Scolytinae) during a sustained outbreak in the Great Lakes region of North America.

5.1 Summary

The eastern larch beetle (*Dendroctonus simplex* LeConte) (Coleoptera: Scolytinae) is distributed throughout the North American boreal forest wherever its primary host the eastern larch (tamarack) (*Larix laricina* (Du Roi) K. Koch) is found. Eastern larch beetles prefer to attack recently dead or stressed tamaracks leading to localized short-lived infestations. Rare, landscape-level outbreaks of eastern larch beetles have occurred following widespread stressing events that have predisposed tamaracks to beetle colonization. Since 2000, an on-going outbreak of eastern larch beetles has caused extensive tamarack mortality throughout the Great Lakes region of North America, including over 86,500 hectares of mature tamarack forests in Minnesota. The current outbreak is not associated with any known biotic predisposing agents weakening host trees, placing enhanced scrutiny on the insect. Previous studies have suggested that eastern larch beetles exhibit a single, reproductive generation composed of spring-emergent adult beetles that establish from one to three “sibling” or “sister” brood groups per year, constrained to univoltinism by an obligate reproductive diapause that is terminated by an over-wintering period. However, recent laboratory studies have discovered that a portion of the population may reproduce without over-wintering, suggesting that a second generation of beetles under natural conditions may be possible. In the present study, we present data of beetle phenology, physiology, and physical characteristics indicating that eastern larch beetles successfully established a second generation under field conditions during the present outbreak in Minnesota in 2012. This is the first report of eastern larch beetles achieving a second generation of offspring under natural conditions. Successful production of a second generation of beetles in some years may partly account for the prolonged nature of the outbreak in the Great

Lakes region. These data have important implications to further tamarack mortality along the southern margin of its range in the face of a changing climate.

5.2 Introduction

In recent decades, a changing climate has been associated with an increased frequency and severity of forest insect outbreaks in North America and Europe (Logan *et al*. 2003, Berg *et al*. 2006, Raffa *et al*. 2008, Weed *et al*. 2015). As ectothermic organisms, forest insects are tightly regulated by their thermal environment. Temperature directly affects reproduction (Amman 1972b, Wagner *et al.* 1981, Régnière *et al.* 2012), development (Wagner *et al*. 1984, Ratte 1985, Dalin 2011, Régnière *et al*. 2012, Chapter 2), survivorship (Bale *et al*. 2002, Robinet and Roques 2010, Amarasekare and Savage 2012), dispersal (Taylor 1963, Kammer and Bernd 1978, Fahrner *et al*. 2015), and phenology (Jenkins *et al*. 2001, Bale *et al*. 2002, Altermatt 2010), and distribution (Netherer and Schopf 2009, Sambaraju *et al*. 2012). Moreover, climate can indirectly affect forest insects through interactions with the host tree species. For example, drought can limited the efficacy of the oleoresin defense systems of conifers to insect colonization, precipitating outbreaks (Mattson and Haack 1987, Bentz *et al*. 2010, Preisler *et al*. 2012, Hart *et al*. 2014).

The bark beetles (Coleoptera: Scolytinae) comprise a large group of economically and ecologically important insects. While the majority are “non-aggressive” species that exist at low, chronic, endemic levels in moribund trees or downed material, a minority may undergo rapid increases in population density concomitant with a shift in behavior from attacking weakened hosts to attacking healthy, vigorous hosts when conditions permit (Baker 1972, Furniss and Carolin 1977, Wood 1982b, Boone *et al*. 2011). Such “aggressive” species of bark beetles can exert biome-level impacts when at outbreak levels (Raffa *et al*. 2008, Bentz *et al*. 2010), influencing forest structure, composition, dynamics, hydrology, as well as carbon dynamics with implications for global climate change (Kurz *et al*. 2008, Raffa *et al*. 2008, Hicke *et al*. 2012, Maness *et al*. 2013).

Among the bark beetles, the genus *Dendroctonus* arguably contains some of the most important agents of forest disturbance from both economic and ecological perspectives. The destructive abilities of the species within this genus vary greatly, however. For example, the mountain pine beetle (*D. ponderosae* Hopkins), spruce beetle (*D. rufipennis* (Kirby)), southern pine beetle (*D. frontalis* Zimmermann), and western pine beetle (*D. brevicomis* LeConte) are among the most aggressive and destructive species, causing severe forest mortality at landscape scales (Baker 1972, Furniss and Carolin 1977, Schmid and Frye 1977, Wood 1982b, Hansen *et al*. 2001b, Berg *et al*. 2006). A hyper-epidemic of mountain pine beetle, for example, has resulted in a major range expansion into novel geographic regions (de la Giroday *et al*. 2012) (*e.g.*, latitudinal gain), and/or novel habitats within regions (*e.g.*, altitudinal gain) (Logan and Powell 2001, Sambaraju *et al*. 2012) that has allowed the beetle to interact with less frequently encountered traditional hosts (Cudmore *et al*. 2010) as well as novel hosts (Safranyik *et al*. 2010, Adams *et al*. 2013, Erbilgin *et al*. 2014). Alternately, species such as round headed pine beetle (*D. adjunctus* Blandford), black turpentine beetle (*D. terebrans* (Olivier)), and red turpentine beetle (*D. valens* (LeConte)) are non-aggressive and not considered serious agents of forest mortality in their native ranges (Baker 1972, Furniss and Carolin 1977). Semi-aggressive species such as the Jeffrey pine beetle (*D. jeffreyi* Hopkins), and Douglas-fir beetle (*D. pseudotsugae* Hopkins) have the potential to cause considerable forest mortality and economic losses, but population eruptions tend to be short-lived (*i.e.*, up to 5 years) and affect forests at a more regional scale (Hopkins 1909, Atkins and McMullen 1960, Furniss and Carolin 1977, Wood 1982b).

In general, scientific efforts to elucidate the biology of the *Dendroctonus* beetles have been undertaken with respect to the economic importance of a particular species. Thus, for many *Dendroctonus* species, the current biological knowledge is insufficient to predict the potential effects of climate change on their population dynamics and capacity to cause large-scale forest mortality (Bentz *et al*. 2010). One such species is the eastern larch beetle *D. simplex* LeConte.

The eastern larch beetle (*D. simplex* LeConte) is distributed throughout the range of its principle host tree, the eastern larch (tamarack) (*Larix laricina* (Du Roi) K. Koch), from Alaska

throughout the Canadian boreal forest including the Great Lakes region eastward to the Maritime provinces of Canada and the New England states (Wood 1982b, Burns and Honkala 1990). Eastern larch beetles prefer to attack physiologically compromised host material, such as tamaracks stressed from flooding, wind or snow breakage, or insect defoliation. As such, the preferred resource of eastern larch beetles is typically of limited quantity, ephemeral, and scattered across the landscape. Occasionally, when beetles exhaust such resources, localized beetle populations will move to proximate healthy trees. Records of eastern larch beetle activity reveal that successful colonization and mortality of healthy tamaracks is usually of short duration (*i.e.*, ≤ 4 years) before beetle populations return to endemic conditions (Hopkins 1909, Wood 1982b, Langor and Raske 1988a, Langor and Raske 1989b, Langor and Raske 1989a). During the 1970s and 1980s, widespread defoliator activity of larch budmoth *Zeiraphera* spp. Treitschke and eastern spruce budworm *Choristoneura fumiferana* (Clemens) precipitated landscape-scale outbreaks of eastern larch beetle affecting 3.3 million ha in Alaska and killing more than 1.4 million m^3 of tamarack on the east coast of Canada, respectively. These outbreaks prompted forest entomologists to re-consider the eastern larch beetle as semi-aggressive bark beetle and an important potential agent of severe, widespread forest mortality (Langor and Raske 1989a).

Over the last 15 years the eastern larch beetle has again demonstrated its capability as a serious agent of forest mortality, with activity increasing throughout the south-western portions of tamarack's range in the Great Lakes region of North America (ONMNR 2012, MIDNR 2013, Phillips *et al*. 2013, WIDNR 2013, MBCFB 2014). Since 2000, for example, an ongoing outbreak in Minnesota, U.S.A. has resulted in the mortality of 86,500 ha of tamarack, representing 22% of the tamarack resource in the state (J. Albers, Minnesota Department of Natural Resources, pers. comm. 2014). Such sustained mortality is highly unusual for this insect, and comprises the first reported landscape-level outbreak of eastern larch beetles in the Great Lakes region. In stark contrast to past outbreaks in eastern and western North America, no predisposing factors such as defoliator activity have been readily apparent.

Descriptions of the insect's ecology have been derived from studies in eastern and western North America. Eastern larch beetles colonize trees early in the spring, when adult

beetles emerge from natal host trees killed the previous year. The emerging beetles disperse, locate a suitable host tree, and bore into the bark. Like many bark beetles, aggregation pheromones attract additional conspecifics to the focal point of attack, aiding both host colonization and mate procurement (Prendergast 1991). Eggs are laid in niches cut into the sides of parental galleries excavated in the phloem of the host tree. After the first set of eggs are laid, the female and male parent beetles may re-emerge, disperse, and establish a second "sibling" or "sister" brood in additional host trees or downed material. The establishment of a third sibling brood is rare. The larvae mine and develop within the phloem tissues. Progeny of the first brood develop to adults by late summer, with second brood adults appearing two to three weeks later. Some progeny emerge from standing hosts and descend to the base of the tree, re-enter the bark, and construct hibernal galleries in which to overwinter. Other brood adults overwinter directly within the pupal chambers (Swaine 1911, Simpson 1929, Prebble 1933, Werner 1986, Langor and Raske 1987a, b, 1988b). In these previous studies, adult progeny of a given year that emerged prior to winter were never observed to attempt reproduction, despite sufficient time putatively remaining in the year for the establishment of a second generation of beetles. This lack of reproductive effort led to the hypothesis that eastern larch beetles had an obligate reproductive diapause that was terminated by an overwintering period.

Thus, the lifecycle of the eastern larch beetle has been reported as uni-voltine with a single reproductive generation (*i.e.*, spring-emergent adults) and up to three sibling brood cohorts (Simpson 1929, Langor and Raske 1987a, b). Characterizing this insect's voltinism is of great interest, as potential climate-induced changes in seasonal phenology and voltinism can have profound consequences to the success (or failure) of other destructive and economically important *Dendroctonus* bark beetles. For example, warmer temperatures facilitating the shift of the spruce beetle *D. rufipennis* from a semi-voltine to a uni-voltine lifecycle have been associated with an increased severity of spruce beetle outbreaks across western North America (Hansen *et al*. 2001a, Berg *et al*. 2006, Bentz *et al*. 2010, Jenkins *et al*. 2014). Conversely, moderate climate warming may accelerate the development of immature mountain pine beetles *D. ponderosae* to

less cold-tolerant life stages by winter periods, resulting in widespread insect mortality (Bentz and Powell 2014).

Recently, the seasonal phenology of eastern larch beetle was characterized in a four-year field study in the area of highest tamarack mortality in Minnesota (Chapter 4). This area encompasses the southern and warmest regions of the range of tamarack, and can portend future insect-plant interactions for this system farther north as the climate warms. The year 2012 was characterized by an early and unusually warm spring, a hot summer, and a long, warm fall. In total, three broods were established in 2012. Trees for the third brood were attacked precisely at the time that young adult offspring were emerging from tamaracks that contained the first brood, suggesting that the third brood was not an additional sibling brood established by the original parent beetles, but an additional beetle generation established by the newly emergent young adults. More than 75% of the insects in the third brood were able to complete development to adult life stages prior to winter (Chapter 4). Simultaneously, laboratory studies demonstrated that a portion of eastern larch beetle adult progeny are reproductively viable prior to overwintering (Chapter 3), suggesting that some insects are not physiologically constrained to univoltinism. A shift from one generation per year, where parent beetles establish two to three sibling broods, to two generations per year, comprised of sibling broods, in addition to progeny reproducing, could result in a significant increase in the number of insects emerging the following spring. The additional conspecifics procuring hosts *en masse* could contribute to extending an outbreak.

This paper presents additional field-based evidence in support of the hypothesis that a second generation of eastern larch beetles was established in 2012, suggesting that expanded growing seasons can result in fractional- or bi-voltinism of eastern larch beetle in the southern portion of its range. This constitutes the first report of a second generation of eastern larch beetles within a single season under natural field conditions (Hopkins 1909, Swaine 1911, Simpson 1929, Prebble 1933, Dodge 1938, Baker 1972, Wood 1982b, Werner 1986, Langor and Raske 1987a, b). We present data on the color of the beetles that attacked the third set of tamaracks and that were removed from egg galleries within the phloem that indicate the beetles

were young brood adults and not older parents. We present lipid content data from re-emergent beetles from the tamaracks colonized for each of the three broods that suggest differences in beetle origin (*i.e.*, spring-emergent parent beetles vs. fall-emergent brood adults). Finally, we quantify beetle development and spring emergence to determine whether the second generation exhibits fractional- or bi-voltinism, and discuss how the distinction is inconsequential to the enhanced tree-killing ability for this insect given its host procurement behavior.

5.3 Methods

Methods for characterizing flight periods, emergence of overwintering adults, attack on cohorts of tamarack, and development of discrete broods from 2011-2014 are detailed in Chapter 4, but summarized in brief below to maintain chapter independence. Additional methods to test hypotheses of the origin of the third brood as a new generation *vs*. additional sibling brood – including new phenological evidence not included in Chapter 4, as well as physical and physiological traits of the parent beetles of the third brood are also described.

5.3.1 Phenology data

Study sites. Four study sites with active populations of eastern larch beetles were selected in or near the Beltrami Island State Forest near Lake of the Woods, Minnesota, U.S.A. on 10 June 2011 (UTMs: 15U 0370411 / 5384452, 15U 0370374 / 5382114, 15U 0370509 / 5390453, and UTM: 15U 0349789 / 5382180, respectively). Sites consisted of between 8-10 recently colonized tamaracks, as indicated by non-coalesced parental galleries that contained eggs and early-instars, as well as parent adults. At each site, up to 42 additional green, apparently healthy tamaracks (bole diameters ≥ 10 cm at 1.4 m) that surrounded the infested tamaracks were selected at random for future weekly monitoring (n = 103 trees total at study initiation). One site was reserved exclusively for monitoring beetle flight, so no colonized tamaracks were sampled there.

Flight periods. Beetle flight at each site was characterized by weekly (7 d) monitoring of 16-unit Lindgren funnel traps (Lindgren 1983) baited with a blend of 50/50 +/- seudenol and α-

pinene (Contech, Victoria, BC, Canada; Baker *et al*. 1977, Werner *et al*. 1981, Prendergast 1991). Seudenol and *α*-pinene dispensers were replaced as necessary. Twelve funnel traps were deployed 3 weeks prior to spring emergence on 24 Mar. 2012 and maintained until 2 Nov. 2012. Funnel traps were collected weekly. Upon collection, the sample from each funnel trap was placed on ice. Later the same day, the samples were transferred to a – 30°C freezer until the samples could be examined.

Emergence from overwintering locations. On 24 Mar. 2012, screen cages fitted with collection cups were affixed to the bark of tamaracks killed in 2011 to capture adult beetles that had overwintered within these trees. Two cages, each measuring 16.5 x 30 cm (W x H) (area = 495 cm^2) were attached to both the north and south bole aspects centered at 0.4 and 1.8 m above ground level. Nine and six tamaracks were caged, respectively, containing the first and second brood groups of 2011. Cages were emptied twice weekly. The second collection (used for a separate study) occurred 48 h after the first. Data from both collections was tallied and pooled for a single weekly value. Collected beetles were catalogued by emergence date, tree, cage aspect, cage height, and sex.

Recording beetle colonization of healthy tamaracks. The green tamaracks were assessed for beetle colonization every 7 d from 31 Mar. to 2 Nov. 2012. These events would include colorizations by both spring-emergent adult beetles and adult beetles re-emerging from fully colonized tamaracks (*e.g.*, those containing the first brood cohort) to establish a subsequent "sibling" brood cohort. The lowest 2.5 m of bole was inspected for orange frass indicative of beetle colonization. Upon finding such evidence, an "observation window" was installed to record weekly attack progression. Observation windows delineated by a string wrapped around four pins encompassing a 40 x 25 cm (H x W) area were centered on the south bole aspect at 1.6 m above ground level.

