Pine Wars: A New Host
Interactions between the mountain pine beetle (*Dendroctonus ponderosae*
Hopkins) and its pine hosts in Canada's boreal forest

by

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Abstract

Mountain pine beetle (MPB) has undergone a climate change facilitated range expansion and has attacked and killed trees at higher latitudes and elevations than has ever been recorded. During outbreaks, MPB attack large healthy pine trees that will fight back against the colonizing beetles using physical and chemical defenses. Attacking beetles communicate with pheromones and will cooperatively "mass attack" and kill these trees. If they win the battle against the tree, the beetles are rewarded with abundant resources under the bark but if they lose, they are poisoned or consumed by pitch. In my PhD research I studied the battle between MPB and its hosts in Alberta. Trees in the expanded range have fewer constitutive defenses and when challenged with simulated MPB attacks, are not able to produce as many toxic chemical defenses as trees in the historic range of MPB. Lodgepole pine is the most common historic host of MPB but the lodgepole pines in Alberta do not have a shared evolutionary history with MPB. I tested the hypothesis that naïve lodgepole pines are more susceptible to mass attack by performing mass attack manipulation experiments in the field. I used aggregation pheromone to attract wild MPB to experimental lodgepole pine trees and stopped the process of attack at different attack densities to determine the minimum density of beetles that can successfully colonize and kill Albertan lodgepole pines. I found variation in the threshold for mass attack across three years of experiments which was best explained by changes in environmental conditions that influenced tree defense. I then performed a similar experiment in jack pine, which is a novel host of MPB. Since there were no wild MPB populations in jack pine stands, I collected MPB from a lodgepole pine forest and transplanted them under caged jack pine trees. The mass attack threshold density in jack pine was half the beetle density typically seen in lodgepole pine which is strong evidence that jack pine is more susceptible to MPB. During the mass attack experiments, I also collected phloem tissue samples to quantify tree chemical

response to MPB attack. I found that neither species increased terpene defenses in the 3−6 weeks after mass attack but all trees had large increases in terpene content the spring following attack, even if the mass attack was unsuccessful. Finally, I performed laboratory experiments to test the preference and performance of MPB that emerged from mass attacked lodgepole and jack pines. I found that beetles that switched hosts from lodgepole pine to jack pine had offspring that were in significantly worse condition compared to beetles that did not switch hosts or switched from jack to lodgepole pine. Although jack pine has a lower mass attack threshold and does not induce defenses quickly, there is a cost to switching from lodgepole pine to jack pine. Jack pine has a thinner phloem layer and so likely has fewer resources available for developing MPB brood. However, adaptation to jack pine could still take place.

Preface

A version of Chapter 2 of this thesis is intended for publication as Musso AE, Shegelski V, Carroll AL, and Evenden ML. Attack threshold and optimal attack density of mountain pine beetle (*Dendroctonus ponderosae*; Coleoptera: Curculionidae) in naïve lodgepole pine (*Pinus contorta* var. *latifolia*). Dr. Allan Carroll and Dr. Maya Evenden conceived of the original experimental design, and I contributed to the final design. I performed the experiment, collected and analyzed the data, and wrote the first draft of the chapter. Dr. Evenden provided feedback on this and further drafts.

A version of Chapter 3 of this thesis is intended for publication as Musso AE, Carroll AL, and Evenden ML. Attack threshold and optimal attack density of mountain pine beetle (*Dendroctonus ponderosae*; Coleoptera: Curculionidae) in the novel host jack pine (*Pinus banksiana*). I designed the experiment with the advice of Dr. Evenden and Dr. Carroll. I performed the experiment, collected and analyzed the data, and wrote the first draft of the chapter. Dr. Evenden and Dr. Carroll provided feedback on this chapter.

A version of Chapter 4 of this thesis was published as: Musso AE, Fortier C, Huber DPW, Carroll AL, and Evenden ML. Naïve pine terpene response to mountain pine beetle (*Dendroctonus ponderosae*) through the seasons. Journal of Chemical Ecology 2023 https://doi.org/10.1007/s10886-023-01418-1 I contributed to the experimental design along with Dr. Dezene Huber, Dr. Evenden, and Dr. Carroll. I performed the experiment and collected the data. I analyzed the data along with Dr. Colleen Fortier. I wrote the manuscript draft and Dr. Evenden, Dr. Huber, Dr. Carroll, and Dr. Fortier provided feedback.

A version of Chapter 5 of this thesis is intended for publication as Musso AE, Carroll AL, and Evenden ML. Performance and preference of the mountain pine beetle, *Dendroctonus ponderosae* Hopk., in lodgepole and jack pine bolts. I designed the experiments with the advice of Dr. Carroll and Dr. Evenden. I performed the experiments, collected and analyzed the data, and wrote the first draft. Dr. Evenden provided feedback on this chapter.

Dedication

For the healthy trees felled for the experiments in this thesis.

Acknowledgements

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A lot of the work in this thesis was done in the field and I was very fortunate to work with three incredible research assistants. Valerie Marshall without whom I would not have survived summer 2017. We did four field experiments in four very different locations, lived off spaghetti, hotdogs, and Crooked Creek donuts, and drove 43 thousand kilometers in four months. It was so, so difficult and somehow, we had fun? In 2018, Felysia Green helped me construct all kinds of solutions to all the wild things we had to do to safely bring to and release 26 thousand MPB in a jack pine forest. In 2019, there were no more beetles all experiment plans collapsed. Dixie Paches, who was excited for a summer of field work,

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Chapter 1.

1.1. General Introduction

One of the most obvious observed effects of climate change is that many living things have shifted and/or expanded their geographic ranges (McCarty 2001). Plants and animals in both terrestrial and aquatic systems have experienced shifts in geographic range to higher altitudes, higher latitudes, and greater depths (Böhning-Gaese and Lemoine 2004, MacLean and Beissinger 2017, Pinsky et al. 2020). Organisms that enter new habitats as environmental conditions become suitable, will only persist in these new habitats if they can establish a niche or ecological fit (Janzen 1985). Insects, for example, have undergone rapid geographic range expansions (Hill et al. 2011) and have increased winter survival (Marshall et al. 2020) due to climate change. Many agricultural and forest pests are herbivorous insects, so range shifts of herbivorous insects that are pests in agricultural and forest ecosystems are of particular interest (Battisti and Larsson 2015). Most insect herbivores are specialists and can only efficiently detect, consume, and reproduce on one, or very few host species (Forister et al. 2015). Herbivorous insects that require a plant host for both food and reproduction are obligate symbionts, and their niche relies completely on biotic interactions with the host plant (Mestre et al. 2020).

Niches are generally divided into two parts, the fundamental and realized niche (Hutchinson 1957, Pulliam 2000). The fundamental niche is a multidimensional space that contains environmental conditions that are suitable for an organism to live in, while the realized niche is the space the organism occupies and is limited by biotic interactions such as competition (Hutchinson 1957). Interpretation of the fundamental niche in biogeography, however, does not limit restrictions on the fundamental niche to biotic interactions but also to dispersal and demographic limitations of the organism (Pulliam 2000, Colwell and Rangel 2009). A specialist insect herbivore's niche can also be also divided into the fundamental niche, which is comprised of both environmental and host traits, but the realized niche can extend past the fundamental niche (Mestre et al. 2020). When host plants have a larger range of tolerance for some environmental condition than the associated herbivore, there can be a mismatch in their distributions and although suitable hosts are present, the environmental requirements of the fundamental niche are not met (Mestre et al. 2020). Climate change can improve the environmental conditions of previously inaccessible host populations and resolve the distribution mismatch (Harms et al. 2021).

Plants have adapted to insect herbivory by evolving specific and complex physical and chemical defense systems (Franceschi et al. 2005, Howe and Jander 2008). Once insects have entered newly suitable habitat, they can encounter plants that are the same species as the plants in their historic range, but the plants are not adapted to tolerate or defend themselves against new herbivory. This can be termed 'defense free space' (Gandhi and Herms 2010) akin to enemy free space (Jeffries and Lawton 1984). Defence-free space could further facilitate the range expansion of an insect herbivore (Jeffries and Lawton 1984), particularly if the insect is able to detect and consume different host species (Bridle et al. 2013). Novel hosts in the expanded range present an opportunity for host switching (Malcicka et al. 2015). Novel host species may have fewer defenses against the specific herbivory pressure applied by a range-expanding insect, but the insect must have additional traits that allow establishment in the area, in a process called ecological fitting (Janzen 1985, Araujo et al. 2015). When they enter new habitat, insects are more likely to switch hosts when the novel host shares a recent evolutionary history with (Mech et al. 2019), or has physical or chemical similarities to, the historic host (Cipollini and Peterson 2018).

Irruptive forest insects are major drivers of forest disturbance and succession (Cooke et al. 2007) and experience range changes and expansions in response to climate change (Aukema et al. 2008, Raffa et al. 2015a, Pureswaran et al. 2018). Insects with irruptive or cyclic population dynamics have two distinct population states: i) low density states, that cause negligible to no host damage and ii) high density states, that result in significant damage to hosts at large spatial scales. Insect herbivores that display cyclical population dynamics occur in an array of insect orders including Lepidoptera (Royama 1984, Berryman 1996), Hemiptera (McClure 1991), Diptera (Isaev et al. 1988), Hymenoptera (Geri 1988), and Coleoptera (Boone et al. 2011, Howe et al. 2022). The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae) is one of the most notorious forest insects in North America and has undergone a climate change-facilitated range expansion (Carroll et al. 2006b, Safranyik et al. 2010, de la Giroday et al. 2012, Sambaraju et al. 2019, Sambaraju and Goodsman 2021). Mountain pine beetles use trees in the genus *Pinus* as hosts and, except for the dispersing adult stage, spend their entire life cycle under the bark of a host tree (Fig 1.1). Mountain pine beetle's main hosts in north-western North America are lodgepole pine (*Pinus contorta* Douglas Ex. Loud. var. *latifolia* Engelman), ponderosa pine (*Pinus ponderosa* Dougl. Ex. P. Lawson & C. Lawson), and western white pine (*Pinus monticola* Dougl. Ex. D. Don; Safranyik and Carroll 2006). Mountain pine beetle has a relatively broad host range and can attack and successfully

reproduce in as many as 22 pine species and two spruce (*Picea*) species (Wood 1963, Furniss and Schenk 1969, Huber et al. 2009).

Mountain pine beetle has an obligatory flight period following adult emergence from the natal host and prior to colonization of a new tree (Wood 1982; Fig. 1.1). Most beetles that disperse only fly short distances under the canopy within a tree stand, but long-distance dispersal occurs when beetles move above the canopy and can be transported over hundreds of kilometers aided by wind (Safranyik et al. 1992, Chen and Walton 2011, de la Giroday et al. 2012). There remains disagreement on how MPB locate and choose hosts during the colonization process (Raffa et al. 2016). Females are the pioneers in host colonization and select suitable hosts before recruiting conspecific beetles for mass attack to overcome tree defenses. Female orientation to preferred host trees could occur by primary attraction through response to long-range chemical (Moeck and Simmons 1991) and visual (Campbell and Borden 2006a) cues, or a combination of both (Campbell and Borden 2006b). Alternatively, females may simply land at random (Byers 1996, Pureswaran and Borden 2003), assess trees using contact chemical and gustatory cues (Raffa and Berryman 1982a), and then accept or reject that host. It is most likely that primary attraction is important when moving between patches of hosts and random landings dominate within host patches (Saint-Germain et al. 2007). After host selection and entry under the bark, females produce the aggregation pheromone *trans*-verbenol which attracts male and more female beetles to the tree (Pitman and Vité 1969, Pureswaran et al. 2000). Males produce their own aggregation pheromone, *exo*-brevicomin, that attracts more females (Rudinsky et al. 1974, Conn et al. 1983). During high population densities, this process of 'mass attack' allows MPB to overcome tree defenses and colonize otherwise healthy trees (Raffa and Berryman 1983a, Safranyik and Carroll 2006).

Host selection by female MPB is dependent on population density (Boone et al. 2011) and population state (Burke and Carroll 2017). Mountain pine beetle have irruptive population dynamics with four defined states (Fig. 1.2). During the endemic population state, when densities are low, females only accept hosts with reduced defenses that are suppressed in some way, by drought, age, or wounding (Carroll et al. 2006a, Safranyik and Carroll 2006, Burke and Carroll 2017). Hosts that are selected by beetles in the endemic phase are characterized by the presence of secondary bark beetles that present interspecific competition to colonizing MPB, such as *Ips* spp. (Carroll et al. 2006a). Beetles at low population densities that attempt to attack healthy trees will face constitutive and induced tree defenses such as resin and terpenes (Franceschi et al. 2005, Keeling and Bohlmann

2006, Chiu and Bohlmann 2022). When beetle populations build to enter the incipient epidemic phase there are enough beetles on the landscape to initiate mass attack and overcome host defenses. In this population phase, MPB prefer to attack trees that are larger and healthier and contain more resources for brood production (Raffa and Berryman 1983a, Boone et al. 2011, Burke and Carroll 2017). In the epidemic population state, landscape-scale tree death occurs as beetles aggregate on the landscape (Howe et al. 2022). During post-epidemic periods, MPB densities crash and return to endemic levels; this can be caused by the depletion of susceptible hosts, unfavourable weather, or pressure from natural enemies (Safranyik and Carroll 2006).

In Canada, MPB populations have spread north and east as far as the Northwest Territories (Nealis and Cooke 2014). In the expanded range, MPB has access to evolutionarily naïve lodgepole pine and the novel host, jack pine (*Pinus banksiana* Lamb.), which is the dominant pine species in the boreal forest (Burns and Honkala 1990). The area where lodgepole pine forests meet jack pine in west-central Alberta is a mosaic hybrid zone (Cullingham et al. 2012, Burns et al. 2019) and MPB has successfully mass attacked both jack and hybrid pines in natural forests (Bentz et al. 2010, Cullingham et al. 2011, de la Giroday et al. 2012). Lodgepole and jack pine differ in constitutive and induced monoterpene profiles (Lusebrink et al. 2011, 2016, Clark et al. 2014, Arango-Velez et al. 2016, Erbilgin et al. 2017b). The most important host monoterpene for MPB is α-pinene as it is the precursor of the female aggregation pheromone, *trans*-verbenol (Hughes 1973, Chiu et al. 2018). Jack pine has three times more α-pinene than lodgepole pine (Clark et al. 2014), which translates into higher amounts of *trans*-verbenol produced by females that colonize jack pines (Erbilgin et al. 2014, Taft et al. 2015a, 2015b) and increases attacks by MPB in the field (Burke and Carroll 2016). Jack pine bolts that are placed in the field during an MPB outbreak are attacked faster and at higher densities than other species, including lodgepole pine (Cerezke 1995).

The minimum density of attacking beetles required to successfully mass attack a lodgepole pine in the historic range is $\approx$40 attacking beetles per square metre (Raffa and Berryman 1983a). Above an optimal mass attack density of $\approx$60 attacking beetles per square metre, MPB brood success begins to decline due to intraspecific larval competition under the bark (Raffa and Berryman 1983a). Lodgepole pines in the expanded range that are naïve to MPB attack support more MPB brood than pines in areas that have historically experienced outbreaks (Cudmore et al. 2010). The reason for this difference could be that evolutionarily naïve trees have fewer defenses such as the monoterpenes limonene and Δ-3-

carene (Clark et al. 2010), which are toxic to MPB (Reid and Purcell 2011, Chiu et al. 2017, Reid et al. 2017). It is not known whether naïve pine hosts, such as jack pine, will be less able to resist mass attacks, so that lower densities of beetles will be required to overcome host defenses in the expanded range. Jack pine trees grow in sandy soil with good drainage which makes them prone to water deficit (Burns and Honkala 1990).

Mountain pine beetles can infest a variety of pine species (Furniss and Schenk 1969, Cook and Martinez 2018a) but it can have differences in preference and performance amongst possible susceptible hosts (Raffa et al. 2013, West et al. 2016). When Hopkins (1916) observed MPB in mixed stands of lodgepole and ponderosa pine, he noted that beetles preferentially colonized lodgepole pine although ponderosa pine was also a suitable host. From these observations, the Hopkins' host selection principle was developed and defined as, "a species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted." This principle has since been tested in many other insect systems with variable results (Barron 2001). Mountain pine beetle host choice and success has been studied in the context of Hopkins' host selection principle in mixed stands of lodgepole and whitebark pine (Raffa et al. 2013, Bentz et al. 2015), lodgepole and ponderosa pine (West et al. 2014, 2016) and in cut bolts of lodgepole pine and interior hybrid spruce (McKee et al. 2013, 2015). Whitebark pine, like jack pine, is currently experiencing higher MPB pressure due to increased climate suitability of its high-altitude habitat (Bentz et al. 2010). In mixed lodgepole and whitebark pine stands, MPB preferentially attacks lodgepole pine as opposed to the relatively naïve whitebark pine, which are less defended and more susceptible to attack (Raffa et al. 2013). In contrast, MPB accepts both hosts readily in no-choice lab experiments, so the preference exhibited in the field is not due to unsuccessful reproduction within the naïve host. Choice bioassays between lodgepole and ponderosa pine conducted in both the field and the lab show MPB preferentially infest ponderosa, which could imply a benefit to choosing a naïve host (West et al. 2016). It is not clear whether MPB has preferences for lodgepole or jack pines and if there are any costs to switching between these hosts.

For this thesis, I examined the interactions between epidemic MPB and its hosts in Alberta, lodgepole pine and jack pine. Chapter 2: I examined the mass attack dynamics of MPB in evolutionarily naïve lodgepole pines, using attack density manipulation experiments in the field with natural MPB populations. I hypothesized that the mass attack dynamics, would differ in lodgepole pine trees in Alberta due to poorly evolved defenses against MPB. I predicted that the mass attack threshold would be lower in naïve lodgepole pine than that

previously measured in the historic range (Raffa and Berryman 1983a). I repeated this experiment over three years with variable results. I discuss how environmental factors could affect attack dynamics and cause changes in MPB success after mass attack.

Chapter 3: I discuss the results of experiments I performed to examine the mass attack dynamics of MPB in jack pine. I released mass reared MPB underneath caged jack pine trees in a natural jack pine forest and quantified the mass attack threshold as well as offspring success and quality. I hypothesized that jack pine would have a reduced mass attack threshold as compared to lodgepole pine due to the lower constitutive and induced defenses in this species (Clark et al. 2014, Lusebrink et al. 2016, Erbilgin et al. 2017b). The mass attack threshold of MPB in jack pine is lower than that of lodgepole pine but phloem thickness plays an important role in offspring success and quality.

Chapter 4: While performing mass attack experiments, I collected phloem samples from trees before mass attack, just after mass attack, and again the following spring. I hypothesized that there would be a change in phloem terpene quantity between the first and second sample and any magnitude of change would be lower in jack pine than lodgepole pine, since it is a novel host. I also hypothesized that the third sample, 10 months after attack, would have the highest quantity of phloem terpenes, and that trees that were not successfully mass attacked would not have an increase in terpenes. I did not find support for the first hypothesis, as there was no change in phloem terpene content between the first two sampling points in either species. The third sample in the spring had the highest terpene content, which supports the second hypothesis but the change in terpenes between summer and the following spring did not depend on attack density. I discuss implications of this result in relation to susceptibility of trees that survive low density attacks.

Chapter 5: I performed laboratory experiments to investigate the preference and performance of MPB that emerge from lodgepole pine and jack pine bolts. I performed a no-choice experiment where beetles that emerged from mass attacked bolts of either species were introduced to uninfested bolts of lodgepole or jack pine. I predicted that MPB would perform best after introduction into a bolt that was the same species as their natal host, in agreement with a simple interpretation of Hopkins' host selection principle. I found that beetles that switched from lodgepole to jack pine produced offspring of lower quality relative to the offspring of beetles that did not switch. I performed choice bioassays using a static air arena that contained lodgepole and jack pine bolts so that beetles could respond to

olfactory, visual, and gustatory cues. I also conducted a walking olfactometer experiment where beetles could respond to only the olfactory cues of lodgepole pine and jack pine phloem. I hypothesized that MPB prefer their natal host in agreement with Hopkins' host selection principle and the preference-performance hypothesis. I found that MPB that emerged from lodgepole pine were more attracted to host volatiles but did not prefer lodgepole or jack pine cues. Beetles that emerged from jack pine responded less to bolts than beetles from lodgepole pine and responded more to controls than host volatiles in the olfactometer experiment. I discuss how the quality of jack pine as a host may result in a change of offspring quality and host-selection behaviour.

1.2. Tables and Figures:
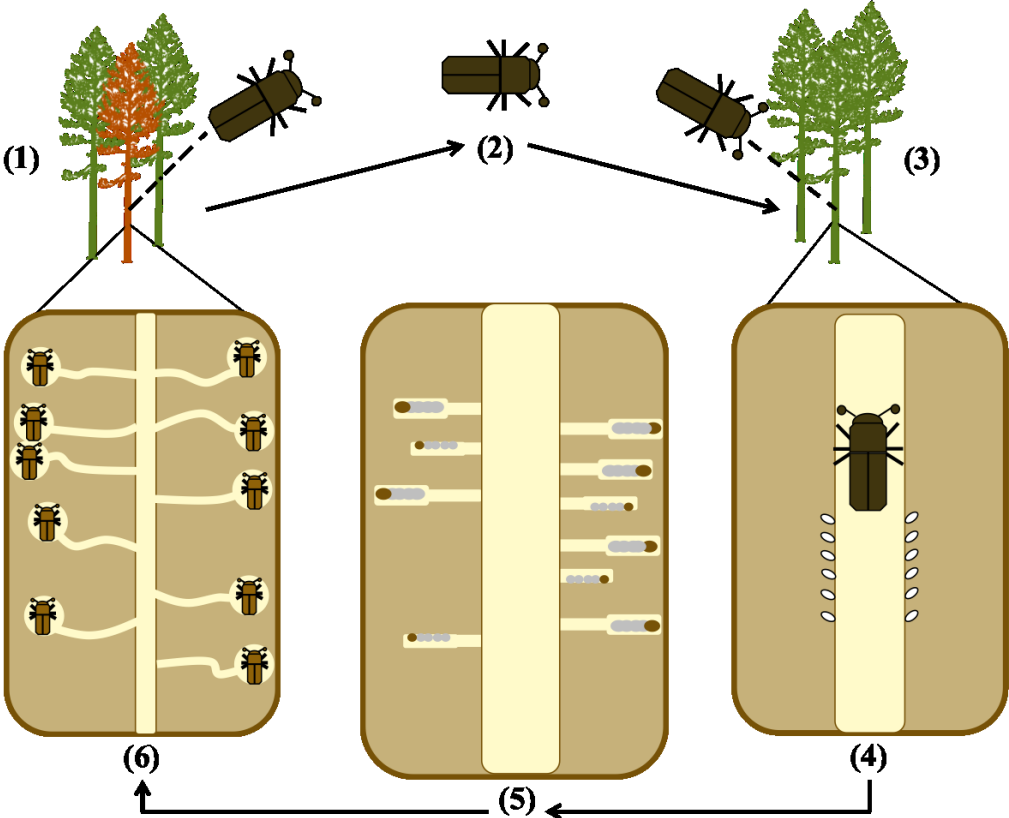
Figure 1.1: Mountain pine beetle life cycle, image by AE Musso, tree silhouettes are Creative Commons. MPB exit their natal host (1) in mid-summer and fly to disperse (2). Female beetles are pioneers, select a new host (3), and release pheromones to call conspecifics. After mating, female MPB lay eggs in niches along a vertical parental gallery (4). Eggs hatch and larvae mine horizontally consuming phloem (5) until temperatures decrease below the developmental threshold. Larvae are the most cold tolerant stage and will overwinter. Larvae complete development the following spring and pupate within oval chambers at the terminus of the larval gallery. Teneral adults eclose from pupae and feed on any remaining phloem tissue and fungal mutualists that grow within the pupal chambers (6) until conditions are ideal for dispersal.
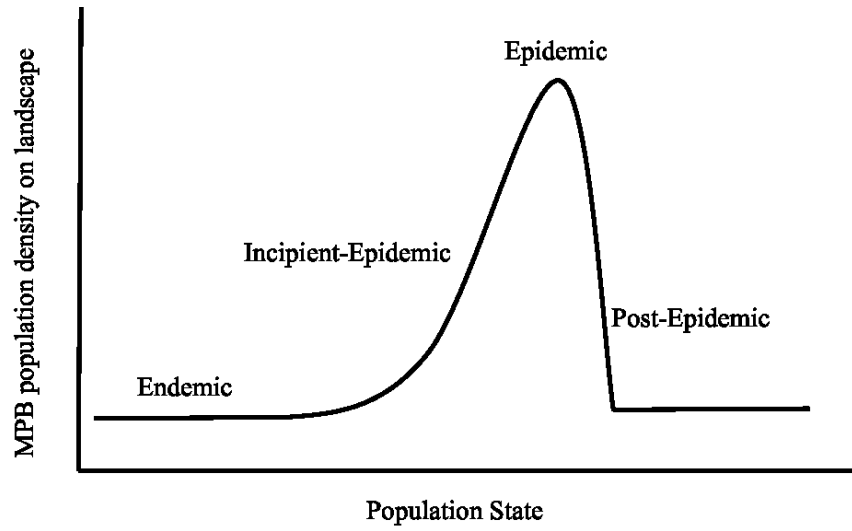
Figure 1.2: MPB population states at relative population densities on the landscape.