Recording the density of attacking beetles. A subset of attacked tamaracks were caged to record beetle attack density and offspring production for a separate study (n = 17, three, and six trees, respectively). Cages were installed as previously described at 1.8 m above ground level when beetle colonization of a tamarack was complete (*i.e.*, no new attacks for two

successive weeks). The number of beetle entrance holes (Fig. 1.2A-D) counted under each cage were pooled and the total number of entrance holes was divided by 9.9 (total caged area = 990 cm^2) to give the density of successful beetle attacks per 100 cm^2 for each tamarack. Eastern larch beetles share entrance holes in host trees (Langor and Raske 1987a). On average, one, two, or three to four conspecific pairs will use the same entrance hole 60, 35, and 5% of the time, respectively (Langor and Raske 1987a). Therefore, to derive the density of attacking beetles per 100 cm^2, the number of attacks per 100 cm^2 on each tree was multiplied by 2.96 per Equation 5.1:

[Equation 5.1]

$$\text{No. beetles per entrance hole} = \Sigma(2(P_N)*O_{\%})$$

Where: 2 = A constant. The number of beetles comprising a beetle pair.

P_N = Number of beetle pairs using an entrance hole on average. Note that three to four beetle pairs was averaged as 3.5

$O_{\%}$ = The percent occurrence of a given number of beetle pairs using the same entrance hole.

Therefore,

$$\text{No. beetles per entrance hole} = (2(1)*0.6) + (2(2)*0.35) + (2(3.5)*0.05)$$
$$= 1.2 + 1.4 + 0.35$$
$$= 2.96$$

Beetle re-emergence from colonized tamaracks. Re-emerging parents were collected from cages twice per week, pooled for a weekly total, and catalogued by emergence date, host tree, trap aspect, and beetle sex. Re-emergent beetles were placed on ice when collected and stored at – 30°C until used for lipid content analysis (see below).

Beetle density within colonized tamaracks. The number of live adult beetles per 100 cm^2 phloem was recorded while sampling trees for brood development beginning with ovipositional activities (see below). This measure was taken every 7 d for all brood cohorts.

Characterizing brood development. Brood development was recorded within a subset of tamaracks colonized for the first, second, and third brood cohorts (*n* = three, three, and six trees, respectively). Sampling began when it was apparent that tree death was likely to occur (*i.e.*, ≥ 10 beetle attacks / 1000 cm^2). A 10 x 10 cm phloem sample was removed from the east and west bole aspect every 7 d. Phloem samples were initially removed over top of an entrance point to ensure that active parental galleries were sampled. As beetle attack densities increased, active beetle entrance points could be sampled using a systematic sampling scheme. Therefore, the phloem of the bole was systematically sampled beginning at 0.5 m and continuing vertically in 10 cm weekly increments up to a height of 2.5 m.. To recover all specimens, eggs and larvae clinging to the sapwood were tallied in the field, immature life-stages falling from the bark sample were captured with an apron and placed in 95% ethanol, and remaining insects in the bark sample were placed in a bag and transported to the laboratory on ice before being counted under a dissecting microscope (Leica MZ6, Leica Microsystems, Wetzlar, Germany) the same day. Only living specimens were collected to reduce temporal error in the data from non-developing (*i.e.*, dead or parasitized) larvae. Sampling continued until all specimens sampled from a brood group consisted of adults for a minimum of two consecutive sampling periods (*e.g.*, first and second broods), or when it became evident that cold temperatures would not permit continued development (*e.g.*, third brood). Sampling of the third brood was resumed in the spring of 2013 (Chapter 4). After sampling was complete, larvae from each brood group were assigned to the appropriate instar using head capsule widths and the methods detailed in Chapter 4.

Pre-winter emergence of beetle progeny. The cages used to capture re-emergent adult beetles were maintained in place to capture emergent beetle progeny (*i.e.*, brood adults) throughout the summer and fall months. Emergent progeny were collected twice per week, pooled for a weekly total, and catalogued by emergence date, host tree, trap aspect, and beetle sex.

5.3.2 Beetle color as a surrogate for age

During sclerotization, eastern larch beetle brood adults progress from white to black as the cuticle becomes increasingly "tanned". During this process, eastern larch beetles pass through a transitory dark brown color phase prior to obtaining the black (body) and maroon (elytra) coloration of the adult beetles. This brown color phase has been used in other bark beetle studies to identify brood adults and separate them from fully sclerotized parent beetles (Harding and Ravn 1985, Hansen and Bentz 2003).

Beetles within the parental galleries of phloem samples removed from the third set of tamaracks were collected after their density was determined. Beetles were collected from between two and five trees depending on the sample date. The beetles were pooled by host tree, catalogued by sample date, placed on ice in the field, and frozen at – 30°C. In the laboratory, samples were assessed for the relative abundance of the "black" versus "brown" individuals. All samples were given a random numeric code, placed in a box, randomized, and then selected individually in a "blind" manner. Beetles from each sample were individually examined in a petri dish using a dissecting microscope (Leica MZ6, Leica Microsystems, Wetzlar, Germany). Beetles were separated into black or brown groups by qualitatively assessing the overall color of the beetles based on the elytra, pronotum, legs, lateral and abdominal sclerites. The relative proportion of beetles of each color was recorded for each sample.

A linear model specifying beetle color as the dependent variable and sample day as the independent variable was fit to test the hypothesis that the composition of beetles colonizing the third set of brood trees was comprised primarily of younger, brown beetles which then declined through time (as opposed to a constant density of older, black beetles that would be consistent with re-emerging parents from the second brood). Proportional data for the prevalence of brown beetles per sample as a function of the sample day post-initiation of beetle attack on the third set of tamaracks was analyzed using regression analysis. The proportional data was transformed ($\text{asin}\sqrt{y}$) to fulfill model assumptions of data homoscedasticity and normality of errors.

5.3.3. Beetle lipid content as a surrogate for age

The lipid content of beetles declines with host colonization activity, such as mating and oviposition (Anderbrant 1988, Hansen and Bentz 2003). As such, if the third brood was a sibling brood instead of a new generation, we would expect lipid contents of re-emerging adults to decline with each successive cohort established throughout the season. A subset of adult beetles re-emerging from colonized tamaracks was analyzed for lipid content. In total, 144, 12, and 17 re-emergent parents from tamaracks attacked for the first, second, and third brood groups, respectively, were analyzed. Beetles were removed from the – 30°C freezer and dried for 24 h at 50°C. Beetle dry mass was measured to 0.01 mg using a Metler-Toledo AX105 Delta range analytical microbalance (Metler-Toledo, Greifensee, Switzerland). Dried beetles were placed in individually, labeled 0.5 mL Eppendorf tubes. Lipids were extracted using a 500 mL Soxhlet extractor with petroleum ether. Each Eppendorf tube was modified to facilitate lipid extraction by drilling a large hole in the cap and by cutting off the tapered portion of the tube. A fine screen that would allow the tube to vent as well as allow the through-flow of petroleum ether was then affixed to the cut end of the tube and the cap. The screen was permanently melded to the tube by slightly melting the plastic of the cut end over a Bunsen burner, laying the screen on a smooth, hard, cool surface, and then pressing the tube onto the mesh so that the hot plastic was forced through the screen and was able to reform before cooling. This process was repeated for the cap. Beetles were divided into three nearly-equal groups for lipid extraction, with 300 mL of petroleum ether per extraction. Each extraction lasted 8 h with the extractor column flushing every hour. Following extraction, beetles were re-dried for 12 h at 50°C and re-weighed for the lean dry mass. Total lipid content (mg) per beetle was calculated as the difference between the dry mass and lean dry mass. Percent lipid content per beetle was calculated as the total lipid content divided by the dry mass.

Lipid data of re-emergent beetles was analyzed using an analysis of variance (ANOVA). Proportion lipid content of the re-emergent beetles was analyzed as a function of the beetle brood group (*i.e.*, first, second, or third). Sample tree nested within study site was included as a random effect. Lipid data for male and female beetles were pooled for statistical analysis. Where significant differences existed, a Tukey's HSD test (α = 0.05) was used to examine means

comparisons. The proportional data was transformed (asin$\sqrt{y}$) to meet model assumptions of homoscedasticity and normality of errors during statistical testing.

5.3.4 Quantifying beetle generation times using degree days

Daily air temperature data (°C) from weather stations at the Baudette International Airport, Baudette, MN, U.S.A. and at the Norris Camp Field Office, Minnesota Department of Natural Resources, Red Lake Wildlife Management Area, MN, U.S.A. were obtained from the NOAA National Climatic Data Center (National Oceanic and Atmospheric Administration, 2014). Study sites were 11.4 – 31.4 and 13.3 – 35.4 km from the Norris Camp and Baudette International Airport stations, respectively. Overall degree day accumulation for the study area was calculated as the mean daily air temperature (minimum + maximum / 2) above a 5°C threshold. The minimum temperature for many adult beetle activities (*e.g.*, spring emergence, host attack, and re-emergence) is 5°C (Langor and Raske 1987a), although the minimum temperature for eastern larch beetle development is 7.5°C (Chapter 2).

To determine the time to produce two putative generations, we calculated the number of days separating the date in the spring of 2012 when 50% of the eggs were laid for the first brood (*i.e.*, the beetles that would develop to establish the third brood (second generation) of 2012) and the date in the spring of 2013 when 50% emergence occurred for adult beetles from the third brood of 2012 (*i.e.*, the putative second generation established by the summer-emergent beetles from the first brood of 2012). Egg deposition by eastern larch beetles occurs within three days of beetles gaining access to the host tissue (FRM pers. obs.) so attack data on the first set of tamaracks was used as a surrogate for the timing of egg deposition and establishment of the first brood of 2012 as follows. Using the phenology data for beetle attack (Chapter 4), the degree days at which 50% of the attacks occurred on each tamarack attacked for the first brood of 2012 was calculated. The mean degree day accumulation of 50% attack density for all trees colonized for the first brood group was then matched the associated 2012 date. The degree days associated with 50% emergence of the third brood of 2012 during the spring of 2013 was determined previously (Chapter 4), and then also matched with the appropriate date. Only the

spring emergence of the third brood of 2012 was used as the second time-point because this brood is the putative second generation that was established by the summer-emergent adults of the first brood of 2012.

5.4 Results

5.4.1 Phenology data

Eastern larch beetles began the main flight at 106 DD and maintained flight activity until 1888 DD. The flight period could be separated into three flight periods. The first flight began in low numbers by 90 DD but became much more pronounced between 151 and 356 DD. The second flight occurred from 451 – 835, DD. Finally, the third flight lasted from 954 – 1888 DD, with the majority of activity being observed between 954 – 1455 DD (Fig. 5.1A).

Three periods of tree colonization by beetles that coincided well with each beetle flight were also observed (Fig. 5.1B). In 2012, adult beetles from the first and second broods of 2011 began emergence in low numbers at 93 and 90 DD, respectively, with mass-emergence beginning by 151 DD and finishing by 317 DD (Chapter 4, Fig. 4.3B). The majority (97.8%) of attacks on the first set of tamaracks, in which the first brood of 2012 was initiated, occurred from 151 – 356 DD. In total, 67 tamaracks were killed for the establishment of the first brood. Data were collected from the cages used to capture the spring-emergent adult beetles until 533 DD and indicated that no additional beetles from either brood emerged after 317 DD to potentially attack tamaracks later in 2012 (Chapter 4, Fig. 4.3B). Furthermore, removal of the bark beneath the emergence cages at 533 DD to measure the ovipositional gallery lengths (for a separate study; Appendix 1) confirmed the absence of additional beetles in the tamaracks attacked and colonized in 2011.

Adult beetles re-emerged from the first set of attacked tamaracks from 451 – 835 DD with 98.9% of re-emergence completed by 715 DD (Fig. 5.1C). Simultaneous with adult re-emergence, adult density within the trees declined steadily through time such that no live adult beetles remained in these host trees to potentially engage in late-season re-emergence and host

attack activities (Fig. 5.1D). Re-emergence of adult beetles from the first set of tamaracks coincided with the onset of beetle attack on the second set of tamaracks (451 – 715 DD) (Fig. 5.1B) and the establishment of the second brood (Fig. 5.1E). Additionally, a second period of beetle flight activity was also recorded from funnel traps throughout the first re-emergence period (Fig. 5.1A). Three tamaracks were killed in the establishment of the second brood.

Adult beetles were captured re-emerging for a second time from the second set of colonized tamaracks beginning at 715 DD and lasting until 1175 DD (Fig. 5.1C), although 74% of beetles re-emerged by 835 DD. Similar to the first period of re-emergence, beetle density data indicates that re-emergence continued until living beetles no longer remained under the bark (Figs. 5.1C&D). Unlike the first re-emergence period, the onset of re-emergence from the second set of brood trees was not associated with a concomitant increase in funnel trap catches (Fig. 5.1A), or with new attacks on the third set of tamaracks (Fig. 5.1B). In fact, the period with the greatest rate of beetle re-emergence (715 – 835 DD) was characterized by a complete lack of new attacks on any of the surrounding, green tamaracks that resulted in a 7 -13 d window that was void of new beetle attacks.

Attack on the third set of tamaracks was observed at 954 DD and continued until 1377 DD (Fig. 5.1B) with seven trees being killed. When these attacks commenced, re-emergence from the second set of tamaracks was nearly complete and restricted to a limited number of beetles (Fig. 5.1C). The pattern and duration of beetle attack on the third set of tamaracks (Fig. 5.1B) closely matched the pattern and initial pulse of pre-winter emergence by the young adult beetles of the first brood (954 – 1390 DD) (Fig. 5.1G). Samples of brood development at 851 DD indicated that young adults of the first brood were present in the host trees (Fig. 5.1F) and the light tan coloration of the young adults indicated that development to this life-stage had occurred perhaps 3 – 4 d previously. In fact, the first collection of young adult beetles from emergence cages took place at 954 DD. Thus, by 954 DD the young adults had matured sufficiently to begin the pre-winter emergence from the pupal chambers. The period of beetle attack on the third set of tamaracks did not continue over the entire duration of pre-winter emergence by the young beetles.

5.4.2 Beetle attack and re-emergence densities by brood tree group

Densities of attacking and re-emerging beetles were recorded to determine if adult beetle survival within each set of brood trees differed. The mean (± SE) density of attacking beetles per 100 cm^2 of caged area was 6.9 ± 0.4, 5.7 ± 0.8, and 7.0 ± 1.2 for the first, second, and third sets of tamaracks, respectively, and did not differ (Fig. 5.2A; ANOVA, $F_{2,20}$ = 1.10, P = 0.35). The overall mean density of attacking beetles was 6.8 ± 0.4 per 100 cm^2. Despite similar initial colonization densities, the number of beetles re-emerging from the different sets of tamaracks was different. The mean (± SE) number of re-emergent beetles per 100 cm^2 of caged bark was greater from the first set of tamaracks (3.5 ± 0.5) than the second (1.4 ± 0.9), but was equal to the third set (2.2 ± 0.2) (ANOVA, $F_{2,20}$ = 6.03, P = 0.0089) (Fig. 5.2B). It is important to note that beetles did not complete re-emergence from the third set of tamaracks prior to winter and a mean (± 0.SE) live beetle density of 0.42 (± 0.15) beetles per 100 cm^2 remaining within the tamaracks at the onset of freezing temperatures (Fig. 5.1D). As such, had these remaining beetles completed re-emergence prior to winter, the mean number of re-emergent beetles per 100 cm^2 would have been more similar as for beetles re-emerging from the first set of tamaracks (Fig. 5.2B). This indicates that beetle survival was the lowest within the second set of tamaracks but equal within the first and third sets of tamaracks. In total, 530, 39, and 117 beetles re-emerged from the first, second, and third set of attacked tamaracks, respectively.

5.4.3 Beetle color suggesting age differences

Mature adult eastern larch beetles have a black body with maroon elytra (Wood 1982b). During fieldwork previous to 954 DD, black and maroon beetles comprised the only type of beetle observed in spring emergence cages, within and re-emerging from the phloem of the first and second sets of attacked tamaracks, and in the pheromone-baited funnel traps. However, at 954 DD the collection cups of the funnel traps contained many beetles with the dark brown coloration (Fig. 5.3A) indicative of young beetles in the latter stages of sclerotization as well as specimens with the black body and maroon elytra of fully sclerotized beetles (Fig. 5.3B). Moreover, from 954

DD onward, both brown and black young adult beetles were observed in the emergence cages on the first set of tamaracks, indicating that both brown and black-bodied individuals of the first brood were in the process of pre-winter emergence. Simultaneous sampling from the first set of tamaracks for larval development indicated that the young adults of the first brood had sclerotized sufficiently for many individuals to possess the black coloration, while many were still dark brown. Positively identifying the black-bodied beetles within the first set of brood trees as being young adults was also possible because the recorded data of parent beetle re-emergence from, and parent beetle density within, the first set of attacked tamaracks (see above) indicated that living parent beetles (also black-bodied) did not exist within these trees by 851 DD at the latest.