Chapter 2. Attack threshold and optimal attack density of mountain pine beetle (*Dendroctonus ponderosae*; Coleoptera: Curculionidae) in naïve lodgepole pine (*Pinus contorta var. latifolia*)

2.1. Introduction:

The fundamental niche of an obligate symbiont is defined by a combination of the traits of its host as well as the host's external environment (Krasnov et al. 2015), whereas the realized niche simply requires that the host is present (Mestre et al. 2020). The overall construction of the symbiont's niche will vary depending on spatial scales (Krasnov et al. 2015) and the symbionts' life history (Mestre et al. 2020). A host with broader thermal tolerance than its symbiont can have a larger geographic distribution than the symbiont and so there is a mismatch between their distributions (Harms et al. 2021). Scolytine bark beetles (Coleoptera: Curculionidae) have an obligate symbiotic relationship with their host plants, as most of their life cycle is spent in the subcortical environment of host trees (Schowalter 2012). They do, however, have a free-living stage when adults disperse to new hosts (Raffa et al. 2015b), and therefore the hosts' external environment is of relatively high importance for bark beetle niches (Mestre et al. 2020). In temperate regions, bark beetle population distributions have tracked those of their host plants during periods of glacier recession (Hill et al. 2011, Schebeck et al. 2018).

The mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins) is a bark beetle native to western North America where its historical distribution follows the distribution of its main host plant, lodgepole pine (*Pinus contorta*) (Zúñiga et al. 2002, Mock et al. 2007). The north-south distribution of lodgepole pine extends from the Yukon and Northwest Territories in Canada south to northern California and New Mexico, USA (Safranyik and Carroll 2006). Historically, the northernmost distribution of MPB has been limited to only part of the range of its host in southern and central British Columbia, Canada (Shore et al. 2006, Safranyik et al. 2010). A climatic barrier inhibited MPB from establishment in the northern populations of lodgepole pine, as environmental conditions were unsuitable for MPB establishment and persistence (Safranyik et al. 1975). The northern parts of the lodgepole pine range were outside the fundamental niche of MPB because environmental conditions made the hosts unsuitable.

Recent climate perturbations have resolved the mismatch between the distribution of MPB and lodgepole pine which has facilitated its range expansion into previously marginal habitats at higher latitudes and higher elevations (Carroll et al. 2006b, Bentz et al. 2016, Sambaraju et al. 2019). Populations of MPB in British Columbia expanded northward as well as eastward into the province of Alberta where they accessed new habitat. Lodgepole pine trees in the expanded MPB range are considered evolutionarily naïve as they have not experienced recent selective pressure from MPB feeding and possess fewer constitutive and induced defenses against MPB herbivory than trees in the native range and (Clark et al. 2010, 2014, Burke et al. 2017).

Mountain pine beetles colonize hosts in different ways depending on population state (Carroll et al. 2006a, Boone et al. 2011, Burke and Carroll 2017). In the endemic population state, low densities of MPB colonize and reproduce in weakened trees that have poor resources for offspring production and are often co-occupied with other bark beetle species (Carroll et al. 2006a, Smith et al. 2011). At epidemic and incipient-epidemic states, high densities of MPB colonize well-defended trees with ample resources for offspring production (Boone et al. 2011) using pheromone-mediated aggregation (mass attack) that overwhelms and kills the host tree (Raffa and Berryman 1983a). High density MPB populations can lead to outbreaks with positive feedbacks (Raffa et al. 2008), as beetles that emerge from mass-attacked trees seek out well-defended trees (Boone et al. 2011, Burke and Carroll 2017) and spatially aggregate on the landscape (Howe et al. 2022) which reduces Allee effects (Goodsman et al. 2016). When MPB mass attack naïve lodgepole pines in the expanded range, more offspring per female are produced than in lodgepole pine trees within the historic range (Cudmore et al. 2010), which suggests naïve lodgepole pine are more suitable for reproduction. It is unknown, however, if naïve lodgepole pine are more susceptible to MPB mass attack due to lower defensive capacity than lodgepole pine in the historic range.

The relative reproductive success of MPB is influenced by the mass attack threshold and the optimal attack density in mass-attacked trees (Raffa and Berryman 1983a). The mass attack threshold, or minimum density of beetles required to overcome lodgepole pine host defenses in the historic MPB range, is ~40 attacking beetles/m^2 (Raffa and Berryman 1983a). Aggregations of beetles below the mass attack threshold density are killed by host tree defenses such as resin (Raffa and Berryman 1982b) and toxic chemical compounds including monoterpenes (Raffa and Berryman 1983b). The number of offspring per female produced increases with density until the optimal attack density (~60 attacking beetles/m^2) is reached (Raffa and Berryman 1983a). Above the optimal attack density, intraspecific

competition reduces reproductive output per MPB female, as offspring compete for finite resources under the bark (Cole 1973b, Goodsman et al. 2018). The attack dynamics of MPB in the expanded range have not been studied, so it is not clear whether entry into this new habitat changes the dynamics between MPB and its host. If naïve lodgepole pines have a reduced defensive capacity against MPB, they should be less able to resist attack. In this study, I hypothesize that lodgepole pine trees in the expanded range should have a lower mass-attack threshold than lodgepole in the historic range. I tested this hypothesis by conducting attack-density manipulation experiments in lodgepole pine in the expanded MPB range in Alberta over three years and predicted that, on average, the mass-attack threshold would be lower than 40 attacks/m^2 in naïve lodgepole pine. Attack density can also negatively affect offspring quality as increases in competition for finite resources can reduce offspring size (Amman and Pace 1976, Amman and Pasek 1986). I also collected data on offspring size and fat content to test the hypothesis that offspring quality is reduced with increased attack density because of intraspecific competition. I predicted that adult offspring that emerge from bolts that have attack densities beyond the optimal attack density would be of reduced quality in size or fat content.

2.2. Methods:

I performed attack density manipulation experiments in lodgepole pine forests south of Grande Prairie Alberta during the MPB flight season in the summers of 2016 (54.469, -118.526) and 2017 (54.487, -118.562), and collected data from another attack density manipulation experiment in the same area performed in 2015 (54.572, -119.343). Weather conditions at each site were obtained from an Environment Canada weather station in Simonette Alberta (54.42, -117.74).

Beetle activity and the initiation of the flight period were monitored weekly at each site with Lindgren 16-funnel traps baited with commercially available MPB lures comprised of aggregation pheromones *trans*-verbenol and *exo*-brevicomin and the host volatile myrcene (Contech Enterprises Inc. Delta BC, Canada). Fifteen trees at each site in each year were randomly assigned to one of three density manipulation treatments (five trees per treatment per year). In 2015 and 2016 attack density treatments were low (20 attacks/m^2), medium (60 attacks/m^2), and high (100 attacks/m^2). In both 2015 and 2016, approximately 80% of the trees in the low-density treatments (20 attacks/m^2) were successfully attacked and had long parental galleries and larval galleries. Therefore, target attack densities were reduced in 2017 to low (10 attacks/m^2), medium (40 attacks/m^2), and

high (80 attacks/m^2). To achieve target attack densities, each tree was baited with one commercial tree bait comprised of a *trans*-verbenol bubble pack and *exo*-brevicomin flex lure (MPB Tree Bait, Contech Enterprises Inc., Delta BC, Canada) at the beginning of the flight period. The diameter at 1.3 m (DBH) and average phloem thickness of each tree was measured before baiting in 2016 and 2017. In 2015, only DBH was measured. Average phloem thickness was measured on four, 10 mm diameter bark/phloem samples taken from the cardinal directions, at ~0.5 m height on the bole of each tree. Phloem tissue was separated from the bark and the thickness of the phloem was measured to the nearest 0.01 mm with digital calipers. Baited trees were checked daily until mass attack was initiated, and then checked several times a day during peak flight and aggregation, and the number of attacks were counted in a 1 m^2 area of the bark at 1 m above the ground. Once target attack densities were reached, trees were wrapped in aluminum wire insect screening (18 × 16 mesh count; PHIFER Inc. Alabama, USA) up to 3 m above the ground to physically prevent further attacks. In 2017, we attached anti-aggregation pheromone (MPB Verbenone, 7 g, Solida Distribution Inc., Saint-Ferréol-les-Neiges QC, Canada) in addition to the insect screening to further discourage attacks on the lower density treatments.

At the end of the beetle flight period, in mid-September of each year, the mesh was removed from the experimental trees, and trees were left until spring to experience natural winter conditions. Trees were felled the following May of each year and two 50-cm bolts from 1 m off the ground were harvested from each successfully attacked tree. Successful attack was determined by the presence of parental galleries >5 cm and the presence of larval galleries in a 15×15 cm^2 sample taken below 1 m height on the bole. Bolts were transported to the University of Alberta, Edmonton, Alberta, Canada where the cut ends were sealed with hot paraffin wax to slow desiccation. Bolts were stored at 5°C for 2–5 months. Bolts were removed from cold storage in groups of 3–5 and placed in vented emergence bins (114 L Rubbermaid Hinged Top Tote) fitted with a glass jar. Bins were checked daily, and emergent beetles were collected and separated by sex.

Beetles were collected throughout the emergence period until no more beetles had emerged for 30 d. Following emergence, bolts were autoclaved, debarked, and gallery characteristics were quantified. The number of parental and larval galleries in 3 randomly selected 15×15 cm samples from one bolt from each tree were counted in trees from the 2015 experiment. Pupal chambers were not counted in 2015. For trees manipulated in 2016 and 2017, all galleries on one bolt per tree were counted and measured by tracing parental, larval, and pupal galleries, onto clear plastic sheets. Gallery traces were photographed and

the number of parental, larval, and pupal galleries were counted using ImageJ (imageJ.nih.gov). For the 2015 data, the number of larvae counted in the three subsamples was divided by the number of parental galleries in the subsample to determine larvae/female and the number of adults/female was calculated by dividing the total number of adults that emerged per bolt and dividing it by the number of attacks on each bolt. For the 2016-2017 data the total number of larval galleries, pupal chambers, and emergent adult offspring were divided by the number of parental gallery starts per bolt and provided larvae/female, pupae/female and adult offspring/female. The attack density for each tree was calculated by counting the total number of attacks on both bolts and dividing this value by the surface area of the two bolts.

A portion of the beetles that emerged from bolts that were attacked during the field experiment were used in flight experiments (data not presented here). The remaining emergent beetles were immediately frozen at −20°C and kept for analysis of body condition. Beetle pronotum width and body length were measured using a stereomicroscope with ocular micrometer at 1.6× on up to 50 females and 50 males from each tree. Pronotum width and body length were used to calculate body volume as a measure of body size using the formula for the volume of an ellipsoid (Reid and Elkin 2005), *a* = half the body length, *b* = half the pronotum width, and *c* = half the pronotum width:

$$V = \frac{4}{3}\pi abc$$

The fat content of each beetle was extracted using a Soxhlet method (Atkins 1969). Beetles were dried in an oven at 60°C for 24 h and then weighed to the nearest 0.01 mg (Mettler Toledo XPE205 Microbalance, Columbus, Ohio). Weighed beetles were placed in individual perforated 0.2 mL PCR tubes (Fisher, Canada) and placed in the Soxhlet apparatus (45/50 Pyrex; Fisher, Canada). Beetles in tubes were washed with warm petroleum ether (Sigma-Aldrich) every 20-30 min for 8 h, dried again at 60°C for 24 h, and re-weighed. Fat content was calculated by subtracting the post-Soxhlet extraction dry weight from the pre-extraction dry weight. Relative fat content was calculated by dividing the fat content by the pre-extraction dry weight. Both body volume and fat content were used to calculate a body condition residual index. The residuals of a linear model of fat content and body volume represented the relative condition of each beetle in the dataset.

All statistical analyses were performed in R (R Core Team 2022) within RStudio (RStudio Team 2022). To determine the mass attack and optimal thresholds of MPB attack

of lodgepole pine in the expanded range, the relationship of beetle attack density on total offspring produced per bolt and offspring produced per female was determined with statistical models for each dependent variable within each study year (Table 2.2). Each dependent variable was fit to a linear model with attack density and tree size characteristic, either DBH or phloem thickness, as independent variables, the lowest density at which offspring production per female was greater than 1 was considered the mass attack threshold and any peaks in the relationship were considered the approximate optimal attack density. Models were checked for violations of assumptions by plotted scaled residuals using *DHARMa* (Hartig 2022). If models violated assumptions, the dependent variable was log + 1 transformed which often improved fit. The model that included the fewest independent variables and did not violate assumptions, was considered the final model. Final models were compared to a null model using likelihood ratio tests and reported if they were significantly different from the null. Some linear models, even with transformations, did not meet assumptions and so alternative models were investigated. The number of larvae produced per bolt predicted by DBH in 2015 was fit using an asymptotic curve using *drc* (Ritz et al. 2015)and *aomisc* (Onofri 2020). Models for the number of larvae per female and number of adult emergent offspring per female were fit using generalized additive models (GAMs) using *mgcv*. GAMs were checked with *DHARMa* and gam.check in *mgcv* (Wood 2017). All regression plots are generated using *visreg* (Breheny and Burchett 2017) and *ggplot2* (Wickham 2016).

The effect of sex, attack density, phloem thickness, and/or DBH on offspring body condition measurements, body size, fat content, and residual condition was analyzed using linear mixed effects models in *lme4* (Bates et al. 2015) with a term for bolt fit as a random effect. Plots of the predicted values of the linear mixed effects models and raw data were constructed using *ggeffects* (Lüdecke 2018) and *ggplot2* (Wickham 2016). Environmental data from the weather station was summarized as mean monthly daily average, and maximum temperatures and total precipitation per month were summarized using *dplyr* (Wickham et al. 2022) and plotted using *ggplot2* (Wickham 2016).

2.3. Results:

A summary of tree DBH and phloem thickness by site can be found in Table 2.1.

Measures of attack success: production of larval, pupal, and adult offspring 2015

All 15 trees appeared to be mass attacked during the 2015 flight season and reached desired attack densities within one week. Bolts from five trees, however, produced fewer than 10 MPB adult offspring. Three of these five trees had high attack densities and many parental and larval galleries, but no exit holes. Galleries were heavily comingled with secondary bark beetle galleries, so these trees were excluded from analyses. The two remaining trees that did not produce any adult offspring were in the low-density treatment, had short (<5 cm) parental galleries with no larval galleries, and so were included in the analysis as true zeros.

Actual attack densities ranged from 24.7 to 86.2 attacks/m^2. The total number of larvae produced per bolt was best predicted as an asymptotic relationship with DBH of the tree (p < 0.001; Table 2.2), the total number of larvae produced increased to a saturation point at 300.7 total larvae per bolt sample (Fig. 2.1a). There was no relationship between attack density and the total number of larvae in the sample from each bolt ($F_{1,10}$ = 0.51, p = 0.49) and including attack density in the model with DBH reduced model fit. The total number of larvae produced per female in the sample from each bolt, however, was influenced by both attack density (p = 0.01; Table 2.2) and DBH (p = 0.03; Table 2.2). The number of larvae per female had a negative relationship with attack density but trees with larger DBH produced more larvae/female than smaller trees (Fig. 2.1b). The total number of adult offspring produced per bolt increased with DBH (p = 0.03, Table 2.2), trees with larger diameters produced more adult offspring than trees with smaller diameters at similar attack densities (Fig. 2.1c). Although the data provide no evidence that DBH was a significant predictor of adult offspring produced per bolt, removal of this term from the model resulted in residuals that were not normally distributed. The number of adult offspring produced per female was not influenced by attack density (p = 0.09, Table 2.2) or DBH (p = 0.2, Table 2.2), but the data show a trend that is similar to the number of larvae per female (Fig. 2.1d). In agreement with the hypothesis that lodgepole pine trees in the expanded range would have a reduced mass attack threshold, five of the six trees with attack densities lower than 40 attacks/m^2 successfully produced more than one adult offspring per female. Further, three of these trees were the most productive producers of adult offspring (Fig. 2.1d), which suggests that the optimal attack density was also less than 40 attacks/m^2.

Measures of attack success: production of larval, pupal, and adult offspring 2016

All 15 trees were mass attacked during the 2016 flight season but due to frequent cool, rainy conditions, some trees were attacked over a long period (7–16 d). More than half of the days during the three-week flight period had rain and rained an average of 8.1 mm. Actual attack densities ranged from 24.6–107.7 attacks/m^2 and in stark contrast to the trees attacked in 2015, the total number of larvae per bolt increased with attack density (p = 0.002, Table 2.2) and bolts from trees with ~50 attacks or fewer/m^2 produced few larvae (Fig. 2.2a). The number of larvae per female also increased with attack density (p = 0.005; Table 2.2; Fig. 2.2b). The total number of pupae per bolt (p < 0.001; Table 2.2) and the number of pupae per female (p = 0.004; Table 2.2) were both linearly related to attack density (Fig. 2.2c,d). The number of adult offspring produced per bolt increased with attack density (p = 0.007; Table 2.2) but followed a more shallow curve than the relationship between larvae per bolt and attack density (Fig. 2.2e). The number of adult offspring produced per female also increased with attack density (p = 0.03, Table 2.2) in 2016, and few trees produced more than 2 offspring per female (Fig. 2.2f). Inclusion of phloem thickness and/or DBH in models did not significantly (p > 0.05) alter the relationship between attack density and the production of any offspring life stages.

Measures of attack success: production of larval, pupal, and adult offspring 2017

Twelve of the 15 baited trees were attacked and caged within 7 d of baiting. Three trees (two medium density, one high density treatment) did not reach the desired attack densities and were monitored for an additional 11 d, but no new attacks were recorded. Actual attack densities ranged from 13–125 attacks/m^2. Unfortunately, woodpecker predation on the manipulated trees was extremely high after the mesh was removed from trees in the fall. All live larvae were killed either by direct predation or from exposure due to extensive bark damage. Only data on the total number of larvae (larval galleries) and number of larvae per female (larval galleries/parental galleries) could be collected. As in 2016, low attack densities did not produce large numbers of larvae. The total number of larvae per bolt (p < 0.001; Table 2.2) and the number of larvae per female (p < 0.001; Table 2.2) increased with attack density (Fig. 2.3 a,b). Neither phloem thickness nor DBH had a significant effect on the total number of larvae per bolt or the number of larvae per female produced (p > 0.05).

Measures of offspring quality: adult offspring body size, fat content, and condition

Although attack density had no effect on the body size of adult offspring produced from trees attacked in 2015 (p = 0.95; Table 2.2), female offspring were larger than male offspring (p = 0.01; Table 2.2; Fig. 2.4a). Offspring that emerged from bolts of trees attacked with high densities in 2015 had less fat than offspring from low density bolts (p < 0.001; Table 2.2, Fig. 2.4b) and females had more fat than males (p = 0.002; Table 2.2; Fig. 2.4b). Relative fat content, however, did not differ by sex (p = 0.31; Table 2.2; Fig 2.4c) but was lower in beetles that emerged from trees with low attack densities (p < 0.001; Fig 2.4c). The relative condition of adult offspring decreased with increased attack density (p < 0.001; Fig 2.4d) and females had a better relative condition than males (p = 0.02, Fig. 2.4d). Female adult offspring that emerged from trees attacked in 2016 were larger and had higher absolute fat and relative fat contents on average than males (p < 0.001; Table 2.2; Fig 2.5a,b,c). Attack density had a significant effect only on body size in offspring from 2016-attacked trees (p = 0.003; Table 2.2, Fig. 2.5a) but it did not influence either absolute or relative fat content (p > 0.05; Table 2.2; Fig. 2.5b). Offspring relative condition from 2016 did not differ with sex or attack density (Table 2.2).

Weather data for years surrounding experiments.

The growing season prior to the 2015 attack density manipulation experiments (2014) was a particularly hot and dry summer. The mean daily temperatures for the months that coincide with MPB flight (July and August) in 2014 trended higher than in the three subsequent years when the experiments were performed (Fig. 2.6a). The maximum daily temperatures for July and August in 2014 were particularly high averaging greater than 30°C for both months (Fig. 2.6b). Monthly precipitation in July and August in both 2014 and 2015 was lower than in 2016 and 2017. There were high levels of precipitation in June and July of 2017, but August was dry (Fig. 2.6c).

2.4. Discussion:

The mass attack threshold of MPB in naïve lodgepole pine in Alberta varied with experimental manipulations across years. In 2015, the mass attack threshold was <40 attacks/m^2, which supported the hypothesis that the mass attack threshold is lower in naïve hosts in the expanded range than the threshold described for lodgepole pine in the historic range (Raffa and Berryman 1983a). Trees attacked at densities below the historic range

mass attack threshold of 40 attacks/m^2 (Raffa and Berryman 1983a) produced the greatest number of offspring per female. Raffa and Berryman (1983a) report ~15 larvae/female produced at attack densities of ~30 attacks/m^2, which is a similar larvae/attack value obtained at similar attack densities in the 2015 experiment. Interestingly, the greatest number of larvae per female produced in 2015 was in the tree with the lowest attack density (~25 attacks/m^2) and all trees with attack densities >25 attacks/m^2 had lower larvae per female. In contrast, the number of larvae/attack decreased only after ~80 attacks/m^2 in lodgepole pine in the historic range of MPB (Raffa and Berryman 1983a).

The mass attack threshold in 2015 was partly related to tree size. Large trees hosted more larvae per female in the 2015 experiment as there was a positive relationship between DBH and larval production per female. In the relationship between attack density and the number of larvae per female, the predicted 25% quantile of DBH crossed 0 around 40 attacks/m^2, so small trees had an attack threshold of 40 attacks/m^2. Increased tree diameter positively influences brood production of MPB in the historic range (Safranyik 1968, Safranyik and Carroll 2006), and lodgepole pine diameter is positively related to bark thickness which also positively influences MPB adult emergence (Amman 1969). Greater MPB brood production in large diameter trees is largely due to the positive relationship of tree diameter and bark thickness with phloem thickness (Amman 1969, Shrimpton and Thomson 1985). Phloem is the main tissue colonized by MPB and its associated fungi (Safranyik and Carroll 2006, Bleiker and Six 2007) and lodgepole pine trees with thick phloem produce more MPB brood than trees with thin phloem (Amman 1972, Amman and Pace 1976). Although we did not directly measure phloem thickness of the trees in the experiment conducted in 2015, the positive influence of DBH on larval brood per female is most likely because large diameter trees had thicker phloem. With the low attack threshold observed in trees attacked in 2015, the negative density dependent effects on larval survival became apparent at low attack densities, especially in small trees. The total number of larvae counted from the random samples from each bolt was not related to the attack density but was positively influenced by DBH. The total number of larval brood increased to a saturation point of ~300 larvae with increasing DBH, regardless of the attack density. The two trees that had the smallest DBHs had no larvae present even though they were attacked at densities similar to, or lower than, other trees that produced many larvae. Larvae mining in thin phloem will mine wider galleries (Cole 1973b, Amman and Cole 1983) so they could have become crowded at lower attack densities. Crowded larvae in thick phloem can mine galleries above or below other larvae but in thin phloem they are more likely to come in physical contact with other larvae (Cole 1973b). Physical contact between

two larvae can result in the death of one or both larvae either by cannibalism or mutual destruction through resource depletion (Cole 1973b, Amman and Cole 1983). These data show strong evidence of the negative density-dependent effect caused by intraspecific competition.

In stark contrast to 2015, in the 2016 and 2017 the number of larvae and the number of larvae per female increased with attack density but did not decrease at high attack densities, as described in Raffa and Berryman (1983a) for beetles in the historic range. In other words, it is not possible to measure the optimal attack density in 2016 and 2017. The estimated attack threshold for larval production per female appeared to be approximately the same as in the historic range (~40 attacks/m^2) in 2016, and greater than the historic range (50 attacks/m^2) in 2017. There was also no evidence of offspring competition caused negative density-dependence in any pupae and adult offspring as they all also increased with attack density in 2016. Neither phloem thickness nor DBH significantly influenced production of any offspring stage (eggs, larvae, pupae), so the lack of attack success at low densities was most likely due to failure of the cooperative effect in mass attack (Goodsman et al. 2016). Since attack densities were not low on average and overlapped with the typically successful range of densities as seen in 2015 and Raffa and Berryman (1983a), it is possible that a change in plant defensive capacity is what caused the glaring change in relationship. Lodgepole pine trees that are able to resist MPB mass attack have a greater capacity for resin and monoterpene production compared to trees that do not resist (Raffa and Berryman 1982b, Erbilgin et al. 2017a). Adult offspring production could not be measured in 2017, but the 2016 results suggests that trees were more defensively capable than those in 2015 since low density attacks did not produce offspring and the total number of offspring per female was lower. The mass attack threshold for larvae per female was higher in 2017 than 2016 so trees in 2017 were more defensively capable even than 2016.

A possible explanation for the differences in attack dynamics in the three years sampled lies in the temperature and precipitation data in the years before and during the experiment. The growing season that preceded the 2015 experiment had relatively high average daily temperatures and high daily maximum temperatures, compared to the other experimental years. In addition, there was low cumulative over winter precipitation leading into the 2015 season. Plants undergoing drought stress close their stomata to reduce water loss, but this also limits CO_2 intake and reduces available carbon for synthesis of defense compounds (Lauder et al. 2019). Limonene, the most toxic monoterpene to MPB (Chiu et al.