All attacked tamaracks were sampled for adult density and brood development beginning the week following the initiation of beetle attack in order to avoid unnecessary injury to the tree that may affect the host defense-beetle colonization dynamics (Chapter 4, Methods). When bark sampling to monitor the development of the third brood began on 21 July (1083 DD), it was immediately apparent that the beetles constructing the parental galleries and ovipositing therein were of two color types. Some beetles exhibited the typical color described for mature adult beetles; *i.e.*, black body with maroon elytra. Others exhibited an atypical mottled chestnut brown colored body and elytra. The proportion of brown to black-bodied beetles sampled from the phloem of each tree in the third set of tamaracks during successive weekly sampling periods declined through time ($F_{1,28}$ = 22.22, P < 0.001) (Fig. 5.4).

5.4.4 Lipid content of re-emerging beetles

The lipid content of the re-emergent adult beetles varied with brood tree group (ANOVA, $F_{2,18}$ = 8.45, P = 0.0026) (Fig 5.5). Beetles that re-emerged from the first set of attacked tamaracks had almost 5% more lipid per individual than parent beetles exiting the second set of tamaracks (17.2 ± 0.5 and 11.5 ± 1.9 % (mean ± SE), respectively). Beetles that re-emerged from the third set of attacked tamaracks, however, averaged 22.3 ± 2.2 % lipid content. These beetles were significantly more lipid-rich than the re-emergent beetles from either the first or second sets of tamaracks.

5.4.5 Time required to complete two generations of beetles

In the spring of 2012, the point at which 50% of the eggs of the first brood were laid was calculated to occur on May 17, 2012 (Julian day 138, 240 DD). In the spring of 2013, the point at which 50% of adults from the third brood of 2012 (*i.e.*, the putative second generation of 2012) emerged was calculated to occur on May 30, 2013 (Julian day 150, 196 ± 26.3 DD). Therefore, 378 days elapsed between the midpoint of spring egg deposition for the first brood of 2012 and the midpoint of the 2013 spring emergence by adults of the third brood of 2012. As such, 2012 was a year of fractional voltinism for eastern larch beetles because the beetles completed two generations in approximately 378 d rather than 365 d. In the spring of 2013, emerging beetles from all 2012 broods appeared fully, and equally, reproductively functional (FRM, unpublished data).

5.5. Discussion

5.5.1 Data interpretation

These data present the first report of two generations within one climatic year (*i.e.*, spring to spring) for a tree-killing species of *Dendroctonus* species supported by physiological and field data. Some *Dendroctonus* beetles such as the mountain pine beetle (*D. ponderosae*) and spruce beetle (*D. rufipennis*) can exhibit 'brood splitting' into uni- and semi-voltine cohorts (DeLeon *et al*. 1934, Reid 1962, Holsten *et al*. 1999, Hansen *et al*. 2001a, Bentz *et al*. 2014). Brood splitting can result in confusing and over-lapping periods of cohort development and adult beetle emergence (Bentz and Powell 2014) that make it difficult to unequivocally determine patterns of bark beetle voltinism based on interpretations of field observations (Mitton and Ferrenberg 2012, Bentz and Powell 2014, Mitton and Ferrenberg 2014), particularly when the cohorts and potential sources of all beetles have not been recorded. Mountain pine beetle can exhibit fractional voltinism in the southern portions of its range (Bentz and Powell 2014) or in particularly warm climes (DeLeon *et al*. 1934, Reid 1962). Recent studies debating the potential for a shift in voltinism of mountain

pine beetles due to a changing climate given putative constraints from evolved traits (Mitton and Ferrenberg 2012, Bentz and Powell 2014, Bentz *et al*. 2014) highlight the importance of empirical observations throughout all stages of brood development as well as detailed records of adult beetle activities.

For the present study, we can dismiss the possibility that a semi-voltine cohort of eastern larch beetles from 2011 emerged during the late summer of 2012 to establish the third brood of beetles (*i.e.*, the putative second generation). Detailed data of brood development throughout 2011 and the spring of 2012 clearly indicate that each brood from 2011 was uni-voltine and that adult beetles emerged from natal trees only during the spring of 2012, completing emergence by 317 DD (Chapter 4). Moreover, the 2012 spring emergence cages were monitored for an additional 216 DD (21 d) past the last observation of adult beetle emergence to ensure that emergence from the tamaracks colonized in 2011 was complete. Finally, removal of the bark beneath the emergence cages indicated that the trees were free of developing larvae and yet-to-emerge adult beetles. We can also dismiss the possibility that the third brood (*i.e.*, second generation) was established by adult parent beetles that re-emerged late from the first set of tamaracks colonized in 2012. Adult re-emergence and density data indicated that no adults remained in the first set of attacked tamaracks after 851 DD and these trees were not a source of adult beetles when attack on the third set of tamaracks began at 954 DD. In summary, we identified and monitored in detail all potential sources of re-emergent adult beetles and the data indicate that these sources did not account for the beetles that attacked the third set of tamaracks.

Many bark beetle species re-emerge from colonized host trees to attack subsequent hosts and establish sibling broods, including eastern larch beetles (Hopkins 1909, Baker 1972, Furniss and Carolin 1977, Wood 1982b, Langor and Raske 1987a, Anderbrant 1989). The initial three weeks of attack on the third set of tamaracks did overlap the final stages of adult beetle re-emergence from the second set of attacked tamaracks. However, we submit that twice re-emergent adult beetles had a minimal, if any, role in attacking the third set tamaracks. The greatest rates of adult re-emergence from the second set of tamaracks occurred at 715 and 835

DD but were not associated with new attacks on surrounding green tamaracks, resulting in a 6 – 13 d attack-free period. Moreover, a latency period before attacking additional hosts after re-emergence from the second set of tamaracks seems unlikely given that this same group of adult beetles did not exhibit this behavior after re-emerging from the first set tamaracks. While eastern larch beetle re-emergence from a second set of hosts is not uncommon (Chapter 4, Hopkins 1909, Simpson 1929, Wood 1982b, Langor and Raske 1987a), the establishment of a third sibling brood is a rare event and is reported only once in the literature (Simpson 1929). Degeneration of thoracic flight muscles of eastern larch beetles occurs rapidly once the beetles invade the host tissues (Langor 1987). Re-generation of the flight muscles that permit dispersal to new hosts occurs in only a small subset of the adult population (*i.e.*, 15% maximum) (Langor 1987), restricting flights to 5 m or less (Langor 1987, Langor and Raske 1987a). Previous studies of twice-emergent adults beetles indicated that they may not even be capable of further reproduction, choosing to forego cut logs placed adjacent to second brood trees (Langor and Raske 1987a). Green tamarack logs placed directly adjacent to infested stumps were attacked by twice re-emergent adult beetles in the only study to report a third sibling brood, but the method negated the need for the parent beetles to disperse and locate new hosts (Simpson 1929).

The capture of brown-bodied beetles within pheromone-baited funnel traps is also indicative that the third set of tamaracks was attacked by young adults from the first brood trees, as the first set of tamaracks was the only identifiable source of brown-bodied beetles at that time of year. Capturing the young adults in the funnel traps confirmed that the beetles were capable of two important aspects related to successful host colonization: successful dispersal from natal hosts via flight, and an ability to detect and orient toward conspecific sex pheromones (*i.e.*, seudenol) and/or host volatiles (*i.e.*, *α*-pinene) consistent with mating and host procurement activities. Although the flight muscles of young eastern larch beetles that emerge pre-winter have been reported to be underdeveloped and incapable of flight (Langor and Raske 1987a), young adults have been observed as flight capable during field collections (FRM, pers. obs.) and laboratory studies (Chapter 3; Erica Nystrom-Santacruz *et al*., unpublished data).

Direct observations of brown-bodied beetles excavating galleries and ovipositing within the phloem of the third set of tamaracks firmly indicate that young adults of the first brood were responsible for establishing the third brood. The significant decline in the preponderance of the brown-bodied beetles when collected over successive weekly samples from the phloem of the third set of tamaracks indicates that these beetles were not yet fully sclerotized, and were still in the process of sclerotizing fully (Figs. 5.3A&B). Such beetles, therefore, would have to have been young adults. Furthermore, the decline of brown-bodied beetles from the population in favor of black-bodied individuals demonstrates that the brown coloration was not a genetically-based color morph, as maybe the case with the spruce beetle (*D. rufipennis*), for example (Linton *et al*. 1984).

Dispersal, host colonization, reproduction, and time spent under the bark of host trees can deplete the energy (*i.e.*, lipid) reserves of bark beetles (Atkins 1969, Botterweg 1982, Anderbrant 1988, Hansen and Bentz 2003, Evenden *et al*. 2014). As such, the lipid reserves of individual eastern larch beetles would be expected to decline as successive host trees are colonized and additional broods are established. In this study, re-emergent beetles from the second set of tamaracks had significantly less lipid than re-emergent beetles from the first set, as expected, and suggest that the same beetles (*i.e.*, spring-emergent adults) attacked both sets of host trees (Fig. 5.5). However, the significantly greater lipid content of the re-emergent beetles from the third set of tamaracks was not as expected and indicates that these were not the same beetles that attacked the two previous sets of host trees and were young adults of the first brood. Overwintering by bark beetles has an apparent energetic cost since bark beetles have greater lipid reserves prior to overwintering (Dworschak *et al*. 2014a, Dworschak *et al*. 2014b, FRM unpublished data). The greater lipid content of re-emergent beetles from the third set of tamaracks relative to beetles from the first and second sets may reflect the absence of an overwintering period by this newly emergent group of young adults.

Additionally, differences in the survival of beetles within the host trees may indicate differences in the fitness of the adult beetles colonizing each set of brood trees. Beetles attacked each set of brood trees in equal densities yet subsequent re-emergence of beetles per 100 cm^2

declined significantly between the first and second sets of brood trees. However, beetle survival was equal in the first and third sets of brood trees. As with beetle lipid content, this pattern of beetle survival suggests that the same beetles (*i.e.*, spring-emergent beetles) attacked the first and second sets of brood trees, while a different group of beetles (*i.e.*, young adults of the first brood) were responsible for attacking the third set of brood trees.

New attacks on the third set of tamaracks ceased by 1377 DD despite continued emergence by young adults of the first brood, and later, the onset of emergence of young adults from the second and third broods. Thus, only a portion of emerging young adults appeared to engage in host colonization, while others proceeded to prepare for overwintering in hibernal galleries constructed at the base of the tree (Werner 1986, Langor and Raske 1987a, FRM pers. obs.). This may reflect a proportion of the population that exhibits a facultative rather than obligate overwintering adult reproductive diapause consistent with recent laboratory studies (Chapter 3). The reproductive success of brown- and black-bodied beetles that emerge prior to winter can be equal to beetles that overwinter prior to reproduction (Chapter 3). A proportion of young adult beetles of the first brood that originated from eggs laid early in the spring may have developed to a critically sensitive life-stage (*e.g.*, pre-pupa) in time to receive sufficient heat units to subvert a reproductive diapause, such as for spruce beetle (*D. rufipennis*) (Hansen *et al*. 2001a, b). Genetic variability among developing larvae within a brood may result in variation in the amount of heat that is required by individual beetles to develop into a reproductive individual without diapause, such as for the large pine weevil (*Hylobius abietis* (L.)) (Tan *et al*. 2010, Inward *et al*. 2012, Wainhouse *et al*. 2014). Thus, some individuals of the first brood of eastern larch beetles in this study may have received enough heat to become reproductively mature within the summer while the majority of individuals of the first brood, and all individuals of the second, and third broods did not. The early and warm spring for the study area in 2012 (Chapter 4, Fig. 4.1) allowed eastern larch beetles to establish the first brood earlier than normal, while the high temperatures throughout the summer and fall allowed the broods to develop rapidly (Chapter 4), allowing some young adults to become reproductively active in that year.

5.5.2 Conclusions and significance

This study adds the eastern larch beetle (*D. simplex*) to a growing list of other important tree killing bark beetles that may exhibit altered patterns of development in response to climate change in North America and Europe (Lange *et al*. 2006, Raffa *et al*. 2008, Faccoli 2009, Bentz *et al*. 2010, Jönsson *et al*. 2011). For southerly distributed species, increases in the number of generations per year may be less important than other biological factors for determining outbreak dynamics. For example, slight increase in voltinism of the southern pine beetle (*D. frontalis*) is a less important predictor of the northward shift in outbreaks compared to the expected increase in overwinter survival of this insect (Ungerer *et al*. 1999). For other species, changes in voltinism and concomitant effects on bark beetle population dynamics can vary geographically. For example, the European spruce bark beetle (*I. typographus*) is predicted to increase the number of generations per year throughout its range under current climate warming scenarios. However, in the colder, northern areas of its range, complete development of the second generation may not be possible prior to the onset of winter, resulting in mortality of the sub-adult life-stages. Conversely, the warmer climate in the southern areas of the range of *I. typographus* may allow additional generations of beetles to complete development prior to winter, or, allow sub-adult life-stages to survive the winter to complete development the following year, potentially adding to insect numbers (Harding and Ravn 1985, Faccoli 2002, Lange *et al*. 2006, Jönsson *et al*. 2007, Faccoli 2009, Jönsson *et al*. 2009, Jönsson and Bärring 2011).

By definition, bi-voltinism is the occurrence of two complete generations of insects within a single year or less (*i.e.*, ≤ 365 d) (de la Torre-Bueno 1989). In this study, using emergence thresholds for entire brood groups, the two generations were only 13 d from achieving true bi-voltinism, although a proportion of individuals laid early in the sequence likely exhibited bi-voltine status. Such proportions will likely increase with expanding growing seasons. From a tree-killing perspective, the effects of bi- vs. fractional voltinism may be similar in this system because spring emergence of the third brood (*i.e.*, second generation) overlapped with the spring emergence periods of the first and second sibling broods (*i.e.*, first generation) in the spring of the subsequent year (Chapter 4). As such, fractional or bi-voltinism facilitating enhanced cooperative

host procurement with mixed generations each spring may be more beneficial to eastern larch beetle than other species such as mountain pine beetle (*D. ponderosae*) or the European spruce bark beetle (*Ips typographus*), where beetles of the second generation have asynchronous spring emergence and potentially greater mortality during winter or host colonization activities (Harding and Ravn 1985, Faccoli 2002, Jönsson *et al*. 2007, Bentz and Powell 2014). Host-caused mortality to eastern larch beetles during colonization by the second generation may be reduced if tamaracks are less well defended, with lower concentrations of defense compounds and higher concentrations of nutrients in the phloem as observed in other *Larix* – bark beetle systems (*e.g.*, Rohde *et al*. 1996). Moreover, reproduction by adult eastern larch beetles of the second generation may be greater than for beetles of the first generation. Beetles of the second generation do not have to expend a portion of their lipid reserves on the over-wintering process (FRM, unpublished data) prior to the opportunity to mate, potentially leaving more lipids available for reproduction. Finally, altered associations with natural enemies reduce mortality in sibling broods of eastern larch beetles that are established later within a year (Langor and Raske 1988b), and may also apply to additional generations of beetles

These results suggest that enhanced numbers of eastern larch beetles brought about by a shift in voltinism, resulting in a second generation established by young adult progeny high in lipid content, could be responsible at least in part for the ongoing activity of eastern larch beetle across the southern margin of the range of tamarack over the past 15 years. Enhanced climatic analysis is needed to determine the frequency with which this might be occurring. Interestingly, Swaine (1911) also described infested tamarack material containing light and dark colored young adults, exit holes indicating the emergence of some young adults, and a proximate set of new host material that was recently colonized but could not identify the source of the adult beetles responsible for the attack. In light of the present findings, it is possible that Swaine may have also documented a second generation of eastern larch beetles in a single year.