2017, Reid et al. 2017), does not increase in concentration in response to inoculation with the MPB fungal symbiont *Grosmannia clavigera* in trees that are experimentally drought stressed, but does increase in trees under well-watered conditions (Lusebrink et al. 2016). Lodgepole pine seedlings inoculated with *G. clavigera* grown under drought conditions produce lower total monoterpene concentrations compared to well-watered seedlings and have decreased stomatal conductance (Arango-Velez et al. 2016). With the trade-off between defense and growth in conifers (Lauder et al. 2019), the combination of low precipitation and high average temperatures in 2014 may have caused trees to invest less in defenses in the 2015 season which was further compounded by the lack of precipitation during the 2015 growing season. Monthly accumulated precipitation between May and August in 2016 was much higher compared to the previous two years. Trees at the 2016 study site had two and a half months of wet weather before the beetle flight period which might have helped them to rebound from the drought stress of the previous two years. Monthly precipitation remained high through July and August and frequent cool, rainy days repeatedly interrupted the mass attack process in 2016. Despite these interruptions in 2016, the timing of mass attack of most trees was similar to 2015, as it took place in less than one week and most trees reached the selected attack density within 3 days. The attack density treatment took 16 days to reach, however, in five of the experimental trees in 2016. Waves of beetle attack, rather than a condensed period of mass attack over a short period, may allow trees to briefly mount a defensive response, particularly when there are ample resources (Glynn et al. 2003).

Delayed induction of defense compounds, such as monoterpenes, can increase in concentration four to six weeks after the initiation of mass attack (Clark et al. 2012, Soderberg et al. 2022). Delays in reaching the mass attack threshold of over one week could have allowed delayed responses to build up and reduce survival of colonizing beetles or their offspring particularly because the tradeoff between defense and growth was eased by greater water availability. Trees with fewer constitutive defenses can host greater brood densities (Cudmore et al. 2010) so a great increase in constitutive defenses prior to the flight period could also have reduced brood survival. Trees in 2017 were attacked within 7 days but the relationship between number of larvae per female and attack density was similar to that in 2016. Monthly precipitation from May–July in 2017 was much higher than in 2014–15, especially in June and July meaning trees could have invested heavily in constitutive defenses. While the mass attack process was not interrupted by rain in the last week of July in 2017, trees could resist attack densities of lower than about 50 attacks/m^2.

Unfortunately, woodpecker predation in 2017 meant that mortality of pupae and adults could not be compared across attack densities.

Attack density differentially affected adult offspring body condition across different years of the study. In 2015, when the attack dynamics were likely driven mostly by intraspecific competition, body size was not affected by attack density, but fat content, relative fat content, and relative condition were all negatively affected by increased attack density. Phloem thickness is more often a better predictor of offspring size than attack density (Amman and Pace 1976, Amman and Pasek 1986, Graf et al. 2012). Since intraspecific competition was high at relatively low attack densities this reduction in fat per body size could be the result of a competitive effect. Interestingly, in 2016 body size was negatively affected by attack density but absolute and relative fat content as well as relative condition were not. A change in body size may not necessarily be detrimental when there is no change to fat content. The body size of MPB can decrease over the span of an outbreak (Graf et al. 2012) but fat content of bark beetles can be related to fecundity (Coppedge et al. 1994). These data provide evidence that intraspecific competition results in a reduction in offspring quality (fat content) but not size in years when attack thresholds are low but in years when attack thresholds are high, there is no cost in offspring quality in terms of fat content.

This study was conducted over three years in naïve lodgepole pine stands in Alberta. Variation among years in weather conditions resulted in variable responses of beetles and subsequent disparity in the effect of attack density on tree response. The mass attack threshold varied from that measured by Raffa and Berryman (1983a) in 2015 and the attack dynamics in 2016 and 2017 prevented measurement of an optimal attack density. These data also highlight the complexity and duality of the obligate symbiont's niche. A symbiont's fundamental niche consists of both host features and environmental conditions and these two features interact to create a niche that is dynamic in space and time (Mestre et al. 2020). More data are needed to explore the effect of varying environmental conditions in the expanded range to determine the likelihood of frequent shifts in the quality of the lodgepole pine niche. The fundamental and realized niche mismatch of MPB and lodgepole pine that has been resolved by climate change warrants the exploration of MPB biology, ecology, and behaviour within the expanded range. Mountain pine beetle's establishment in previously unsuitable habitat was mediated by changes in environmental conditions, but once a symbiont enters a new niche and establishes a sustainable population, further range expansion can take place following an evolutionary facilitation. Evolutionary facilitation

occurs when symbionts are introduced to a new niche in numbers large enough that random mutations and natural selection allow the symbiont to move further through the same host species or, through niche construction, switch host species (Mestre et al. 2020).

2.5. Tables and Figures

Table 2.1: Mean and standard deviation of diameter at breast height (1.3m; DBH) and phloem thickness for 15 trees per site selected at each site in each study year.

Year	Location	Mean (SD) DBH (cm)	Mean (SD) Phloem Thickness (mm)
2015	54.572, -119.343	30.1 (4.03)	n/a (n/a)
2016	54.487, -118.562	27.3 (2.76)	2.00 (0.76)
2017	54.469, -118.526	25.1 (1.99)	2.08 (0.41)

Table 2.2: Statistical models for the relationships between attack density (Density) and diameter at breast height (DBH) and measures of offspring production on a per bolt or per female scale. NLS = non-linear least squares, with equation specified. GAM = Generalized additive model, independent variable with (smooth) was designated the smoothing term, other terms are linear predictors. LM = Linear model, transformations of the dependent variable are denoted in brackets after the term. NLS, GAM, or LM Estimates, test statistics, estimated degrees of freedom (edf), degrees of freedom and/or p-values from summaries of models in R.

Year	Model Type	Dependent Variable	Independent Variable(s)	Statistics
2015	NLS Asymptote	# Larvae/Bolt	DBH	x Intercept: Estimate 16.77, SE = 5.18, p = 0.01
				Plateau Intercept: Estimate 300.66, SE = 43.31, p < 0.001
	GAM	# Larvae/Female	Density (smooth)	F = 8.92, edf = 3.09, p = 0.001
			DBH	t = 2.85, p = 0.03
	LM	# Adult offspring/Bolt (log +1)	DBH	$F_{1,10} = 6.38$, p = 0.03
	GAM	# Adult offspring/Female	Density (smooth)	F = 3.33, edf = 1.93, p = 0.09
			DBH	t = 1.30, p = 0.23
2016	LM	# Larvae (log +1)/Bolt	Density	$F_{1,13} = 15.84$, p = 0.002
	LM	# Larvae/Female (log +1)	Density	$F_{1,13} = 11.18$, p = 0.005
	LM	# Pupae/Bolt	Density	$F_{1,13} = 30.14$, p < 0.001
	LM	# Pupae/Female	Density	$F_{1,13} = 12.39$, p = 0.004
	LM	# Adult offspring/Bolt (log +1)	Density	$F_{1,13} = 10.05$, p = 0.007
	LM	# Adult offspring/Female (log +1)	Density	$F_{1,13} = 6.27$, p = 0.03
2017	LM	# Larvae/Bolt	Density	$F_{1,13} = 41.75$, p < 0.001
	LM	# Larvae/Female	Density	$F_{1,13} = 25.21$, p < 0.001
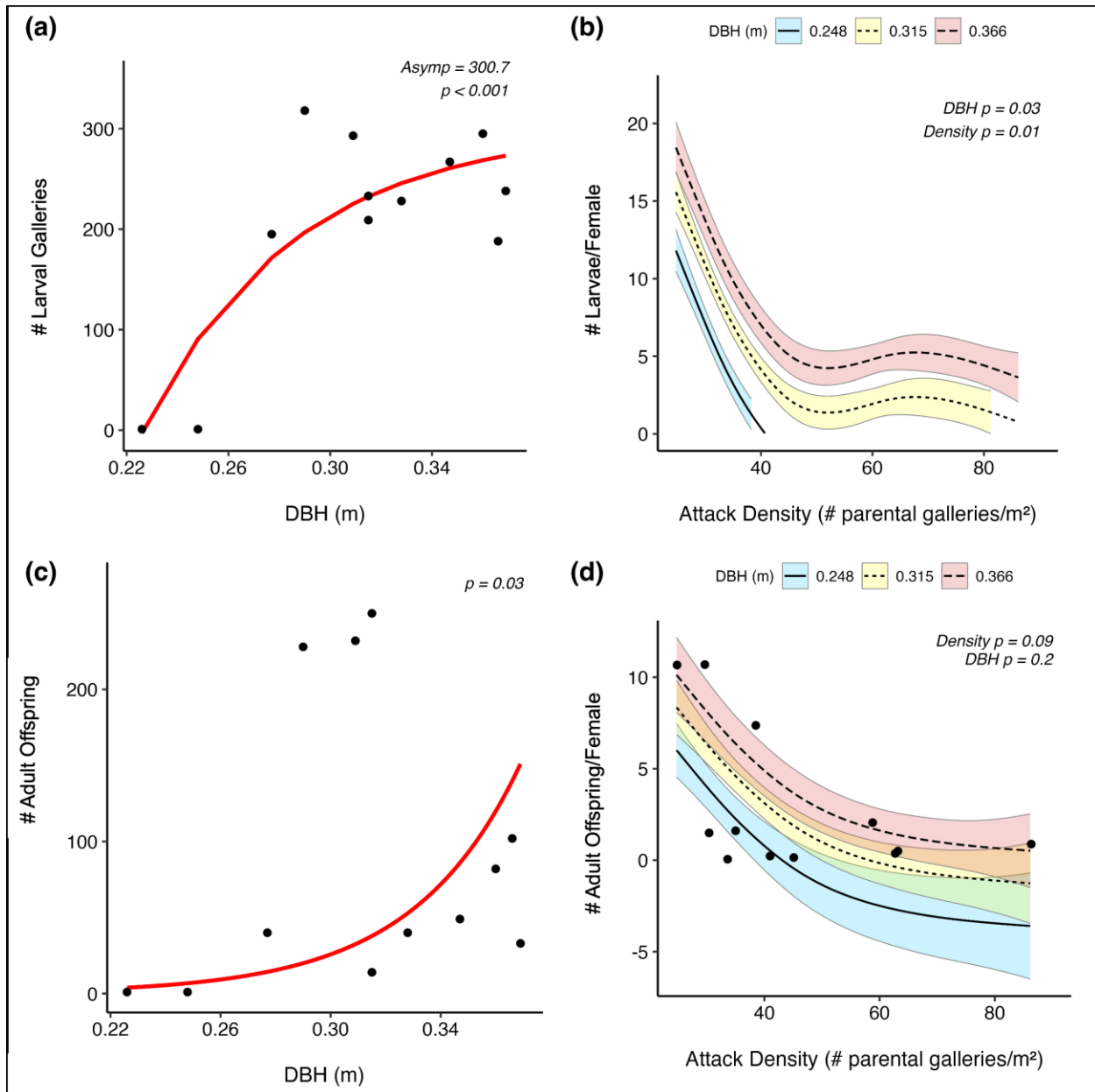
Figure 2.1: Offspring production from trees attacked at variable densities in 2015. (a) The vertical axis represents the total number of larvae counted from three 15 × 15 cm samples from one 50 cm bolt from each tree and the horizontal axis represents the diameter at breast height (DBH) of each tree. The raw data (black points) were fit to an asymptotic curve (red line), the estimated y-intercept for the asymptote (Asymp) with the p-value for that estimate are reported within the plot. (b) The vertical axis represents the number of larvae per female that emerged from one 50 cm bolt from each tree and the horizontal axis represents the attack density of each tree estimated as the number of parental gallery starts per m^2 of bolt surface area. Fit lines are the predicted values of a linear model where

the log transformed number of adults was predicted by attack density and DBH, which was separated into 10%, 50% and 90% quantiles, data are presented on the response scale. (c) and (d) show the raw data in black points with curves of the predicted values of generalized additive models with density as a smoothing term and DBH as a linear predictor which was also separated into 10%, 50%, and 90% quantiles. P-values for smoothing and linear terms within (c) and (d) were generated by the respective GAMs.
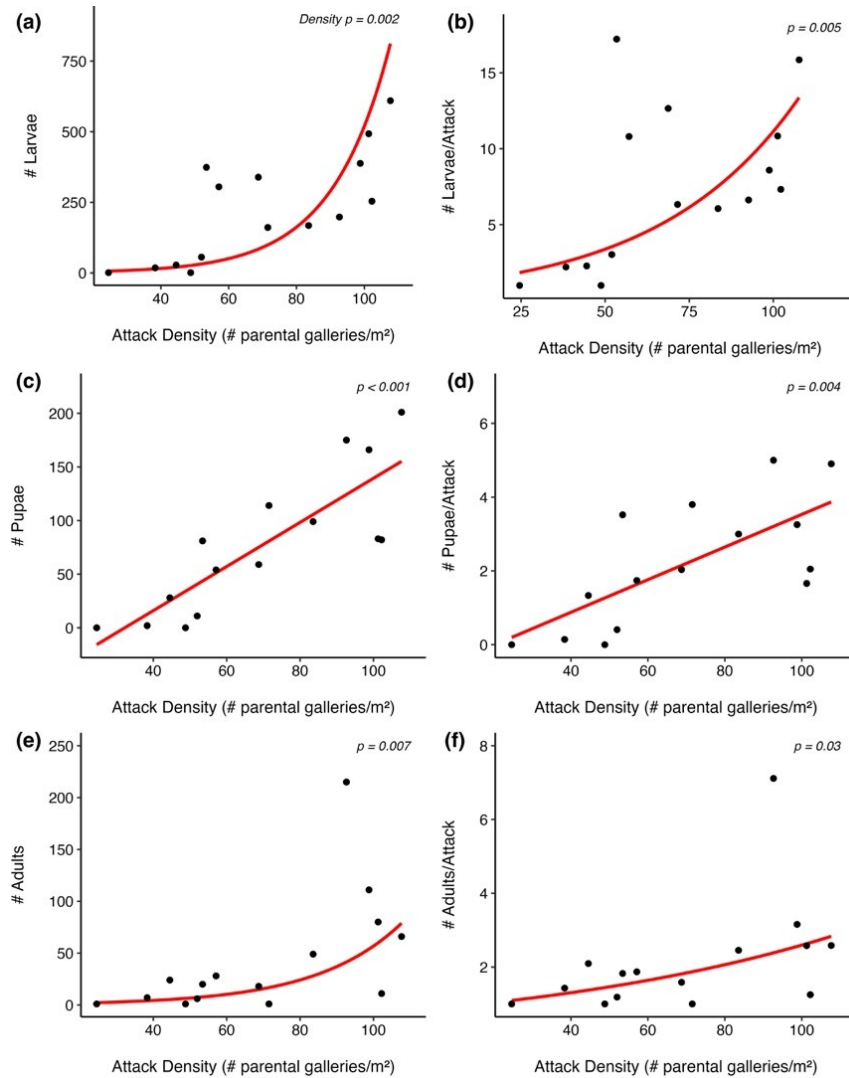
Figure 2.2: Offspring production from trees attacked at variable densities in 2016. Horizontal axes all represent the attack density of each tree estimated by the number of parental galleries started per m^2 of surface area. Vertical axes in (a) and (b) represent the total number of larvae and number of larvae per female, respectively, counted from the entire surface area of one 50 cm bolt from each tree. Vertical axes in (c) and (d) represent the total number of pupae counted and total number of pupae per female produced from one 50 cm bolt from each tree. Vertical axes in (e) and (f) represent the total number of adults that emerged and the number of adult offspring per female in one 50 cm bolt from each tree. The number of larvae, pupae and adult offspring per female were estimated by dividing the total number of each life stage by the number of parental gallery starts. Black points in all figures are raw data. Fit lines in (a), (b), (e) and (f) are the predicted values of linear models with the dependent variable log + 1 transformed, values are all shown on the

response (back-transformed) scale. Fit lines in (c) and (d) are the predicted values of general linear models with density as the independent variable.
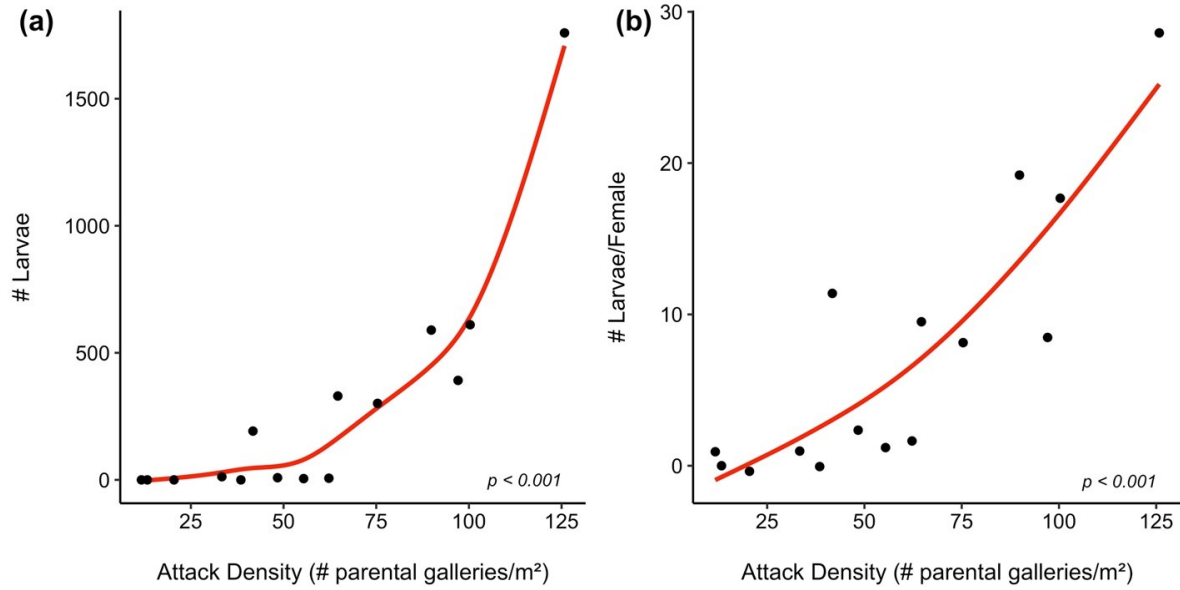
Figure 2.3: Offspring production from trees attacked at variable densities in 2017. Horizontal axes in both panels represent the attack density of each tree estimated by the number of parental galleries started per m^2 of the surface area of each 50 cm bolt. Vertical axes represent the total number of larvae (a) and the number of larvae per female (b) counted from one 50 cm bolt from each tree. Number of larvae per female was estimated by dividing the total number of larvae by the number of parental gallery starts in that bolt. Black points are raw data and fit lines are the predicted values of linear models on the response (back-transformed) scale.
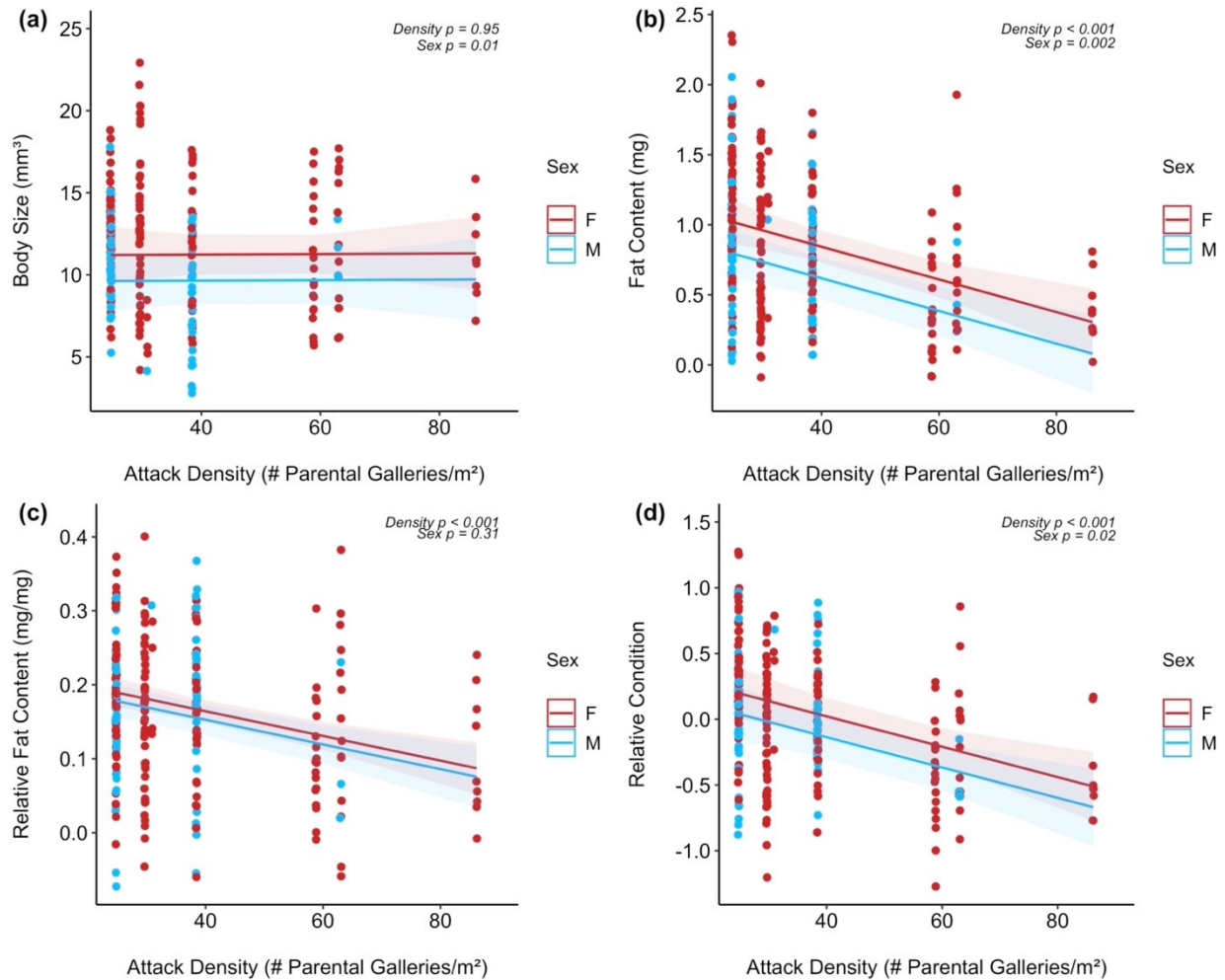
Figure 2.4: Offspring condition data from adult offspring collected from the 2015 attack density manipulation experiment. Relationship of attack density (horizontal axis) and sex (legend) with adult offspring body size (a), fat content (b), relative fat content (c), and relative condition (d). For each dependant variable (y-axis) displayed, raw data are plotted with fit lines with confidence bands from linear mixed effects models with attack density and sex as independent variables, and a term for bolt fitted as a random effect. P-values for each independent variable were generated with ANOVA tables of the linear mixed effects models.
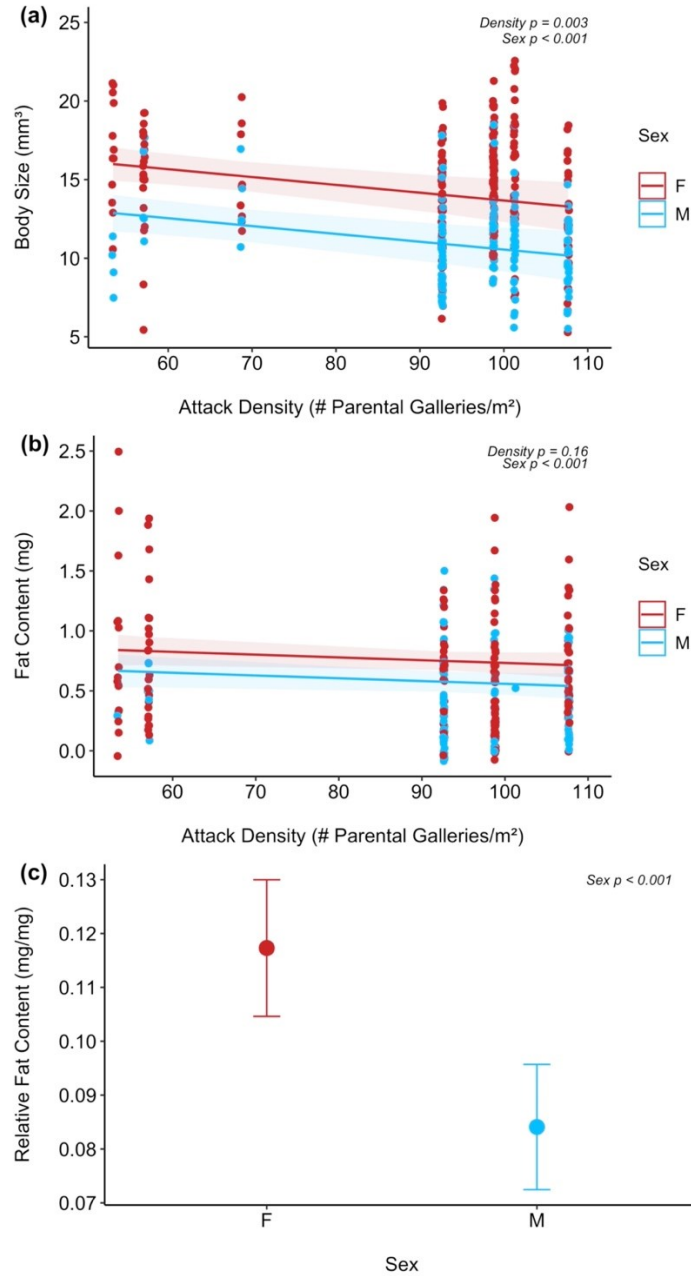
Figure 2.5: Offspring condition data from adult offspring collected from the 2015 attack density manipulation experiment. Relationship of attack density (horizontal axis) and sex (legend) with adult offspring body size (a), and fat content (b). For body size (a) and fat content (b), raw data are plotted with fit lines with confidence bands from linear mixed effects models with attack density and sex as independent variables, and bolt as a random effect. P-values for each independent variable were generated with ANOVA tables of the linear mixed effects models. In (c) the relationship between adult offspring sex and relative fat content is displayed as a single point representing the marginal mean for each sex