5.6 Acknowledgements

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5.7 Figures

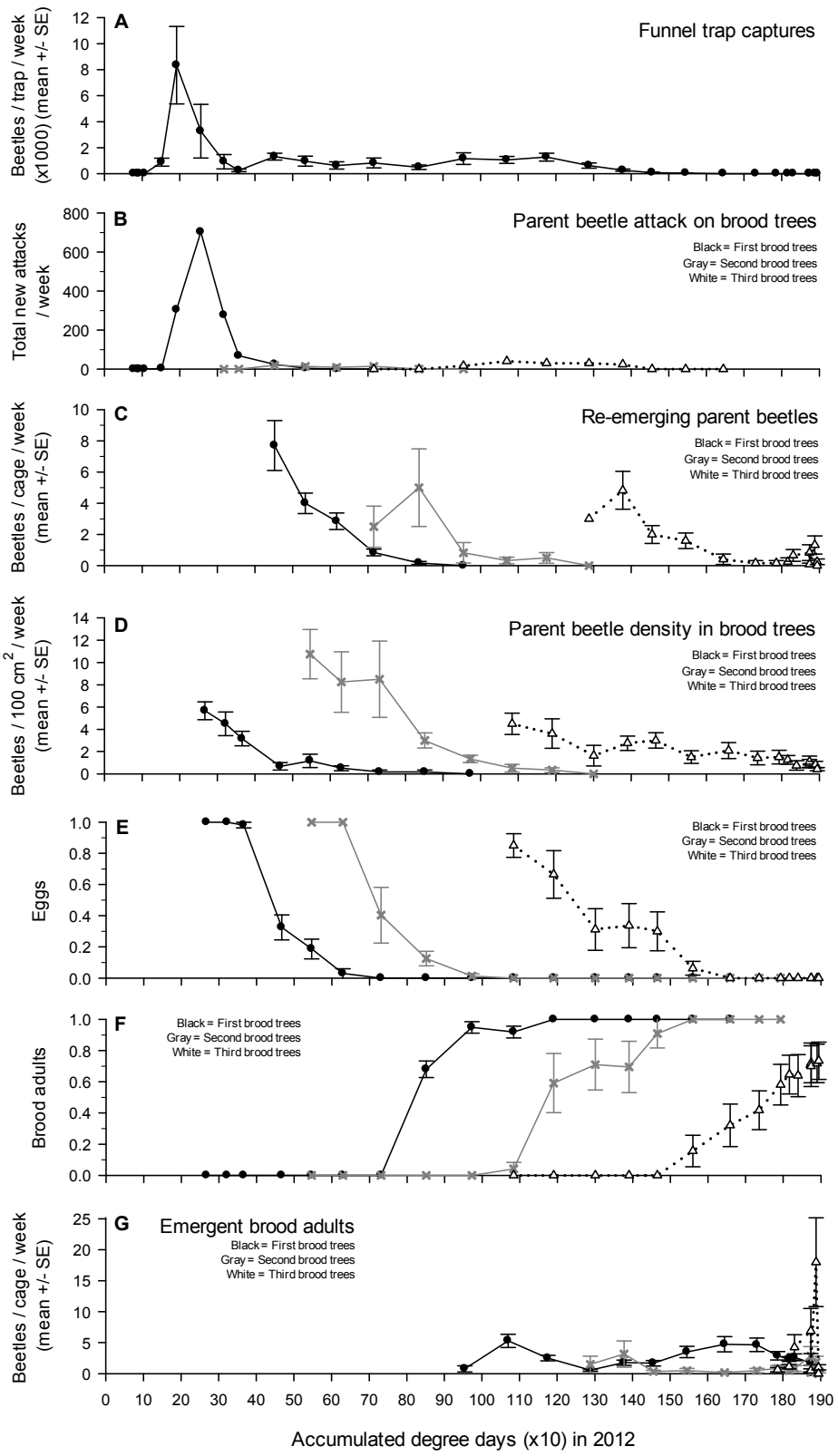
A
Funnel trap captures
Beetles / trap / week (x1000) (mean +/- SE)
B
Parent beetle attack on brood trees
Black = First brood trees
Gray = Second brood trees
White = Third brood trees
Total new attacks / week
C
Re-emerging parent beetles
Beetles / cage / week (mean +/- SE)
D
Parent beetle density in brood trees
Beetles / 100 cm2 / week (mean +/- SE)
E
Eggs
F
Brood adults
G
Emergent brood adults
Beetles / cage / week (mean +/- SE)
Accumulated degree days (x10) in 2012

Figure 5.1. (*Continued from previous page*) (A) Captures of adult eastern larch beetles in funnel traps, (B) Attacks by parent beetles on tamaracks for each brood, (C) Re-emergence of parent beetles from attacked tamaracks, (D) Parent beetle density in tamaracks attacked for each brood, (E – F) Proportion (mean ± SE) of eastern larch beetle eggs and brood adults, respectively, relative to all life-stages present per sample per week within each tamarack colonized for each brood, and (G) Number (mean ± SE) of emergent brood adults per cage per week on each tamarack colonized for each brood. All data are relative to accumulated degree days (5°C base) from 30 Mar. to 2 Nov., 2012 (first and last data point, respectively).

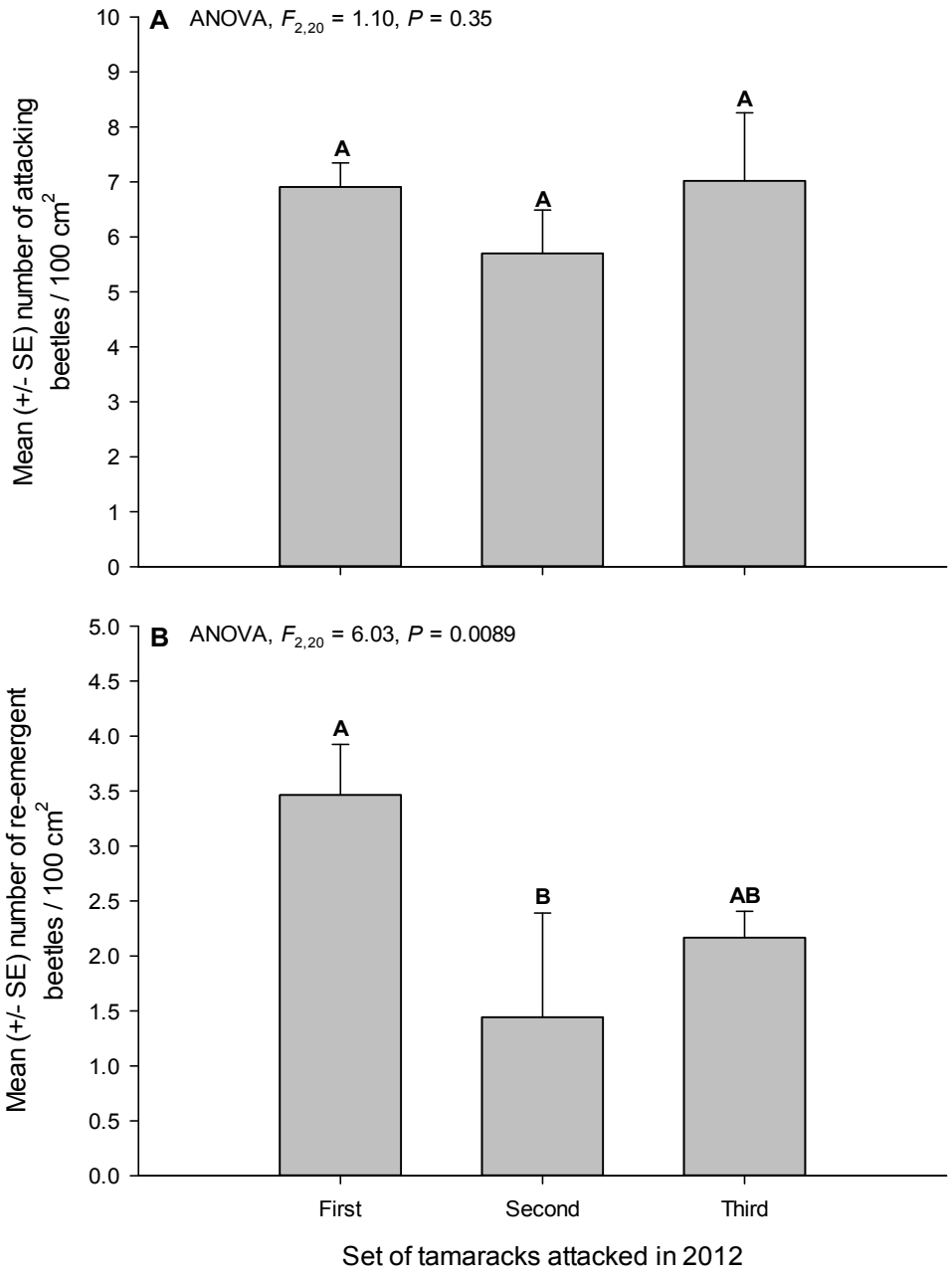

Figure 5.2. (A) Mean (± SE) number of attacking eastern larch beetle per 100 cm^2 of bark on the first, second, and third sets of tamaracks colonized in 2012 for the first, second, and third beetle broods. (B) Mean (± SE) number of re-emergent eastern larch beetles per 100cm^2 of bark from tamaracks colonized for the first, second and third broods of eastern larch beetles in 2012. Within each figure, different letters represent significant differences between groups (Tukey HSD test, α = 0.05).

Figure 5.3 (A) Young, brown bodied eastern larch beetle adults that have not become fully sclerotized and that represent such beetles that were captured in pheromone-baited funnel traps as well as observed excavating parental galleries and ovipositing within the phloem of the third set of tamaracks to establish a putative second generation of eastern larch beetles in 2012, (B) Fully sclerotized adult eastern larch beetles with black bodies and maroon elytra that are typical of mature adult beetles that emerge from over-wintering hosts in the spring of the year and establish the first larval brood. (Photo credit: Aubrey Wilke).

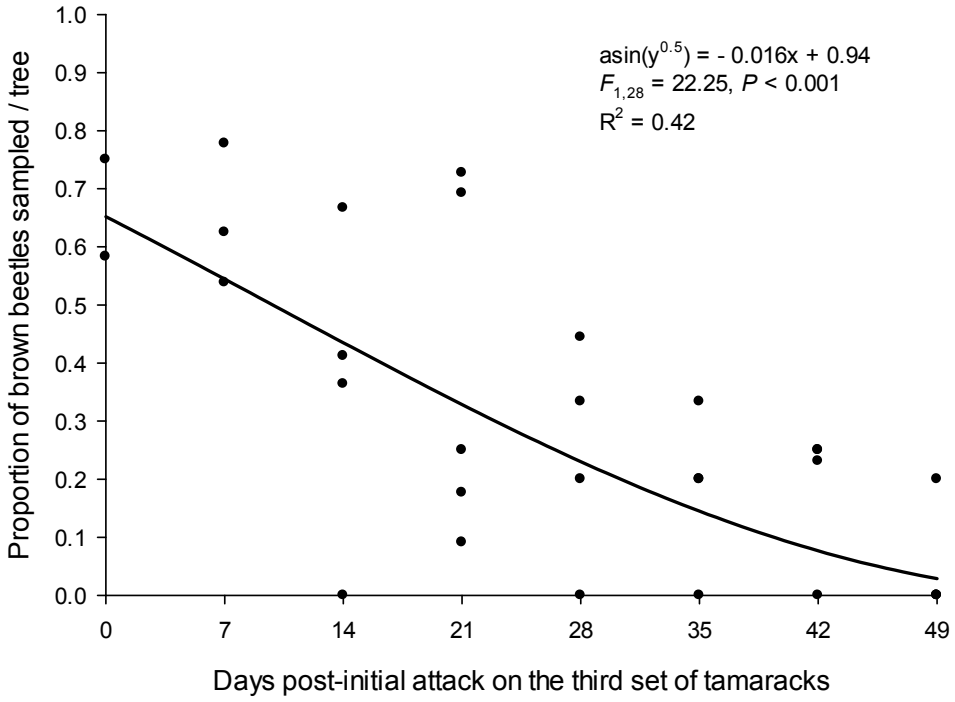

Figure 5.4 Proportion of brown- relative to black-bodied eastern larch beetle adults per 100 cm^2 of bark within tamaracks attacked for the third brood of 2012 (*i.e.*, the putative second generation) sampled at 7 d intervals following mass attack. *n* = six tamaracks attacked for the third brood of 2012.

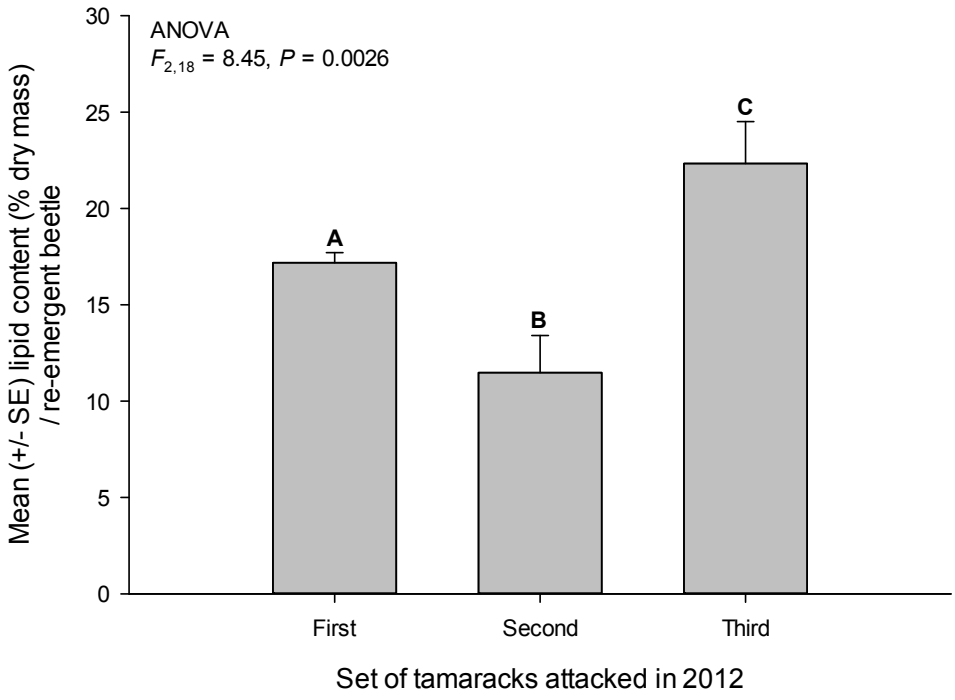

Figure 5.5 Mean (± SE) lipid content as a percentage of beetle dry mass for adult eastern larch beetles captured in cages during re-emergence from the first, second, and third set of tamaracks attacked for the first, second, and third brood groups of 2012. Female and male beetles were pooled for analysis. n = 144, 12, and 17 re-emergent beetles sampled for lipid content from tamaracks attacked for the first, second, and third brood groups, respectively. Different letters represent significant differences between groups (Tukey HSD test, α = 0.05).

Dissertation conclusions

1) The minimum and optimal temperature for the development of eastern larch beetles is 7.5 and 27.9°C, respectively. Temperatures during beetle development between 20 and 22°C maximize the fitness of young adult beetles. The minimum developmental temperature threshold of all life-stages is less than 9.9°C. The potential trade-off between developmental rate and beetle fitness may help to maximize beetle survival and reproductive potential over a wide range of temperatures and environmental conditions. Minimum developmental temperatures of less than 9.9°C for all life-stages may allow eastern larch beetles to shift from uni- to bi-voltinism more readily than other *Dendroctonus* species.

2) Laboratory studies indicate that a subset of eastern larch beetles within a population possess a facultative rather than obligate reproductive diapause. These results indicate for the first time that eastern larch beetles may not be restricted to a single reproductive generation per year under natural condition as was previously thought. These results redefine what is known of the reproductive biology of eastern larch beetles.

3) Eastern larch beetles in the Great Lakes region consistently produce two broods per year. A third brood is possible in some years. The first brood consistently develops to adults prior to winter; whereas this is inconsistent with the second brood. Broods that do not complete development prior to winter resume development the following spring. Widespread overwinter mortality of the second and third broods was not observed. Spring emergence by adult beetles from all broods is highly synchronous allowing cooperative attack on new host trees. Eastern larch beetle broods that are established following the first brood appear to be important contributors to the adult beetle population and overall population dynamics of the eastern larch beetle.

4) For the first time on record, eastern larch beetles established a second generation of offspring under natural field conditions resulting in fractional voltinism (*i.e.,* 1+ generations / year) in 2012. This observation corroborates laboratory studies indicating that these insects are not restricted to a uni-voltine lifecycle. The shift by eastern larch beetles from uni- to fractional-voltinism may be climate related given the early, warm spring, hot summer, and warm, extended fall season that characterized 2012. It is possible that a larger portion of the eastern larch beetle population will shift from a uni-voltine to a fractional or bi-voltine lifecycle under predicted future climate warming scenarios. Additional generations of eastern larch beetles per year would have important implications for the insect's population dynamics and ability to cause widespread forest mortality.

5) Tamaracks that were "challenged" in the recent past by unsuccessful eastern larch beetle attacks were subsequently colonized successfully at lower densities than unchallenged or "naïve" tamaracks. Reproductive success per female within challenged trees, however, can be almost 2-fold greater than that of similar sized naïve tamaracks. However, offspring that completed development in challenged tamaracks had reduced fitness compared to offspring that developed in naïve hosts. Tamaracks challenged or stressed by an event appear to be an advantageous substrate for eastern larch beetles since these hosts are more easily colonized. Moreover, the high reproductive success attained by eastern larch beetles in challenged hosts may allow the rapid build-up of beetle populations and have important roles in allowing this insect to transition from endemic to incipient population phases when beetle numbers in a population are more important than individual beetle fitness.

6) During outbreak conditions eastern larch beetles preferred to attack the largest, most vigorous tamaracks available. Beetle reproduction was greater in larger versus smaller tamaracks. Beetle offspring were larger in size when development occurred in larger host trees, and in trees with thicker phloem. Female offspring had greater lipid content when development occurred in larger host trees and in trees with thicker phloem. The behavior

of eastern larch beetles to attack the largest and most vigorous host trees demonstrates that this insect can behave in a manner similar to the traditionally recognized “aggressive” *Dendroctonus* species. Further, this system appears to operate on a positive feedback mechanism whereby parent beetles prefer to attack the largest trees, which in turn increase the reproductive success of the parent beetles. Moreover, the utilization of larger host trees also increases the fitness of the resulting offspring, potentially increasing the dispersal ability, reproductive success, and survivorship, of the next generation of beetles.

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Appendix 1.

Host selection, colonization dynamics, reproductive success, and offspring fitness of the eastern larch beetle *Dendroctonus simplex* LeConte in relation to host quality during a large-scale outbreak in the Great Lakes region of North America.

A1.1 Introduction

Bark beetles (Coleoptera: Scolytinae) are important insect herbivores and agents of ecosystem disturbance ((Kurz *et al.* 2008, Raffa *et al.* 2008, Hicke *et al.* 2012). The economic importance of many bark beetle species, particularly species within the *Dendroctonus* genus, has precipitated a large number of studies devoted to understanding the ecology of these insects. There are numerous biotic and abiotic factors that are important regulators of bark beetle population dynamics (Raffa *et al.* 2008, Bentz *et al.* 2010). However, an understanding of the interactions between bark beetles and their respective host trees are an important foundation for understanding the host-centric mechanisms involved in influencing the population dynamics of a given bark beetle species and its potential to cause large-scale damage to forest ecosystems.

There are four areas of focus related to bark beetles and their host trees that are important for understanding the ecology of a bark beetle species: host selection, colonization dynamics, reproductive success, and offspring fitness, all of which may be regulated by host characteristics or quality. Numerous studies of bark beetle ecology have focused on the effect of host quality and its influence on bark beetle host selection (Cole *et al.* 1976, Cole and Amman 1980, Lih and Stephen 1996, Shore *et al.* 1999, Bleiker *et al.* 2003, Steed and Wagner 2004, Fettig *et al.* 2007, Björklund *et al.* 2009, Boone *et al.* 2011, Knapp *et al.* 2013, Johnson *et al.* 2014, Meddens *et al.* 2015), colonization dynamics (Berryman 1976, Waring and Pitman 1980, Raffa and Berryman 1983, Berryman *et al.* 1985, Mulock and Christiansen 1986, Christiansen *et al.* 1987, Raffa 1988, Raffa 2001, Reid and Glubish 2001, Bleiker *et al.* 2014, Raffa *et al.* 2015), beetle reproductive success (Reid 1963, Cole and Amman 1969, Amman 1972a, Cole 1973b, Berryman 1976, Cole *et al.* 1976, Fargo *et al.* 1979, Haack *et al.* 1984, Anderbrant *et al.* 1985,

Anderbrant and Schlyter 1989, Lessard and Schmid 1990, Raffa 2001, Graf *et al.* 2012), or offspring fitness (McGhehey 1971, Cole 1973a, Amman and Pace 1976, Botterweg 1982, Anderbrant *et al.* 1985, Amman and Pasek 1986, Anderbrant 1988, Anderbrant and Schlyter 1989, Awmack and Leather 2002, Graf *et al.* 2012). However, such studies tend to focus on the interrelated effects of one or two, and occasionally three, of the four areas previously mentioned. Studies designed to examine the influence of host plant quality on bark beetle host selection, colonization dynamics, reproductive success, and offspring fitness in a complete and inter-related holistic framework are much less common.

In this experiment, the interactions between the eastern larch beetle (*Dendroctonus simplex* LeConte) (Coleoptera: Scolytinae) and its host tree the eastern larch (tamarack) (*Larix laricina* (Du Roi) k. Koch) is investigated in detail. To initiate the study, three tamarack stands were selected that each contained a small epicenter of between 8 and 10 tamaracks that were infested with eastern larch beetles. Around each infestation, between 28 and 32 apparently healthy tamaracks were selected. In total, measurements of host tree diameter, phloem thickness, growth rate, age, phloem resin pocket density, competition from nearby trees, and history of previous unsuccessful eastern larch beetle attack were recorded on 132 tamaracks. First, this study records the host attributes that are most important to eastern larch beetles during host selection process. The hierarchy of tamarack characteristics associated with eastern larch beetle host selection is recorded across five separate periods of beetle host selection between the spring of 2011 and summer of 2013. Changes to the preferences of attacking beetles for certain host attributes as the host pool declines is recorded. Additionally, how the colonization dynamics and attack behavior of eastern larch beetles is mediated by the traits of a host tree under attack is also measured. Further, as eastern larch beetles successfully colonize tamaracks, the manner in which the traits of host trees affect the reproductive success of the attacking beetles is examined. Finally, this study examines the emergent offspring that successfully developed within a subset of the tamaracks that were attacked to investigate how host traits influence the fitness of the resultant offspring. The density of attacking beetles on successfully colonized tamaracks is also examined to measure the effect of intraspecific

competition on the reproductive success of eastern larch beetles as well as on the fitness of resultant offspring.

A1.2 Methods

A1.2.1 Study site selection

Study sites were selected within the Red Lake Wildlife Management Area, near Lake of the Woods, MN, on 10 June 2011. Three sites, PS1, PS2, and PS3, were located on the Pitt Grade Forest Road in the Beltrami Island State Forest, Lake of the Woods County, MN, U.S.A (UTMs: 15U 0370411 / 5384452, 15U 0370374 / 5382114, and 15U 0370509 / 5390453, respectively).

Each study site was based around small epicenters of eight to ten tamaracks colonized approximately 2-3 weeks previously (determined by brood maturity, see Chapter 4). Each epicenter of beetle activity was surrounded by a healthy tamarack stand in which the host selection behavior of eastern larch beetles could be monitored throughout the multi-year study.

A1.2.2 Selecting healthy tamaracks to monitor eastern larch beetle host selection

On 10 June 2011, 28, 33, and 42 (n = 103 total) green, non-attacked, apparently healthy tamaracks with bole diameters ≥ 10 cm at 1.4 m (*i.e.*, diameter at breast height (DBH)) were randomly selected at sites PS1, PS2, and PS3, respectively, from the tamarack stand surrounding each epicenter of beetle activity. The healthy tamaracks were selected during the lull in beetle attack on host trees following the establishment of the first sibling brood and preceding parent beetle re-emergence in large numbers. Each tamarack was assigned a unique identification tag.

A1.2.3 Monitoring healthy tamaracks for beetle attack

Tagged tamaracks were monitored every 7 d for beetle attack from 10 June – 22 October 2011, 24 March – 2 November 2012, and 23 April to 28 October 2013. With the exception of

2011, monitoring for attacks began in the spring at least 2 weeks prior to emergence by over-wintering beetles. Monitoring ended in the fall after at least 2 weeks without beetle activity. Tamaracks were monitored in the spring for attack by reproductively mature, spring-emergent adult beetles (*i.e.*, the would-be "parent" beetles), then monitored throughout the early- and mid-summer for attack by the same beetles re-emerging from fully colonized tamaracks after establishing either the first or second sibling brood, and, finally, monitored during the late-summer and early-fall for attacks by brood adults emerging from natal host trees prior to winter, of which a portion of the population is known to be reproductively mature and capable of establishing a partial second generation of beetles (Chapters 2 & 5). The healthy tamaracks were assessed for beetle attack by visually inspecting the lower 2.5 m of bole for the frass produced by beetles boring into the bark.

Observation windows installed on attacked tamaracks were used to record beetle attack dynamics during assessments every 7 d and to determine when colonization was complete. Observation windows were centered at 1.6 m above ground-level on the south aspect of the bole and measured 20 x 20 cm (H x W) in 2011 and 40 x 25 cm (H x W) in 2012 and 2013. Attacks were marked with pins to prevent double counting during weekly assessments.

A1.2.4 Tamarack characteristics associated with host quality

Diameter at breast height (DBH). Tree DBH was measured to the nearest 0.1 cm at 1.4 m using a DBH tape. The DBH of the tamaracks comprising the infestation epicenters as well as the healthy tamaracks were recorded. Measurements of DBH were taken 10 June 2011.

Phloem thickness. A 5 x 2 cm (H x W) phloem sample was removed from each healthy tamarack. Phloem samples were not taken from the tamaracks comprising the epicenters since these trees were already attacked when the study began. Phloem samples were collected from the healthy tamaracks on 12 June 2011, 24 March 2012, and 5 May 2013. In 2011, phloem samples were collected from the healthy tamaracks prior to parent beetle re-emergence and attack. In 2012 and 2013, phloem samples were collected prior to eastern larch beetle spring emergence from tamaracks that survived the previous year(s) and remained on the landscape as

potential hosts. A new phloem sample was removed from each healthy tamarack each year since phloem thickness can vary annually due to changes in environmental conditions (*e.g.*, drought). Phloem samples were removed at 1.4 m on the bole. In 2011 and 2012, samples were taken from the east bole aspect 5 cm apart. In 2013, the phloem was sampled from the west aspect to avoid excessive damage to one area of the bole that may influence tree vigor and the natural host selection behaviors of the eastern larch beetles in each site. Phloem thickness was measured to the nearest 0.01 mm using a Leica MZ6 microscope with real-time camera and digital micrometer (Leica Microsystems, Wetzler, Germany). Phloem thickness was recorded as the mean value after measuring at 0.5, 2.5, and 4.5 cm along the 5 cm length.

Phloem resin pocket density. The density of constitutive resin pockets per cm^2 within the phloem sample of each healthy tamarack was recorded. To calculate resin pocket density for a given phloem sample, the number of resin pockets present on the longitudinal section (*i.e.*, the same surface used to measure mean phloem thickness) of the sample were counted. The resin pocket count was then divided by the longitudinal cross sectional area (cm^2) of the sample calculated using the mean thickness (converted to cm) multiplied by the sample length (*i.e.*, 5 cm). The density of resin pockets for a given healthy tamarack was re-calculated when a new phloem sample was collected each spring, as applicable.

Tamarack age & growth rate. Increment cores were used to estimate tree age and to calculate annual growth rate for each tamarack that was either killed by eastern larch beetles or that escaped attack during each study year from 2011 to 2013. Two increment cores were removed from each tamarack 20 – 25 cm above the ground, at least 90 degrees apart, and from locations where intercepting the pith seemed likely. The increment borer was a Haglöf three-thread borer with a 5.15 mm diameter bore (Haglöf Sweden AB, Långsele, Västernorrland, Sverige, Sweden). For 2011 and 2012, only tamaracks killed by eastern larch beetles in each respective year were cored. In 2013, tamaracks killed by beetles in that year and the tamaracks that ultimately escaped attack were cored. Coring occurred in late October of all years. Coring only the killed tamaracks in 2011 and 2012 avoided having to repeatedly (and unnecessarily) core the healthy tamaracks that could have been killed (necessitating re-coring) the following

year. Also, repeated coring has the potential to negatively impact tree vigor and potentially alter the natural host selection behavior of the eastern larch beetles.

Cores were handled and processed for cross dating using standard dendrochronological techniques (Stokes and Smiley 1996, Fritts 2001). Cores were cross-dated using standard manual and statistical cross-dating techniques (Stokes and Smiley 1996, Fritts 2001). Cross-dating is required to identify and account for anomalies in tree growth patterns present in each core (*e.g.*, missing or false growth rings) and to assign an exact calendar year to each annual growth ring present in each core. The following methods were used for cross-dating. First, individual skeleton plots were made of each "sibling" core removed from the same tamarack and compared to identify anomalies between the sibling cores. If sibling cores lacked growth anomalies then a composite skeleton plot for the tamarack was constructed using the skeleton plots of each sibling core. Next, site-specific master chronologies were constructed using the composite skeleton plot of each tamarack within each site. Although the site-specific master chronologies had excellent agreement among sites, a regional master chronology that incorporated all sites was not created because, while certain marker years were present across study sites, the presence of additional site-specific marker years enhanced cross dating on a site-by-site basis. Site-specific master chronologies were created for each year of the study using tamaracks killed in each year. Site-specific master chronologies demonstrated excellent alignment between study years. After constructing the site master chronologies, the skeleton plot of each sibling core was then re-checked against the appropriate site chronology to determine the accurate alignment of growth rings specific to each calendar year.

Finally, each core was statistically cross-dated to the site master chronology to validate the manual cross-dating process. The width of each growth ring on each core was measured to the nearest 0.001 mm on a Velmex model TA4030H1-S6 and data was input to MeasureJ2X v4.2 software (VoorTech Consulting, Holderness, NH, U.S.A.). Each sibling core from each study year was then statistically cross dated to the year-specific site master chronology using COFECHA v6.06P. Within COFECHA, ring width series were analyzed with a segment length of 23 years and a lag of 11 years. The cubic smoothing spline was maintained at 32 years.

After the growth rings in each core were assigned calendar years via cross-dating, it was possible to calculate the rate of growth exhibited by each tamarack during each year of life. Annual growth rate was based on the mean ring width (mm) associated with each calendar year of tamarack growth. For each tamarack, the mean width of each growth ring was calculated by averaging the ring width for the same calendar year present on both sibling cores. Annual growth rates for each tamarack were calculated only for the years in common between the sibling cores. For example, if one sibling core contained growth rings from 1955 onwards until the year of tree death while the other sibling core contained rings from 1960 onwards, then the annual growth rate based on mean ring width was only calculated for the years 1960 onwards.

The annual growth rate of each tamarack was expressed as the basal area increment (BAI) of new wood accrued each year (cm^2 / yr.). Tree growth rate was expressed as the mean BAI over the two years prior to, but not including, the year of beetle attack, or, potential beetle attack, for tamaracks killed *vs*. escaping attack, respectively. The two year averaging window for growth rate was based on an analysis of growth patterns that determined that the tamaracks in this study exhibited a mean (± SD) length of autocorrelation in growth of 2.3 (± 1.2) years, with 56% of the tamaracks having a two year autocorrelation.

History of previous eastern larch beetle attack. In the summer of 2011, six tamaracks were attacked by eastern larch beetles unsuccessfully. In the spring of 2012, each of the unsuccessfully attacked tamaracks were re-attacked and successfully killed. The legacy of being previously challenged by eastern larch beetles prior to being successfully colonized was recorded.

Tamarack association with inter- and intraspecific competition. The basal area of inter- and intraspecific trees growing in the immediate vicinity of each tamarack in this study was recorded as a measure of microsite competition. Each tamarack served as the center of a variable-radius plot. A Jim-Gem Cruz-All (Forestry Suppliers, Jackson, MS, U.S.A., Prod. # 59795) with basal area factor (BAF) 10 ft^2 / ac was used to survey the basal area of competitive trees surrounding each tamarack. All tree species with bole diameters at breast height (1.4 m) large enough to fill the BAF 10 gauge on the Cruz-All were tallied. The basal area (ft^2 / ac) of

competitive trees surrounding each tamarack was calculated by multiplying the number of boles tallied by a factor 10. The ft^2 / ac basal area was multiplied by 0.2296 to convert the measurement to m^2 / ha [*i.e.*, (1 ft^2 / ac) x (1 m^2 / 10.76 ft^2) x (1 ac / 0.4047 ha) = (1 / (10.76 x 0.4047)) = 0.2295 m^2 / ha].

A1.2.5 Recording eastern larch beetle reproductive success

Beetle reproductive success was recorded from 53 tamaracks killed by eastern larch beetles from 2011 – 2013 and included tamaracks comprising the original epicenters of beetle infestation as well as selected individuals from among the group of 103 healthy tamaracks. Beetle reproductive success was defined as the number of emergent offspring produced per parent female, and, as the number of emergent offspring produced per 100 cm^2 of infested bark.