estimated by a linear mixed effects model with a term for bolt fit as a random effect. Error bars above and below points are the estimated mean $+/-$ the standard error. P-values in all figures are generated by ANOVA tables of the respective linear mixed effects models.
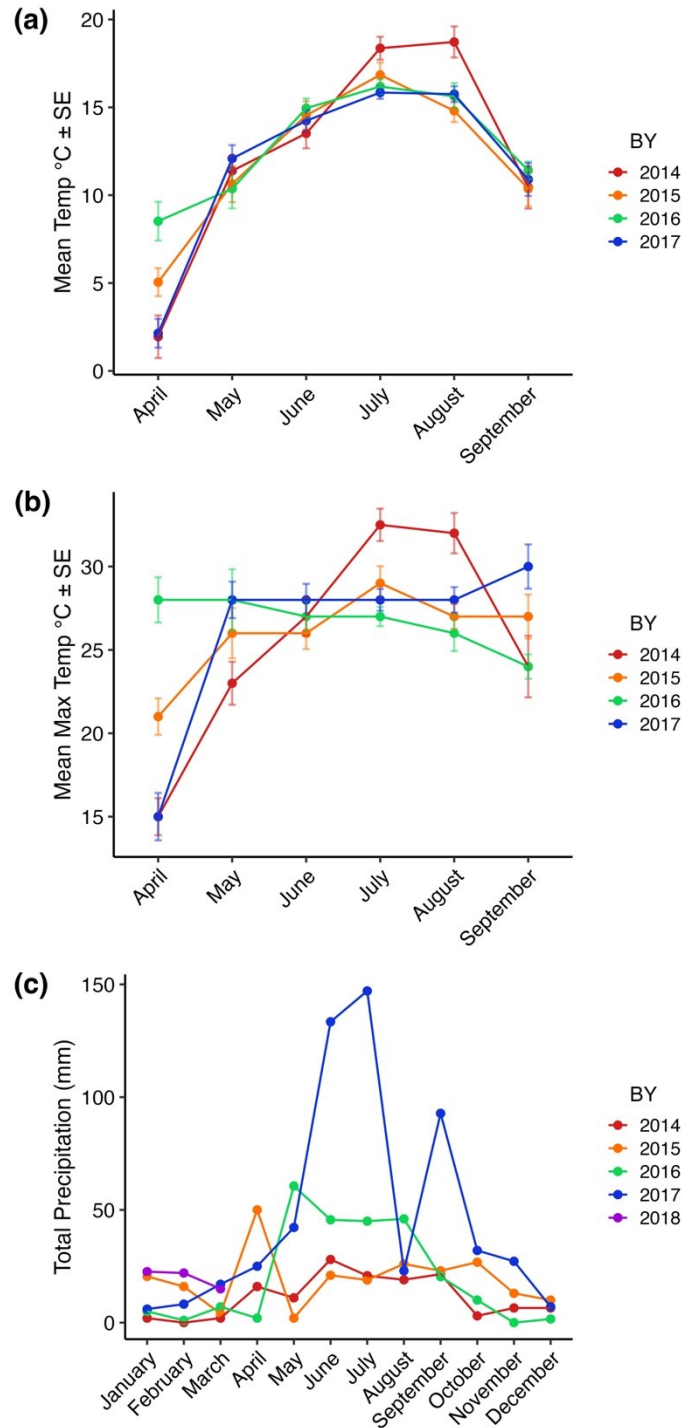
Figure 2.6: Data from Environment Canada Simonette Alberta weather station separated by year (BY). The weather station was decommissioned at the end of March 2018. (a) mean average ± SE daily temperatures per month, (b) mean daily maximum temperatures ± SE per month, and (c) monthly total precipitation.

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Chapter 3. Attack threshold and optimal attack density of mountain pine beetle (*Dendroctonus ponderosae*; Coleoptera: Curculionidae) in the novel host jack pine (*Pinus banksiana*)

3.1. Introduction:

Climate change has facilitated the range expansion of many insects (Hill et al. 2011) and has worsened outbreaks of irruptive herbivores in forests (Raffa et al. 2008, Pureswaran et al. 2018). In a new habitat, an insect must find a suitable niche to establish and persist. Insects can have traits that ease its establishment in a new area, a process deemed ecological fitting (Janzen 1985, Agosta and Klemens 2008). For herbivorous insects, this means they must find detectable and susceptible host plants in the new environment (Malcicka et al. 2015, Mestre et al. 2020). New habitat in the expanded range can provide feeding opportunities on novel host plant species which may result in host-switching behaviour. Phylogenetic or chemical similarity between the historic and novel host can aid insect establishment in the new habitat (Cipollini and Peterson 2018, Mech et al. 2019). Novel hosts may additionally present "defense-free space" in that they are easier for the herbivore to detect or digest, further facilitating the insects' establishment in the expanded range (Gandhi and Herms 2010). Together, a shared evolutionary history and chemical similarity of hosts in the historic or native range, along with reduced defenses in new hosts, can mediate the range expansion of irruptive insect herbivores in particular (Erbilgin 2019, Mech et al. 2019).

The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae) is an irruptive bark beetle that specializes on trees in the genus *Pinus* (Pinales: Pinaceae; Safranyik and Carroll 2006). It is a subcortical herbivore that feeds and reproduces within the phloem tissues of the main stem of its hosts. Mountain pine beetle has distinct population phases, including an endemic (non-outbreak, low density), and epidemic (outbreak, high density) phase. Beetles in the epidemic population phase preferentially colonize healthy, defensive host trees that have an abundance of resources to support beetle reproduction, whereas endemic beetles preferentially attack moribund trees with impaired defenses. The switch in colonization behaviours is driven by density-dependent cues (Boone et al. 2011; Burke and Carroll 2017). Well defended host trees have

an array of physical and chemical defenses that interfere with MPB colonization (Franceschi et al. 2005, Keeling and Bohlmann 2006, Chiu and Bohlmann 2022). Epidemic MPB recruit conspecifics with aggregation pheromones that are produced by both females and males to overcome host defenses (Safranyik and Carroll 2006). Females initiate colonization and release *trans*-verbenol to attract males and recruit more females (Pitman and Vité 1969). Males that enter the tree and join the female release *exo*-brevicomin which attracts even more females to the tree (Rudinsky et al. 1974, Conn et al. 1983). The result is a "mass attack", the success of which depends on the density of beetles that are recruited to the tree (Raffa and Berryman 1983a). If MPB are unable to reach a threshold density for successful mass attack, the beetles are killed by tree defenses during the colonization attempt, whereas a successful mass attack results in the death of all or part of the tree (Safranyik and Carroll 2006).

Lodgepole pine (*Pinus contorta*) is the most common host of MPB in its historic range (Safranyik and Carroll 2006). The threshold density for successful mass attack of lodgepole pine requires a minimum of ~40 attacking MPB/m^2 (Raffa and Berryman 1983a). At lower attack densities, the beetles are physically "pitched out" by resin defenses or killed by exposure to high concentrations of induced monoterpenes (Raffa et al. 2005, Chiu et al. 2017, Reid et al. 2017). Beyond the mass attack threshold density, the reproductive output of MPB that enter the tree increases until it reaches an optimal attack density of ~60 attacking MPB/m^2 (Raffa and Berryman 1983a). As attack densities increase above the optimum, there is a decline in offspring production per female as larvae compete for finite space and resources under the bark (Raffa and Berryman 1983a). Females that join a tree after the optimal attack density has been reached will have reduced fitness compared to beetles that avoid these trees (Raffa et al. 2016).

Mountain pine beetle has evolved to respond to antiaggregation cues to avoid trees that are already densely attacked. Antiaggregation pheromones are produced by both females (Hunt et al. 1989, Hunt and Borden 1990) and males (Rudinsky et al. 1974), as well as by some fungal mutualists (Cale et al. 2019). In a successfully mass attacked tree, females mine a small chamber in the phloem just under the bark where they mate with arriving males. Once mated, they construct vertical parental galleries through the phloem, lay eggs in niches on either side of the gallery and cover them with boring dust. Larvae hatch from eggs and develop through four instars while mining through the phloem horizontally at right angles to the parental gallery. Larvae overwinter and complete development in the spring, pupating within oval chambers. After eclosion, teneral adults

spend a period of time feeding on remaining phloem and the spores and hyphae of fungal mutualists until conditions are right for dispersal (Safranyik and Carroll 2006). Parental gallery construction, egg laying, and larval mining behaviour are influenced by temperature, moisture, phloem thickness, and the magnitude of competition with conspecifics (Amman and Cole 1983). The actual number of offspring that survive through the various stages of the lifecycle is also related to various host tree factors such as diameter, growth rate, phloem thickness (Berryman 1976), and a shared evolutionary history between MPB and the host tree (Cudmore et al. 2010).

Mountain pine beetle has a relatively wide host breadth within the genus *Pinus* and can mass attack and reproduce in as many as 22 different pine species and even two spruce (*Picea*) species (Wood 1963, Furniss and Schenk 1969, Smith et al. 1981, Huber et al. 2009, Cook and Martinez 2018b). In Canada, MPB has expanded its geographic range north and east from its historic range in British Columbia and into Alberta (Carroll et al. 2006b, Safranyik et al. 2010, de la Giroday et al. 2012). Once established in Alberta, MPB spread eastward through lodgepole pine forests and into the boreal forest where it successfully attacked and reproduced in the novel host species jack pine (*Pinus banksiana*) and lodgepole × jack pine hybrids (Cullingham et al. 2011). Terpenes are an important chemical defense for pines (Keeling and Bohlmann 2006, Chiu and Bohlmann 2022) and while lodgepole and jack pine evolved from a common ancestor and have similar phloem terpene defenses, the concentrations and ratios of those phloem terpenes differ between species (Dancik and Yeh 1983, Pollack and Dancik 1985). One of the most significant differences is that jack pine phloem has two to three times more α-pinene and much less β-phellandrene than lodgepole pine (Clark et al. 2014). α-Pinene is the precursor for the aggregation pheromone *trans*-verbenol and females that colonize jack pine bolts in the lab produce more *trans*-verbenol than those that colonize lodgepole pine bolts (Erbilgin et al. 2014, Taft et al. 2015a). Aggregation of MPB is directly related to relative α-pinene content (Burke and Carroll 2016) and wild populations of MPB attack jack pine bolts at higher densities than lodgepole pine bolts (Cerezke 1995). Jack pine can be considered evolutionarily naïve to MPB attack as it has fewer constitutive and induced defenses than lodgepole pine in response to simulated herbivory by MPB (Clark et al. 2014, Arango-Velez et al. 2016, Lusebrink et al. 2016, Erbilgin et al. 2017b). Lodgepole pine trees also induce more toxic phloem monoterpenes (Chiu et al. 2017, Reid et al. 2017) than jack pine trees after inoculation with the phytopathogenic fungal mutualist of MPB, *Grosmannia clavigera* (Arango-Velez et al. 2016, Lusebrink et al. 2016, Erbilgin et al. 2017b). Boreal jack pine forests therefore potentially comprise defense-free space and the chemical and phylogenetic

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similarity of lodgepole, and jack pine might allow range expanding MPB to establish via ecological fitting (Erbilgin 2019).

To date, studies of jack pine suitability as a host for MPB have only been performed in cut bolts (Safranyik and Linton 1982, Cerezke 1995, Erbilgin et al. 2014, Klutsch et al. 2016, Lusebrink et al. 2016, Rosenberger et al. 2017b, 2017a, 2018) and not in living trees. If jack pine trees are more susceptible to mass attack due to a lack of insect-host coevolution, as has been demonstrated for evolutionarily naïve lodgepole pine populations (Burke et al. 2017), then the relationship between MPB offspring production and attack density would differ to that observed in historic lodgepole pines. To investigate this, I simulated mass attack of jack pine by introducing MPB to living jack pine trees in the field. I manipulated the attack densities, and quantified beetle success and reproductive output. As jack pine has fewer constitutive and induced defenses, I predicted that the mass attack threshold would be lower than 40 attacks/m^2. I also examined offspring performance by rearing MPB from jack pine trees attacked *in situ* as an additional measure of jack pine suitability.

3.2. Methods:

Beetle collection

In mid-June 2018, four 50-cm bolts from each of 20 MPB-infested lodgepole pine trees (80 bolts in total) were collected across three sites near Hinton, Alberta (53.380, −117.543; 53.343, −117.587; 53.275, −117.665; Fig. 3.1). The cut ends of the bolts were sealed with hot paraffin wax to reduce desiccation, and then they were brought to the University of Alberta. Twenty bolts were immediately placed in bins (Rubbermaid Hinged Top Tote, 114 L, fitted with glass jars) to collect emergent beetles. The remaining 60 bolts were stored at 5°C and removed in batches of 20 per week. Beetles that emerged each day were counted, pooled, and placed in vented 1 L plastic containers filled with jack pine wood chips and kept at 5°C until use in the field (21 July–13 August 2018).