To determine parent beetle reproductive success, screen cages used to capture emergent brood adults were installed on the bark of tamaracks once beetle colonization was complete (*i.e.*, no new attacks occurring in the observation window between two successive sample dates). Each cage was fitted with a collection cup. Each tamarack was fitted with two cages, one on each the north and south bole aspect centered at 1.8 m above the ground and each measuring 16.5 x 30 cm (W x H) and each covering 495 cm^2 of bark (990 cm^2 bark total). The number of attack points on the bark area to be covered by each cage was counted prior to cage attachment. Re-emergent parent eastern larch beetles captured in the cages were accounted for using methods (*e.g.*, phenology data) to distinguish parent beetles from emergent brood adults (see Chapter 4) and to ensure accurate counts of brood adults from under each caged area. Emergent brood adults were captured throughout the summer and fall months with collections ending in late fall after 2 weeks without captures. Cages were emptied twice per week, with the second collection occurring 48 h after the first. Brood adults from the second collection were collected alive and placed on ice in the field and frozen for analyses of fitness (see below). Beetles from both collections were summed for a weekly total of emergent offspring.

Since eastern larch beetle brood adults do not complete emergence from natal host trees prior to winter, collections were resumed the following spring. Cages were re-installed on 24

March 2012, 22 April 2013, 28 April 2014 over the areas of the bark that were caged the previous year. Cages were re-installed at least 2 weeks prior to beetle emergence. Emergence cages were emptied twice per week identical to the fall collections and summed for a weekly total. Spring-emergent brood adults were not used for analyses of fitness since the effect of host quality was confounded with an over-wintering period. Emergent brood adults were catalogued by emergence date, host tree, trap aspect, and sex for the fall and spring collections and summed to get a total number of emergent offspring for each caged area.

Number of brood adults per parent female. Eastern larch beetles share an entrance hole in host trees with approximately one, two, or three to four conspecific pairs 60, 35, and 5% of the time, respectively (Langor and Raske 1987a). To get an accurate count of the number of brood adults per parent female, the number of entrance holes under each cage was converted to the number of parent females associated with that number of entrance holes via multiplying by 1.48 per Equation A1.1:

[Equation A1.1]

$$\text{No. females per entrance hole} = \Sigma[(2(P_N)*O_{\%})] * P_F$$

Where: 2 = A constant. The number of beetles per pair.

P_N = number of beetle pairs using an entrance hole on average. Note that three to four beetle pairs was averaged as 3.5

$O_{\%}$ = the percent occurrence of a given number of beetle pairs using the same entrance hole.

$P_F = 0.5$ = the proportion of the beetle pair being female

Therefore:

$$\text{No. females per entrance hole} = [(2(1)*0.6) + (2(2)*0.35) + (2(3.5)*0.05)] * 0.5$$
$$= [1.2 + 1.4 + 0.35] * 0.5$$
$$= 1.48$$

To calculate the number of brood adults produced per parent female beetle, the number of emergent brood adults captured under each cage on each study tree was summed to provide a total number of emergent brood adults for the entire 990 cm^2 caged area. Similarly, the number of parent females expected to be present under each cage was summed for a total number of parent females under the entire 990 cm^2 caged area. Finally, the total number of emergent brood adults was divided by the total number of parent females.

Number of brood adults per 100 cm^2 of infested bark. To record the reproductive success of eastern larch beetles per 100 cm^2 of infested tamarack bark, the number of brood adults that emerged from under each cage on each study tree was recorded. For each study tree, the total number of brood adults that emerged under each cage was summed for the total emerged from 990 cm^2 of infested bark. The total number of emergent brood adults per 990 cm^2 bark was then divided by 9.9 to yield the number of emergent offspring per 100 cm^2.

A1.2.6 Measuring brood adult fitness

Brood adult size (*i.e.*, pronotal width) and lipid content were used as proxies for fitness. Body size in bark beetles is associated with a variety of fitness attributes such as greater survival, fecundity, mating success, and dispersal potential (McGhehey 1971, Roff 1980, Anderbrant 1988, Honěk 1993, Kingsolver and Huey 2008, Williams and Robertson 2008, Evenden *et al.* 2014). Similarly, lipid content has been associated with increase dispersal, survival, host attack, and fecundity in bark beetles (Atkins 1966, Thompson and Bennett 1971, Anderbrant 1988, Jactel 1993, Wallin and Raffa 2000, Elkin and Reid 2005, Williams and Robertson 2008, Evenden *et al.* 2014). Pronotal width of brood adults was measured to the nearest 0.01 mm using a Leica MZ6 microscope with real-time camera and digital micrometer (Leica Microsystems, Wetzlar, Germany). Natal host and sex was recorded for each brood adult measured.

Once measured for size, beetles were dried for 24 h at 50°C to determine their dry mass to the nearest 0.01 mg using a Metler-Toledo AX105 Delta range analytical microbalance. Lipids were extracted using a 500 mL Soxhlet extractor with petroleum ether. Dried beetles were placed in individual, screened, and labeled 0.5 mL Eppendorf tubes. Sixty-four beetles were processed

per lipid extraction using 300 mL of warm petroleum ether. Extractions lasted 8 h with the extractor column flushing once per hour. Following extraction, beetles were re-dried for 12 h at 50°C and re-weighed to the nearest 0.01 mg to obtain the lean dry mass. Total lipid content (mg) per beetle was calculated as the difference between the dry mass and the lean dry mass. Proportion lipid content for each beetle was calculated as a proportion of beetle dry mass prior to lipid extraction.

In total, 802 beetles (408 females, 394 males) were analyzed for size and lipid content. Between 3 and 61 fall-emergent beetles were sampled from each of the 44 (of 53) attacked tamaracks that exhibited brood adult emergence prior to winter.

A1.2.7 Statistical analyses

Host selection. Tamaracks were analyzed as either attacked vs. not attacked for each period of eastern larch beetle host selection in which the beetles attacked tamaracks to establish a brood. In total, the data was analyzed for five rounds of host selection; for the first and second sibling broods of 2011, the first sibling brood for the first as well as second generation of beetles (see Chapter 4) of 2012 (host selection for the second sibling brood of 2012 was not analyzed as only three tamaracks were attacked and did not provide adequate replication for comparing the attributes of attacked vs. non-attacked tamaracks), and finally for the first sibling brood of 2013 (host selection for the second sibling brood could not be included since beetles attacked tamaracks that were not included as part of the original group of 103 study trees).

For each period of beetle attack, host selection was analyzed using generalized linear mixed effects models (GLMER) with a binomial response for whether a healthy tamarack was attacked or whether it escaped attack. The host attributes for tamarack DBH, phloem thickness, resin pocket density, age, growth rate, attack history, and associated competition were all analyzed separately as fixed effects to determine which factors were significantly associated with whether a tamarack was attacked for escaped attack. All analyses of beetle host selection used study site as the random effect.

Eastern larch beetle attack density. A linear mixed effects model was used to determine if eastern larch beetle attack densities differed for the areas under the north and south cages. Bole aspect was the fixed effect while study site nested within beetle brood nested within study year served as the random effect. Attack densities were similar between bole aspects (ANOVA, $F_{1,79}$ = 0.0764, *P*-value = 0.783) so attacks were pooled for each tamarack. Attack density was scaled to the number of attacks per 100 cm^2 for convenience.

Individual linear mixed effects models were used to regress beetle attack density against the fixed effects of DBH, phloem thickness, resin pocket density, attack history, age, growth rate, and associated competition. Study site nested within beetle brood nested within study year served as the random effect term. For analyses, beetle attack density was transformed as needed (*e.g.*, $\sqrt{y}$) to satisfy model assumptions of homoscedasticity and normality of errors.

Parent female reproductive success. An ANOVA was used to test if the number of emergent brood adults differed between the cages on the north and south bole aspects. Brood adult emergence was similar between bole aspects (ANOVA, $F_{1,78}$ = 0.9650, *P*-value = 0.3289) so data was pooled to give the number of emergent brood adults per tamarack.

To calculate the number of brood adults per parent female, the total number of emergent brood adults from the entire caged area of each tamarack (990 cm^2) was divided by the number of parent females expected to have colonized the caged area (*i.e.*, number of attacks multiplied by 1.48). To calculate the number of brood adults per 100 cm^2 of infested bark, the total number of emergent brood adults from the entire caged area of each tamarack (990 cm^2) was divided by 9.9 to yield the number of emergent brood adults per 100 cm^2.

Individual linear mixed effects models were used to regress the number of emergent brood per parent female against the fixed effects of DBH, phloem thickness, resin pocket density, attack history, age, growth rate, and associated competition. Identical methods were also used to analyze the number of emergent brood adults per 100 cm^2. In addition, all independent variables were fit into a model and backwards elimination as well as the AIC score (Akaike 1973) was used to determine the variables that most significantly affected each measure of eastern larch beetle reproductive success. A random effect with study site nested within beetle brood nested within

study year was used to account for potential variation in parent female reproductive success due to multiple study years, beetle broods, and study sites. The response variable was transformed as needed (*e.g.*, $\sqrt{y}$) in order to satisfy model assumptions of homoscedasticity and normality of errors.

Brood adult fitness. Brood adult size was first analyzed using a linear mixed effects model to test if the pronotal width of the brood adults varied by sex. The model incorporated a random effect of tamarack nested within study site within beetle brood within study year. Because there was not a sex-related size difference (ANOVA, $F_{1,790} = 0.8113$, $P = 0.368$) female and male beetles were pooled for further analysis.

For lipid content analysis, separate linear mixed effects models tested for sex-related differences in total and proportion lipid content of the brood adults. Each model used a random effect of tree nested within study site within beetle brood within study year. Significant sex-related differences existed for the total lipid content (ANOVA, $F_{1,787} = 15.552$, $P < 0.001$) and proportion lipid content (ANOVA, $F_{1,784} = 9.989$, $P = 0.00164$). As such, male and female beetles were analyzed separately for each measurement of lipid content.

Analyses of beetle pronotal width, total lipid content, and proportion lipid content were each analyzed with individual linear mixed effects models that regressed the appropriate response variable against the fixed effects of DBH, phloem thickness, resin pocket density, attack history, age, growth rate, and associated competition. Each response variable was transformed as needed (*e.g.*, $\sqrt{y}$, $\text{asin}\sqrt{y}$) in order to satisfy model assumptions of homoscedasticity and normality of errors.

A1.3 Results

A1.3.1 Beetle host selection

For the first round of eastern larch beetle host selection (*i.e.,* first generation, first sibling brood of 2011), tamarack DBH was the only tree character associated with eastern larch beetle attack. No other predictor variable tested was associated with eastern larch beetle host selection

(Table A1.1, Section A). The DBH of attacked (n = 28) tamaracks was significantly larger than that for the non-attacked counterparts (n= 104) (Table A1.2, Section A). In all, 28 tamaracks were attacked to create epicenters of beetle activity compared to the 104 randomly selected tamaracks that comprised the surrounding forest cover.

Similarly, tamarack DBH was associated with eastern larch beetle attack during the second period of beetle host selection (*i.e.,* first generation, second sibling brood of 2011) while all other measured variables were not significant (Table A1.1, Section B). Once again, attacked (n = 13) tamaracks were larger than tamaracks that were not attacked (n = 91) (Table A1.2, Section B). Among the attacked tamaracks, there were several significant, although somewhat weak differences between the trees that survived and those that were killed. In 2011, the surviving tamaracks tended to have a smaller DBH than the tamaracks that were killed, 16.1 ± 0.7 *vs.* 20.1 ± 1.7 cm (mean ± SE), respectively, although this difference was not significant (ANOVA, $F_{1,9}$ = 4.95, P = 0.053). However, the surviving relative to killed tamaracks had thinner phloem (2.3 ± 0.3 *vs.* 3.2 ± 0.3 mm, respectively) (ANOVA, $F_{1,9}$ = 5.68, P = 0.041), lower mean annual growth rate (BAI) (7.3 ± 1.8 *vs.* 17.9 ± 4.9 cm^2 / yr., respectively) (ANOVA, $F_{1,9}$ = 6.36, P = 0.033), lower densities of phloem resin pockets (4.4 ± 1.1 *vs*. 5.6 ± 1.3 per cm^2, respectively) (ANOVA, $F_{1,9}$ = 5.56, P = 0.043), and lower beetle attack densities (0.6 ± 0.2 *vs*. 1.8 ± 0.3 per 100 cm^2, respectively) (ANOVA, $F_{1,9}$ = 9.96, P = 0.012).

More host characteristics were significant to eastern larch beetles for the third period of host selection (*i.e.*, first generation, first sibling brood of 2012). Tamaracks that were larger were preferentially attacked, as observed previously. In addition, attacked tamaracks also possessed thicker phloem, greater growth rates, older age, and lower density of phloem resin pockets than tamaracks that were not attacked. A history of unsuccessful eastern larch beetle attack did not affect whether a tamarack was attacked for a second time. All tamaracks with a history of unsuccessful beetle attack were killed during this period of eastern larch beetle attack. Finally, inter- and intra-specific competition surrounding each tamarack was not associated with eastern larch beetle attack (Table A1.1, Section C) as competitive basal area was not different

surrounding attacked vs. non-attacked tamaracks (Table A1.2, Section C). In total, 70 tamaracks were attacked and killed while 27 tamaracks escaped attack.

The tamaracks that were attacked and killed by eastern larch beetles for the second generation, first sibling brood of 2012, were not associated with any of the measured tree characteristics relative to the tamaracks that escaped attack (Table A1.1, Section D) as these measures did not differ between the tamarack groups (Table A1.2, Section D). In total, seven tamaracks were attacked and killed while 17 remained non-attacked.

Likewise, tamaracks attacked during the final period of larch beetle host selection (*i.e.*, the first generation, first sibling brood of 2013) were also not associated with any measured tree characteristics (Table A1.1, Section E) as the measured variables were similar between tamarack groups (Table A1.2, Section E). Six tamaracks were attacked while 11 escaped attack.

A1.3.2 Tamarack characteristics associated with tree size

When all tamaracks that were attacked and killed throughout all years of the study were considered, tree DBH had a significant, positive association with phloem thickness (t_{39} = 6.22, P < 0.001) such that the largest trees had the thickest phloem (Fig. A1.1A). Similarly, tamarack DBH also had a positive relationship with the mean annual BAI (t_{46} = 6.59, P < 0.001) (Fig. A1.1B). Tree age had a weak relationship with DBH (t_{50} = 2.11, P = 0.040) (Fig. A1.1C). However, the phloem resin pocket density, a putative measure of tamarack constitutive defense, was not associated with DBH (t_{40} = -0.52, P = 0.61) (Fig. A1.1D).

A1.3.3 Beetle attack density

Across tamaracks attacked by eastern larch beetle in all years of the study, the density of beetle attacks per 100 cm^2 increased significantly with increases in tamarack DBH, phloem thickness, and growth rate but declined if a tamarack had been previously attacked unsuccessfully. The density of phloem resin pockets, tree age, and inter- and intraspecific competition did not influence beetle attack density (Table A1.3).

A1.3.4 Beetle reproductive success

Number of brood adults per female parent beetle. The number of offspring produced by each parent female within all successfully killed tamaracks from all study years was not dependent on tamarack DBH, phloem thickness, growth rate, or competitive basal area of each colonized tamarack. In addition, the density of attacking beetles did not affect beetle reproductive success (Table A1.7, Section A). In contrast, the reproductive success of parent females was found to increase with increasing tree age and when a host tree had a previous occurrence of being attacked unsuccessfully. However, greater densities of phloem resin pockets were associated with reduced numbers of offspring per female and a decline in parent female reproductive success (Table A1.4).

Number of brood adults per 100 cm^2 infested bark. The number of offspring produced per 100 cm^2 of bark increased significantly with increased tamarack DBH and declined with increasing phloem resin pocket density. Tamarack phloem thickness, growth rate, age, attack history, and surrounding competitive basal area did not influence offspring production per unit area (Table A1.5). The density of attacking parent beetles also had no effect on offspring production per unit area (Table A1.7, Section B). It should be noted, however, that the mean (± SE) total length (cm) of excavated parental gallery per 100 cm^2 was 52.8 ± 1.7 cm and did not vary with tamarack diameter (t_{44} = 1.90, P = 0.064).

A1.3.5 Comparisons of brood adult production in "challenged" and "naïve" tamaracks

For the tamaracks attacked and killed in the spring of 2012 for the first generation, first sibling brood, it was possible to compare the reproductive success of parent female eastern larch beetles within "challenged" tamaracks (*i.e.*, trees that had been unsuccessfully attacked in 2011) and "naïve" tamaracks (*i.e.*, trees with no recorded history of eastern larch beetle attack).

Naïve and challenged tamaracks had a similar mean (± SE) total length of parental gallery per 100 cm^2 of bark was 57.1 ± 3.2 and 48.5 ± 5.0 cm, respectively (ANOVA, $F_{1,18}$ = 1.04, P = 0.32). The parental gallery in naïve tamaracks was entirely excavated during 2012, was completely "clear" (*i.e.*, did not exhibit resin-filled portions), and female reproduction was

successful for the entire length of each gallery, as indicated by the presence of larval mines and pupal chambers. In contrast, the challenged tamaracks had a mean (± SE) length of 11.3 ± 2.3 cm of reproductively unsuccessful, resin-filled gallery per 100cm^2 (from the unsuccessful attacks of 2011). The resin-filled galleries were also characterized by the presence of callous tissue bordering the entire length of the gallery. In addition, to the resin-filled gallery, 37.2 ± 4.9 cm of clear, reproductively successful gallery was also present per 100 cm^2 (from the successful attacks of 2012). Therefore, the naïve tamaracks had significantly more reproductively successful parental gallery per 100 cm^2 relative to the challenged tamaracks (ANOVA, $F_{1,18}$ = 11.66, P = 0.0029).

Eastern larch beetle attack density was significantly lower on challenged tamaracks relative to naïve conspecifics with 1.4 ± 0.1 and 2.3 ± 0.1 attacks per 100 cm^2, respectively, (ANOVA, $F_{1,20}$ = 13.43, P = 0.0015) resulting in fewer parent females per 100 cm^2 in challenged versus naïve tamaracks (2.0 ± 0.1 *vs*. 3.5 ± 0.2, respectively) (ANOVA, $F_{1,20}$ = 13.43, P = 0.0015). Overall, the length of successful parental gallery per parent female was equal within challenged and naïve host trees (18.6 ± 2.8 *vs*. 17.3 ± 0.8 cm, respectively) (ANOVA, $F_{1,20}$ = 0.11, P = 0.75). Strikingly, however, female beetles within challenged tamaracks produced nearly 2-fold more offspring per female than the females within the naïve tamaracks (5.5 ± 0.8 *vs*. 2.9 ± 0.4, respectively) (ANOVA, $F_{1,19}$ = 7.21, P = 0.014). The number of offspring produced per 100 cm^2, remained the same between the challenged and naïve tamaracks, however at 10.7 ± 1.0 *vs.* 9.2 ± 1.2, respectively (ANOVA, $F_{1,21}$ = 1.32, P = 0.26). Physical host attributes for challenged and naïve tamaracks were similar between the two host tree groups. For challenged and naïve tamaracks, DBH measured 16.1 ± 0.7 and 18.8 ± 1.0 cm, respectively, (ANOVA, $F_{1,19}$ = 2.36, P = 0.14), phloem thickness measured 2.3 ± 0.3 and 2.9 ± 0.2 mm, respectively, (ANOVA, $F_{1,20}$ = 2.19, P = 0.15), phloem resin pocket density measured 4.4 ± 1.1 and 5.6 ± 0.6 pockets / cm^2, respectively, (ANOVA, $F_{1,20}$ = 1.64, P = 0.21), and tree growth rates (BAI) were 7.3 ± 1.8 and 19.8 ± 3.4 cm^2 / yr., respectively (ANOVA, $F_{1,19}$ = 1.13, P = 0.30).

A1.3.6 Fitness of emergent brood adults

Size of brood adults. The pronotal width of fall-emergent female and male offspring did not vary (ANOVA, $F_{1,790}$ = 0.81, P = 0.37) and was 1.64 ± 0.0036 mm (mean ± SE) when pooled. The pronotal width of the brood adults was positively influenced by greater host tree DBH and phloem thickness. Conversely, offspring size was not affected by the growth rate or age of the host tree, the amount of competitive stem basal area surrounding a host tree, or by the density of phloem resin pockets. Whether a tamarack had been previously attacked before being successfully colonized also did not influence offspring size (Table A1.6, Section A). The size of beetle progeny was similarly not affected by the density of colonizing parent beetles within the natal host trees (Table A1.8, Section A).

Total lipid content of brood adults. Male and female brood adults differed significantly in total lipid content (ANOVA, $F_{1,787}$ = 15.55, P < 0.001) with mean (± SE) values of 0.80 ± 0.02 and 0.93 ± 0.02 mg for males and females, respectively. For male beetles, total lipid content was only affected by the attack history of the natal host tamarack such that tamaracks that had been challenged previously by an unsuccessful beetle attack were associated with beetles with lower total lipid contents. No other tamarack metric measured in this study affected the total lipid content of male brood adults (Table A1.6, Section B). The total lipid content of female brood adults was affected by more host attributes than for male beetles. For female brood adults, tamaracks with a history of a previous unsuccessful attack resulted in lower total lipid content, while total lipid content increased with greater tamarack DBH and phloem thickness. Similar to male brood adults, the growth rate, density of phloem resin pockets, tree age, and competitive basal area was not associated with the total lipid content of the female brood adults (Table A1.6, Section C). The total lipid contents of either male or female progeny was affected by the density of the attacking parent beetles (Table A1.8, Sections B&C).

Proportion lipid content of brood adults. The proportion of dry mass attributed to the lipid content of the brood adults was significantly different between males and females. The mean ± SE proportion of beetle dry mass composed of lipid was 0.247 ± 0.005 for males and 0.271 ± 0.005 for females (ANOVA, $F_{1,784}$ = 9.99, P = 0.0016). For both male and female brood adults, proportion lipid content was affected only by the attack history of natal host trees. Brood adults

that emerged from tamaracks that had been unsuccessfully attacked prior to being successfully attacked and killed had lower proportional lipid content then brood adults that emerged from tamaracks with no previous beetle attack history prior to being killed. No other metrics of tree quality measured in this study influenced the proportional lipid content of brood adults of either sex (Table A1.6, Sections D&E). Similarly, parent beetle attack density was not found to influence the proportion lipid content of male and female offspring (Table A1.8, Sections D&E).

A1.4 Summary

Outbreak populations of eastern larch beetles preferentially attacked the largest host tamaracks during the initial stages of local infestations. However, once the largest host trees were killed, beetle host selection became random on the remaining host trees and was not associated with any recorded tamarack characteristics. Tamarack size was highly correlated with phloem thickness; however, phloem thickness was similar between attacked and non-attacked tamaracks from each period of eastern larch beetle host selection. Tamarack growth rate also did not influence beetle host selection. However, when all attacked and killed tamaracks were analyzed as a group, tree growth rate was highly correlated with tree size with the largest tamaracks being the most vigorous. Thus, by preferentially attacking the largest tamaracks in the stands, eastern larch beetles were also attacking the most vigorous specimens. This is the first study to demonstrate that eastern larch beetles will preferentially attack the most vigorous host trees available during outbreak conditions. Due to the low correlation of phloem resin pocket density with tree size as well as tree vigor, by preferentially attacking the largest and most vigorous trees, eastern larch beetles did not necessarily display a preference for attacking the tamaracks with the greatest level of constitutive defense. However, studies relating the induced resin response of tamaracks to tree vigor would be required to better understand how tamarack defense mediates the host selection behavior of eastern larch beetles. Nevertheless, the behavior of eastern larch beetles during this study of preferentially attacking the largest and most vigorous host trees during outbreak conditions is consistent with some of the most aggressive *Dendroctonus* bark beetles.

Eastern larch beetle attack density increased with increasing tamarack size, phloem thickness, and growth rate such that greater attack densities were recorded on larger and more vigorous host trees. Tamaracks that were challenged in 2011 with an unsuccessful beetle attack prior to being re-attacked and killed in the spring of 2012 had significantly lower attack densities relative to naïve tamaracks killed during the same period in 2012. This difference was apparently not due to tree size, phloem thickness, phloem resin pocket density, or growth rate, as these measurements were similar between the two tamarack groups.

Per capita reproductive success of female eastern larch beetles was not related to tamarack size, phloem thickness, or growth rate. However, phloem resin pocket density, tree age, and attack history did significantly affect female reproduction. Thus, there appears to be a disjunct relationship between the host attributes that are important for eastern larch beetle host selection and those that are important for reproduction. The total length of excavated egg gallery per 100 cm^2 did not vary with host size. Since attack density was greater in larger trees, each female beetle, therefore, apparently excavated proportionately less gallery. To achieve equal per capita reproductive success, female beetles using shorter galleries may have increased the number of eggs laid per centimeter of gallery. Alternately, females may have maintained a constant rate of linear egg deposition but increased brood survivorship in larger trees owing to thicker phloem may be responsible for the equal per capita reproductive rates despite the use of shorter egg galleries by females in larger trees with greater attack densities.

Offspring production per 100 cm^2 increased significantly as host size increased. The static per capita reproductive rate of eastern larch beetle females within hosts of all sizes, coupled with greater attack densities on larger tamaracks likely resulted in the increased offspring per 100 cm^2 observed in the larger tamaracks. The rate of increase in the number of offspring per 100 cm^2 displayed a linear rather than exponential trend indicating intraspecific competition for phloem resources. Although larger tamaracks produce more eastern larch beetle offspring both in total number per tree and per unit area within a tree, it appears that for eastern larch beetles to successfully overwhelm healthy trees and secure a successful breeding substrate, the

beetles are required to attack these hosts at densities that ultimately promote intraspecific competition.

One interesting aspect of the reproductive success of female eastern larch beetles is that the beetles were able to achieve population replacement and growth in trees as small as 10 cm DBH, with equivalent reproduction in hosts up to 30 cm. The ability of eastern larch beetles to achieve relatively good reproductive success in small diameter hosts may make epidemic populations of eastern larch beetles more resilient to population crashes once the largest host trees are exhausted within a stand. Moreover, this ability may be an adaptation for survival when generally having to utilize the phloem of weakened or downed host material which may often have thinner, desiccated phloem.

Within challenged tamaracks, eastern larch beetles achieved per capita reproductive success that was almost twice that of what was observed in naïve hosts, despite these groups of host trees being similar in many other apparent aspects (*e.g.*, size, phloem thickness, growth rate). The reproductive success of beetles per 100 cm^2 was equivalent between challenged and naïve hosts, however, because the colonization density was much lower on the challenged tamaracks. Nevertheless, this study demonstrates the potential importance of stressed and moribund tamaracks for eastern larch beetle reproductive success and the rapid build-up of beetle populations that could potentially lead to outbreaks.

The size (pronotal width) of emergent brood adults increased with increasing tamarack size and phloem thickness. Thus, the selection of larger hosts by parent beetles allows the production of larger offspring. Total lipid content of emergent male and female offspring was reduced when development occurred in challenged tamaracks. Further, the total lipid content of female offspring increased with both increased host size and phloem thickness. The proportion lipid content of both male and female emergent offspring declined when development occurred in challenged tamaracks. No other measured host characteristic influenced the proportional lipid content of eastern larch beetle offspring. Regarding challenged tamaracks, the results of this study suggest that such trees are more easily colonized by eastern larch beetles and allow greater per capita reproductive rates for parent female beetles. However, offspring produced

within challenged tamaracks appear to contain fewer lipids in total and as a proportion of body size, potentially reducing the fitness of such offspring. Offspring size and lipid content was not influenced by the density of parent female beetles within successfully attacked and colonized tamaracks.

A1.5 Acknowledgements

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A1.6 Tables

Table A1.1 Effect of tamarack host quality on eastern larch beetle host selection. Each regression is fit using a generalized linear model with a logit link (*i.e.,* back-transform *y* as $e^y / (1 + e^y)$).

Predictor variable	Intercept statistics			Predictor statistics		
	Estimate (Std. error)	*z*-value	*P*- value	Estimate (Std. error)	*z*-value	*P*- value
Section A: Host selection for the first generation, first sibling brood of 2011						
DBH (cm)	**-7.62 (1.53)**	**-4.96**	**< 0.001**	**0.33 (0.077)**	**4.31**	**< 0.001**
Phloem thickness (mm)	.	.	.	.	.	.
Mean BAI (cm^2/yr.)	-1.87 (0.37)	-4.98	< 0.001	0.033 (0.017)	1.92	0.055
Resin pocket density (no./cm^2)	.	.	.	.	.	.
Age (yr.)	-2.73 (1.45)	-1.89	0.059	0.033 (0.033)	1.00	0.32
Competitive basal area (m^2/ha.)	-1.12 (0.49)	-2.28	0.023	-0.011 (0.026)	-0.42	0.67
Attack history (0/1)	.	.	.	.	.	.
Section B: Host selection for the first generation, second sibling brood of 2011						
DBH (cm)	**-6.51 (2.00)**	**-3.26**	**0.0011**	**0.21 (0.10)**	**2.15**	**0.032**
Phloem thickness (mm)	-4.81 (1.80)	-2.68	0.0074	0.75 (0.55)	1.36	0.17
Mean BAI (cm^2/yr.)	-2.93 (0.67)	-4.37	< 0.001	0.023 (0.031)	0.73	0.46
Resin pocket density (no./cm^2)	-2.22 (0.78)	-2.86	0.0042	-0.055 (0.12)	-0.46	0.64
Age (yr.)	-3.77 (2.71)	-1.39	0.16	0.028 (0.063)	0.45	0.65
Competitive basal area (m^2/ha.)	-2.02 (0.84)	-2.42	0.016	-0.034 (0.048)	-0.69	0.49
Attack history (0/1)	--	--	--	--	--	--

Table A1.1 (Continued)

Predictor variable	Intercept statistics			Predictor statistics		
	Estimate (Std. error)	*z*-value	*P*- value	Estimate (Std. error)	*z*-value	*P*- value
Section C: Host selection for the first generation, first sibling brood of 2012						
DBH (cm)	**-8.31 (2.37)**	**-3.51**	**< 0.001**	**0.58 (0.14)**	**4.11**	**< 0.001**
Phloem thickness (mm)	**-1.86 (1.56)**	**-1.19**	**0.24**	**1.17 (0.47)**	**2.48**	**0.013**
Mean BAI (cm^2/yr.)	**-0.35 (1.04)**	**-0.33**	**0.74**	**0.11 (0.044)**	**2.57**	**0.010**
Resin pocket density (no./cm^2)	**3.15 (0.93)**	**3.38**	**< 0.001**	**-0.20 (0.068)**	**-2.94**	**0.0032**
Age (yr.)	**-7.33 (2.50)**	**-2.93**	**0.0034**	**0.21 (0.062)**	**3.30**	**<0.001**
Competitive basal area (m^2/ha.)	1.41 (0.96)	1.46	0.14	-0.017 (0.039)	-0.43	0.67
Attack history (0/1)	1.052 (0.63)	1.67	0.096	19.69 (512.00)	0.038	0.97
Section D: Host selection for the second generation, first sibling brood of 2012						
DBH (cm)	-6.08 (2.96)	-2.05	0.041	0.36 (0.20)	1.81	0.071
Phloem thickness (mm)	-0.78 (1.77)	-0.44	0.66	-0.040 (0.61)	-0.065	0.95
Mean BAI (cm^2/yr.)	-0.67 (0.81)	-0.84	0.40	-0.020 (0.066)	-0.31	0.76
Resin pocket density (no./cm^2)	-0.85 (1.12)	-0.76	0.45	-0.004 (0.10)	-0.040	0.97
Age (yr.)	-2.09 (2.64)	-0.79	0.43	0.032 (0.069)	0.47	0.64
Competitive basal area (m^2/ha.)	-1.63 (1.09)	-1.49	0.14	0.051 (0.066)	0.77	0.44
Attack history (0/1)	--	--	--	--	--	--

Table A1.1 (Continued)

Predictor variable	Intercept statistics			Predictor statistics		
	Estimate (Std. error)	*z*-value	*P*- value	Estimate (Std. error)	*z*-value	*P*- value
Section E: Host selection for the first generation, first sibling brood of 2013						
DBH (cm)	-1.97 (3.14)	-0.63	0.53	0.10 (0.23)	0.44	0.66
Phloem thickness (mm)	-1.69 (2.54)	-0.66	0.51	0.40 (0.92)	0.44	0.66
Mean BAI (cm^2/yr.)	-0.14 (2.36)	-0.057	0.95	-0.26 (0.26)	-1.01	0.31
Resin pocket density (no./cm^2)	0.40 (1.17)	0.34	0.73	-0.091 (0.099)	-0.92	0.36
Age (yr.)	-47.77 (24.96)	-1.91	0.056	0.73 (0.42)	1.73	0.084
Competitive basal area (m^2/ha.)	1.79 (1.49)	1.20	0.23	-0.19 (0.12)	-1.64	0.10
Attack history (0/1)	--	--	--	--	--	--

Table A1.2 Means (± SE) for tamarack host quality across five different periods of eastern larch beetle host selection.

Predictor variable	Predictor mean ± SE	
	Attacked	Non-attacked
Section A: Host selection for the first generation, first sibling brood of 2011		
DBH (cm)	**20.8 ± 0.4a**	**17.1 ± 0.3b**
Phloem thickness (mm)	.	.
Mean BAI (cm^2/yr.)	19.3 ± 2.5a	14.5 ± 1.1a
Resin pocket density (no./cm^2)	.	.
Age (yr.)	43.4 ± 1.2a	42.0 ± 0.6a
Competitive basal area (m^2/ha.)	16.6 ± 1.2a	17.4 ± 0.8a
Attack history (0/1)	.	.
Section B: Host selection for the first generation, second sibling brood of 2011		
DBH (cm)	**20.1 ± 1.7a**	**16.9 ± 0.4b**
Phloem thickness (mm)	3.2 ± 0.3a	2.9 ± 0.07a
Mean BAI (cm^2/yr.)	17.9 ± 4.9a	14.7 ± 1.1a
Resin pocket density (no./cm^2)	5.6 ± 1.3a	6.3 ± 0.4a
Age (yr.)	42.9 ± 1.9a	41.7 ± 0.7a
Competitive basal area (m^2/ha.)	15.1 ± 3.8a	17.4 ± 0.9a
Attack history (0/1)	.	.
Section C: Host selection for the first generation, first sibling brood of 2012		
DBH (cm)	**17.9 ± 0.4a**	**14.1 ± 0.5b**
Phloem thickness (mm)	**3.1 ± 0.1a**	**2.8 ± 0.1b**
Mean BAI (cm^2/yr.)	**14.9 ± 1.2a**	**10.6 ± 1.4b**
Resin pocket density (no./cm^2)	**6.7 ± 0.4a**	**10.1 ± 0.9b**
Age (yr.)	**43.7 ± 0.7a**	**37.4 ± 1.2b**
Competitive basal area (m^2/ha.)	18.5 ± 1.0a	15.1 ± 1.6a
Attack history (0/1)	.	.
Section D: Host selection for the second generation, first sibling brood of 2012		
DBH (cm)	15.6 ± 1.0a	13.4 ± 0.5a
Phloem thickness (mm)	2.8 ± 0.4a	2.8 ± 0.2a
Mean BAI (cm^2/yr.)	9.9 ± 2.2a	11.0 ± 2.0a
Resin pocket density (no./cm^2)	10.2 ± 1.7a	10.2 ± 1.1a
Age (yr.)	38.3 ± 0.9a	36.9 ± 1.9a
Competitive basal area (m^2/ha.)	15.7 ± 3.8a	13.4 ± 1.3a
Attack history (0/1)	.	.
Section E: Host selection for the first generation, first sibling brood of 2013		
DBH (cm)	13.8 ± 1.0a	13.3 ± 0.7a
Phloem thickness (mm)	2.8 ± 0.2a	2.6 ± 0.2a
Mean BAI (cm^2/yr.)	6.1 ± 1.3a	8.8 ± 1.8a
Resin pocket density (no./cm^2)	9.8 ± 2.3a	12.4 ± 1.8a
Age (yr.)	37.8 ± 0.7a	36.5 ± 2.9a
Competitive basal area (m^2/ha.)	10.3 ± 1.0a	15.0 ± 1.8a
Attack history (0/1)	.	.

Table A1.3 Effect of tamarack host quality on eastern larch beetle attack density. Attack density is transformed log(*y*+1).

Predictor variable	Intercept statistics				Predictor statistics			
	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value
DBH (cm)	**0.16 (0.17)**	**1,37**	**0.93**	**0.36**	**0.032 (0.0090)**	**1,41**	**3.49**	**0.0012**
Phloem thickness (mm)	**0.24 (0.16)**	**1,38**	**1.51**	**0.14**	**0.15 (0.052)**	**1 39**	**2.94**	**0.0055**
Mean BAI (cm^2/yr.)	**0.57 (0.062)**	**1,14**	**9.11**	**< 0.001**	**0.011 (0.0028)**	**1,46**	**3.72**	**< 0.001**
Resin pocket density (no./cm^2)	0.62 (0.11)	1,32	5.53	< 0.001	0.013 (0.016)	1,37	0.80	0.43
Age (yr.)	1.13 (0.37)	1,32	3.07	0.0044	-0.0097 (0.0086)	1,30	-1.13	0.27
Competitive basal area (m^2/ha.)	0.88 (0.096)	1,19	9.14	< 0.001	-0.0088 (0.0048)	1,48	-1.83	0.074
Attack history (0/1)	**0.78 (0.048)**	**1,2**	**16.24**	**0.0013**	**-0.44 (0.12)**	**1,46**	**-3.77**	**< 0.001**

Table A1.4 Effect of tamarack quality on eastern larch beetle reproductive success (no. of emergent brood adults per parent female). Reproductive success is transformed log(*y*).

Predictor variable	Intercept statistics				Predictor statistics			
	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value
DBH (cm)	0.33 (0.48)	1,34	0.68	0.50	0.034 (0.026)	1,39	1.33	0.19
Phloem thickness (mm)	0.96 (0.49)	1,30	1.97	0.058	-0.018 (0.16)	1,39	-0.11	0.91
Mean BAI (cm^2/yr.)	0.93 (0.18)	1,26	5.16	< 0.001	$8.37x10^{-4}$ (0.0084)	1,47	0.099	0.92
Resin pocket density (no./cm^2)	**1.51 (0.28)**	**1,12**	**5.38**	**< 0.001**	**-0.11 (0.043)**	**1,38**	**-2.51**	**0.017**
Age (yr.)	**-0.85 (0.83)**	**1,51**	**-1.01**	**0.32**	**0.043 (0.020)**	**1,51**	**2.16**	**0.035**
Competitive basal area (m^2/ha.)	0.61 (0.23)	1,31	2.59	0.014	0.022 (0.013)	1,49	1.73	0.090
Attack history (0/1)	**0.84 (0.17)**	**1,1**	**4.98**	**0.076**	**0.86 (0.33)**	**1,51**	**2.61**	**0.012**

Table A1.5 Effect of tamarack quality on the reproductive success of female eastern larch beetles (no. emergent brood adults per 100 cm^2). Reproductive success is transformed log(*y*).

Predictor variable	Intercept statistics				Predictor statistics			
	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value
DBH (cm)	**0.81 (0.45)**	**1,51**	**1.81**	**0.076**	**0.069 (0.024)**	**1,51**	**2.89**	**0.0057**
Phloem thickness (mm)	1.53 (0.49)	1,33	3.10	0.0040	0.014 (0.16)	1,39	0.90	0.38
Mean BAI (cm^2/yr.)	1.87 (0.25)	1,2	7.38	0.014	0.0089 (0.0081)	1,50	1.09	0.28
Resin pocket density (no./cm^2)	**2.48 (0.33)**	**1,4**	**7.43**	**0.0012**	**-0.095 (0.044)**	**1,36**	**-2.16**	**0.037**
Age (yr.)	0.97 (0.87)	1,45	1.12	0.27	0.025 (0.020)	1,50	1.22	0.23
Competitive basal area (m^2/ha.)	1.81 (0.30)	1,3	6.05	0.0040	0.013 (0.013)	1,49	1.01	0.32
Attack history (0/1)	1.96 (0.26)	1,1	7.63	0.030	0.40 (0.34)	1,50	1.17	0.25

Table A1.6 Effect of tamarack quality on eastern larch beetle brood adult fitness.

Predictor variable	*y* trans.	Intercept statistics				Predictor statistics			
		Estimate (Std. error)	Num,Den df	*t*-value	*P*- value	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value
Section A: Pronotal width (mm) of male and female brood adults (pooled)									
DBH (cm)	**log(*y*)**	**0.44 (0.019)**	**1,7**	**23.82**	**< 0.001**	**0.0025 ($9.82x10^{-4}$)**	**1,10**	**2.58**	**0.027**
Phloem thickness (mm)	**log(*y*)**	**0.45 (0.013)**	**1,25**	**34.11**	**< 0.001**	**0.013 (0.0046)**	**1,24**	**2.76**	**0.011**
Mean BAI (cm^2/yr.)	log(*y*)	0.48 (0.0077)	1,5	62.52	< 0.001	$2.94x10^{-4}$ ($3.24x10^{-4}$)	1,50	0.91	0.37
Resin pocket density (no./cm^2)	log(*y*)	0.48 (0.010)	1,27	47.26	< 0.001	$1.36x10^{-4}$ (0.0017)	1,26	0.079	0.94
Age (yr.)	log(*y*)	0.49 (0.033)	1,40	15.00	< 0.001	$-4.30x10^{-5}$ ($7.6x10^{-4}$)	1,41	-0.057	0.96
Competitive basal area (m^2/ha.)	log(*y*)	0.50 (0.010)	1,15	49.67	< 0.001	$-4.12x10^{-4}$ ($5.2x10^{-4}$)	1,50	-0.80	0.43
Attack history (0/1)	log(*y*)	0.49 (0.0072)	1,2	67.40	<0.001	0.011 (0.012)	1,40	0.98	0.33
Section B: Total lipid content (mg) of male brood adults									
DBH (cm)	log(*y*+1)	0.46 (0.085)	1,18	5.44	< 0.001	0.0056 (0.0046)	1,18	1.21	0.24
Phloem thickness (mm)	log(*y*+1)	0.48 (0.069)	1,24	6.90	< 0.001	0.020 (0.025)	1,24	0.80	0.43
Mean BAI (cm^2/yr.)	log(*y*+1)	0.58 (0.041)	1,2	14.13	0.0012	-0.0012 (0.0015)	1,50	-0.78	0.44
Resin pocket density (no./cm^2)	log(*y*+1)	0.56 (0.049)	1,8	11.47	< 0.001	-0.0052 (0.0078)	1,24	-0.67	0.51
Age (yr.)	log(*y*+1)	0.25 (0.16)	1,35	1.57	0.12	0.0073 (0.0037)	1,35	1.96	0.058
Competitive basal area (m^2/ha.)	log(*y*+1)	0.53 (0.050)	1,13	10.64	< 0.001	0.0020 (0.0024)	1,48	0.84	0.41
Attack history (0/1)	**log(*y*+1)**	**0.58 (0.023)**	**1,1**	**24.86**	**0.0031**	**-0.12 (0.047)**	**1,32**	**-2.46**	**0.020**

Table A1.6 (Continued)

Predictor variable	*y* trans.	Intercept statistics				Predictor statistics			
		Estimate (Std. error)	Num,Den df	*t*-value	*P*- value	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value
Section C: Total lipid content (mg) of female brood adults									
DBH (cm)	**log(*y*+1)**	**0.45 (0.077)**	**1,27**	**5.85**	**< 0.001**	**0.0096 (0.0040)**	**1,23**	**2.36**	**0.027**
Phloem thickness (mm)	**log(*y*+1)**	**0.44 (0.071)**	**1,27**	**6.23**	**< 0.001**	**0.059 (0.024)**	**1,25**	**2.40**	**0.024**
Mean BAI (cm^2/yr.)	log(*y*+1)	0.64 (0.037)	1,4	17.50	< 0.001	-6.14×10^{-4} (0.0015)	1,30	-0.41	0.69
Resin pocket density (no./cm^2)	log(*y*+1)	0.63 (0.053)	1,26	11.86	< 0.001	-0.0052 (0.0091)	1,24	-0.57	0.57
Age (yr.)	log(*y*+1)	0.48 (0.15)	1,21	3.23	0.0040	0.0035 (0.0034)	1,20	1.04	0.31
Competitive basal area (m^2/ha.)	log(*y*+1)	0.60 (0.044)	1,13	13.72	< 0.001	0.016 (0.0023)	1,36	0.68	0.50
Attack history (0/1)	**log(*y*+1)**	**0.65 (0.018)**	**1,7**	**37.07**	**< 0.001**	**-0.16 (0.041)**	**1,27**	**-3.84**	**< 0.001**
Section D: Proportion lipid content of male brood adults									
DBH (cm)	asin(√*y*)	0.51 (0.049)	1,17	10.46	< 0.001	-3.55×10^{-5} (0.0027)	1,16	-0.013	0.99
Phloem thickness (mm)	asin(√*y*)	0.48 (0.044)	1,18	10.87	< 0.001	0.011 (0.015)	1,26	0.73	0.47
Mean BAI (cm^2/yr.)	asin(√*y*)	0.53 (0.019)	1,15	28.34	< 0.001	-0.0012 (8.71×10^{-4})	1,46	-1.35	0.18
Resin pocket density (no./cm^2)	asin(√*y*)	0.51 (0.031)	1,10	16.44	< 0.001	-4.83×10^{-4} (0.0048)	1,24	-0.10	0.92
Age (yr.)	asin(√*y*)	0.35 (0.092)	1,28	3.79	< 0.001	0.0037 (0.0021)	1,27	1.74	0.092
Competitive basal area (m^2/ha.)	asin(√*y*)	0.50 (0.025)	1,20	19.88	< 0.001	2.68×10^{-4} (0.0014)	1,31	0.20	0.85
Attack history (0/1)	**asin(√*y*)**	**0.52 (0.0099)**	**1,33**	**52.56**	**< 0.001**	**-0.085 (0.025)**	**1,30**	**-3.44**	**0.0017**

Table A1.6 (Continued)

Predictor variable	*y* trans.	Intercept statistics				Predictor statistics			
		Estimate (Std. error)	Num,Den df	*t*-value	*P*- value	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value
Section E: Proportion lipid content of female brood adults									
DBH (cm)	asin(√*y*)	0.53 (0.048)	1,36	11.11	< 0.001	3.62×10^{-4} (0.0025)	1,33	0.14	0.89
Phloem thickness (mm)	asin(√*y*)	0.48 (0.044)	1,29	11.08	< 0.001	0.020 (0.015)	1,29	1.29	0.21
Mean BAI (cm^2/yr.)	asin(√*y*)	0.55 (0.017)	1,46	32.80	< 0.001	-7.41×10^{-4} (8.30×10^{-4})	1,46	-0.89	0.38
Resin pocket density (no./cm^2)	asin(√*y*)	0.55 (0.031)	1,29	17.91	< 0.001	-0.0028 (0.0053)	1,27	-0.52	0.61
Age (yr.)	asin(√*y*)	0.53 (0.079)	1,35	6.66	< 0.001	2.54×10^{-4} (0.0018)	1,33	0.14	0.89
Competitive basal area (m^2/ha.)	asin(√*y*)	0.53 (0.023)	1,40	23.19	< 0.001	1.91×10^{-4} (0.0013)	1,42	0.15	0.88
Attack history (0/1)	**asin(√*y*)**	**0.55 (0.011)**	**1,3**	**48.62**	**< 0.001**	**-0.10 (0.024)**	**1,34**	**-4.32**	**< 0.001**

Table A1.7 Effect of female parent eastern larch beetle density (no. per 100 cm^2) on the reproductive success of female eastern larch beetles. Female parent density is transformed log(*y*).

Predictor variable	Intercept statistics				Predictor statistics			
	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value
Section A: Number of emergent brood adults per parent female								
Density of female parent beetles (no. per 100 cm^2)	1.43 (0.41)	1,16	3.50	0.0031	-0.17 (0.11)	1,50	-1.49	0.14
Section B: Number of emergent brood adults per 100 cm^2 bark								
Density of female parent beetles (no. per 100 cm^2)	1.56 (0.41)	1,15	3.82	0.0017	0.14 (0.11)	1,50	1.24	0.22

Table A1.8 Effect of female parent eastern larch beetle density (no. per 100 cm^2) on the fitness of emergent brood adults.

Predictor variable	*y* trans.	Intercept statistics				Predictor statistics			
		Estimate (Std. error)	Num,Den df	*t*-value	*P*- value	Estimate (Std. error)	Num,Den df	*t*-value	*P*- value
Section A: Pronotal width (mm) of male and female brood adults (pooled)									
Density of female parent beetles (no. per 100 cm^2)	log(*y*)	0.49 (0.015)	1,24	33.10	< 0.001	$4.18x10^{-4}$ (0.0042)	1,39	0.10	0.92
Section B: Total lipid content (mg) of male brood adults									
Density of female parent beetles (no. per 100 cm^2)	log(*y*+1)	0.50 (0.064)	1,19	7.74	< 0.001	0.20 (0.018)	1,28	1.12	0.27
Section C: Total lipid content (mg) of female brood adults									
Density of female parent beetles (no. per 100 cm^2)	log(*y*+1)	0.56 (0.063)	1,18	8.98	< 0.001	0.020 (0.018)	1,27	1.09	0.29
Section D: Proportion lipid content of male brood adults									
Density of female parent beetles (no. per 100 cm^2)	asin(√*y*)	0.47 (0.035)	1,16	13.57	<0.001	0.012 (0.010)	1,26	1.13	0.27
Section E: Proportion lipid content of female brood adults									
Density of female parent beetles (no. per 100 cm^2)	asin(√*y*)	0.51 (0.034)	1,35	14.79	< 0.001	0.0087 (0.010)	1,34	0.85	0.40