Field experiment

Three sites within a jack pine forest north of Lac La Biche, Alberta were selected (site A: 54.984, −112.009; site B: 55.170, −112.038; site C: 55.021, −111.980; Fig. 3.1). At each site, nine jack pine trees, free of obvious disease and insect herbivory, were selected

and the diameter at 1.3 m (DBH) and average phloem thickness were measured. Average phloem thickness was measured using a 10 mm diameter leather punch to obtain four phloem samples, one from each of the cardinal directions at ~50-cm height on the tree bole. Phloem tissue was separated from the bark for each sample and measured with digital calipers to the nearest 0.1 mm.

Experimental trees at each site were fitted with conical cages constructed from standard aluminum wire mesh insect screening (18 × 16 mesh size: PHIFER Inc. Alabama, USA). Cages were secured just above the 2 m mark on the tree bole so that the lower 2 m of each tree was exposed to the MPB released within the cage (Fig. 3.2). To limit beetles from walking out of the top of the cage through the bark ridges, a sheet of soft packing foam (15.24 cm width, ULINE, Edmonton AB) was placed between the mesh and the tree bark, and the top of the cage was secured in place with a plastic cable tie and ~five staples into the outer bark (Fig. 3.2). The bottom of the cage was buried in the sand ~1 m away from the base of the tree with an opening for experimenter access to introduce the beetles.

One thousand MPB were randomly selected from a pool of all beetles that emerged over the previous 10 days in the lab. The sex ratio of the random selection of beetles was not determined, but the average sex ratio of MPB ranges for 2:1 to 4:1, female:male (Safranyik and Carroll 2006). Selected beetles were placed into a 4 L paint bucket that was half full of jack pine wood chips and sealed with a vented lid. Buckets were transported from the University of Alberta to the field sites in refrigerated containers. Beetle release occurred on August 1, 8, and 13, 2018 for sites A, B, and C, respectively. Immediately before beetle release, to facilitate mass attack, the experimental trees were baited within the cage with commercially available MPB lures that consist of aggregation pheromones *trans*-verbenol and *exo*-brevicomin as well as the host volatiles myrcene and terpinolene (Mountain pine beetle lure- "California blend" from Synergy Semiochemicals Corp. Burnaby BC; Klutsch et al. 2017). After baiting, beetle buckets were placed within the cage under each experimental tree, the cage opening was sealed with staples along a seam, and the beetles were allowed to emerge from the wood chips naturally to attack the caged trees. Only eight of the nine trees that were selected at site C were manipulated, which made a total of 26 experimental trees across the three sites. To achieve a range of attack densities, without changing the initial density condition of the beetles in the buckets, buckets were removed from three randomly selected trees at each site 24 h after the initial release and were added within the cage of three different randomly selected trees. At each site there were three trees that had the bucket of beetles removed after 24 h (low density), three trees that had

that bucket added after 24 h (high density), and three trees (two at site C) which had no removals or additions (medium density).

Quantification of attack success and gallery characteristics

In late-October 2018, six trees at each site were felled and two 50-cm bolts from 1 m above the ground were harvested from each tree. Cut ends of the bolts were immediately sealed with hot paraffin wax and the bolts were brought back to the University of Alberta and stored at 5°C. Between November 2018 and July 2019, bolts were removed from storage in groups of two to four and placed individually into vented opaque emergence bins (Rubbermaid Hinged Top Tote, 114 L, fitted with glass jars) to collect emerging beetles. Emergent beetles were collected daily and placed individually into 1.5 mL microcentrifuge tubes for use in other experiments (data not reported here) or frozen immediately to measure body condition. Once emergence was completed (no emergent beetles for 30 d), bolts were autoclaved and debarked. Gallery characteristics were documented by either photographing the inner bark or, when the bark did not come off in a solid piece, galleries were traced onto clear plastic sheets directly from the bolt surface and the sheets were photographed. From the photographs, galleries were quantified in ImageJ 1.53k (NIH, USA) and the number and length of parental galleries, number of larval galleries, and the number and area of pupal chambers were measured. Pupal chambers were not as distinct as typically observed in lodgepole pine, so counts of pupal chambers were difficult to standardize. The number of 'pupal chambers' was based on the area and shape of the enlarged portion of the distal ends of larval galleries. The attack density for each tree was calculated by dividing the total number of parental galleries in both bolts by the surface area of each of the two bolts and taking the average.

Offspring condition

Up to 50 female and 50 male beetles that were immediately frozen after emergence from each experimental tree were assessed to determine body size and fat content. Pronotum width and body length were measured using a dissecting microscope with an ocular micrometer at 1.6×. The pronotum width and body length of each beetle were used to calculate body size as volume with the equation for the volume of an ellipsoid, a = half the body length, b = half the pronotum width, and c = half the pronotum width:

$$V = \frac{4}{3}\pi abc$$

The fat content for each beetle was quantified using a Soxhlet extraction method. Beetles were dried in an oven at 60°C for 24 h, weighed to the nearest 0.01 mg, and then placed individually in perforated 0.2 mL microcentrifuge tubes. Tubes with beetles were placed in a Soxhlet apparatus and washed every 20 min with warm petroleum ether (Sigma-Aldrich) for 8 h. Beetles were then dried again for 24 h at 60°C before being weighed. Pre-extraction dry weight was subtracted from post-extraction dry weight to determine lipid content. A residual body condition index was used to calculate the relative condition of beetles. A least-squares regression of fat content against body size was generated and the residual of each beetle, the difference between each data point and the values of the predicted relationship between body size and fat content, represented the residual condition of the beetle. Beetles with a positive residual value had a better body condition than predicted by the linear regression and those with a negative residual value had a poorer condition than predicted by the linear regression.

Overwintering success

Eight experimental trees (3 at site A, 3 at site B, 2 at site C) were left standing in the field until May 2019 when they were harvested and two 50-cm bolts from 1 m above the ground from each tree were brought back to the lab, placed in emergence bins, and galleries were quantified as described above.

Statistical analysis

All analyses were conducted in R (version 4.0.4; R Core Team 2022) within RStudio (version 1.2.5001; RStudio Team 2022). Data wrangling and cleaning were done using packages within *tidyverse* (Wickham and Grolemund 2016, Wickham et al. 2019). Data were analyzed with linear models. Statistical model fit was assessed by plotting scaled residuals and q-q plots using the *DHARMa* package (simulateResiduals; Hartig 2022). The Kolmogorov-Smirnov test for overdispersion, outlier test, and Shapiro-Wilk test for normality of residuals were also performed to assess model fit and whether assumptions were met. Independent and dependent variables were transformed to meet assumptions of the model if necessary. Final models were compared to a null model using likelihood ratio tests and results of the models were only interpreted if they differed significantly from the null model. Model summaries provided F-statistics and adjusted r^2 values for whole models and ANOVA tables were generated using *car* (Fox and Weisberg 2019). Figures of raw data and fitted values of linear models were made using *visreg* (Breheny and Burchett 2017) and

ggplot2 (Wickham 2016). Raffa and Berryman (1983a) presented their mass attack manipulation data as eight density groups with a mean and standard error, so for a simpler comparison, the data from trees that had attack densities within 5 attacks/m^2 were pooled and the mean and standard errors of those groups were plotted along with the fit line of the linear model of the ungrouped data.

The relationship between the independent variables of body condition measurements, body size, absolute fat content, relative fat content, and residual condition, with the dependent variables attack density, phloem thickness and sex, were initially analyzed using linear mixed effects models with a term for bolt fit as a random effect. Linear mixed models with the raw body condition measurements had extreme variation between trees (Appendix A Fig. A3) which violated the assumption of homogeneity of variance across groups. To combat this, raw body condition measurements were averaged by sex and by tree (N = 14 trees that produced more than 3 individuals of each sex) and the averages were analyzed using linear mixed effects models with tree as a random factor. Predicted values of the linear mixed models and raw data were plotted using *ggpredict* (Lüdecke 2018) and *ggplot2*.

3.3. Results

Attack success and gallery characteristics

Tree DBH ($F_{2,23} = 0.19$, p = 0.83) and average phloem thickness ($F_{2,23} = 0.52$, p = 0.60) did not differ significantly among trees located at the three different sites. The mean DBH for trees across all sites was 28.98 cm $\pm$ 2.77 cm (SD) and mean phloem thickness was 1.40 mm $\pm$ 0.29 mm (SD).

The gallery architecture of MPB in jack pine was markedly different from that typically seen in lodgepole pine (Fig. 3.3a; Wood 1963, Safranyik and Carroll 2006). Distinct oval pupal chambers were rare and larval galleries often fused with pupal chambers and created large areas of consumed phloem (Fig. 3.3b). Early-instar larval galleries that were immediately adjacent to the parental gallery were often more distinct than late-instar larval galleries further away from the parental gallery. Larval galleries were also very long in some bolts with low attack densities (Fig. 3.3c), too few bolts had low densities and displayed this type of gallery architecture, so this could not be compared quantitatively. The average parental gallery length was not affected by beetle attack density ($F_{1,15} = 1.31$, p = 0.27), or

phloem thickness ($F_{1,15}$ = 2.44, p = 0.14). The total number of larval galleries per bolt was positively influenced by attack density ($F_{1,15}$ = 35.67, p < 0.001; Fig. 3.4a), but the number of larvae per female increased with phloem thickness ($F_{1,15}$ = 6.60, p = 0.02; Fig. 3.4b) and not attack density ($F_{1,15}$ = 1.24, p = 0.28). The total number of pupae per bolt increased with attack density ($F_{1,15}$ = 10.02, p = 0.006; Fig. 3.4c), but the number of pupae per female increased only with increasing phloem thickness ($F_{1,15}$ = 7.36, p = 0.02; Fig. 3.4d). The total number of adult offspring that emerged from bolts from attacked jack pine trees was positively related to attack density ($F_{1,15}$ = 24.35, p < 0.001; Fig. 3.4e). The number of adult offspring per female was also positively influenced by attack density ($F_{1,15}$ = 5.10, p = 0.04; Fig. 3.4f), and was greater in trees with thicker phloem but this difference was not significant at α = 0.05 ($F_{1,15}$ = 3.83, p = 0.07; Fig. 3.4f).

The relationship between the estimated number of pupal galleries per bolt and the total number of adult offspring that emerged per bolt was positively correlated but the number of pupae was often an underestimate of the number of adult offspring that emerged from the bolt (Fig. 3.5a). Although Raffa and Berryman (1983a) compared pupae per female to attack density to determine the mass attack threshold and optimal attack density, the number of adult offspring that emerged per female was a better measure to estimate the mass attack threshold and optimal attack density in jack pine. As predicted, the mass attack threshold in jack pine was lower than 40 attacks/m^2, with successful adult offspring production at densities as low as 20 attacks/m^2 (Fig. 3.4f, 3.5b). The relationship between attack density and adult offspring per female did not peak but was lower on average than in Raffa and Berryman (1983a). Higher attack densities resulted in a greater percentage of the under-bark area consumed ($F_{1,15}$ = 13.64, p = 0.002; Fig. 3.6a) and as the percent area consumed increased, the number of adult offspring that emerged increased in a log-log relationship ($F_{1,15}$ = 40.94, p < 0.001; Fig. 3.6b). The number of adult offspring per female produced increased with the log-percent area consumed ($F_{1,15}$ = 12.13, p = 0.003), the number of offspring per female increases quickly with the percent of under bark area consumed, but then saturates (Fig. 3.6c).

Body condition measurements

When included in linear models, attack density did not affect any of the body condition measurements of adult offspring that emerged from the attacked trees. Adult offspring body size was affected by both sex and phloem thickness (Table 3.1). Females were larger than males (χ^2 = 102.06, df = 1, p < 0.001; Fig. 3.7a) and phloem thickness

had a positive effect on body size of both sexes (χ^2 = 10.43, df = 1, p = 0.003; Fig. 3.7b). Similarly, absolute and relative fat content was influenced by both sex and phloem thickness (Table 3.1). Phloem thickness had a positive effect on absolute fat content (χ^2 = 6.61, df = 1, p = 0.01; Fig. 3.7b) but only a marginal effect on relative fat content (χ^2 = 3.03, df = 1, p = 0.08; Fig. 3.7c), and females had more absolute (χ^2 = 32.06, df = 1, p < 0.001 Fig. 3.7b) and relative (χ^2 = 16.73, df = 1, p < 0.001; Fig. 3.7c) fat content. Residual body condition was not significantly affected by either phloem thickness or sex (p > 0.05).

Overwintering success

All bolts harvested from the eight mass-attacked jack pine trees that were harvested in May 2019 produced zero adult offspring. Cool late summer temperatures and extreme cold during winter led to the mortality of all juvenile offspring. Galleries under the bark in bolts from the overwintered trees at site A indicated that juveniles were at the second instar larval stage, or younger, and juveniles in bolts from the overwintered trees at sites B and C were a mix of first instar larvae and eggs. The Environment Canada weather station in Lac La Biche recorded that, after August 20, 2018, daily average temperatures did not rise above 15°C and after September 7, 2018, they did not rise above 10°C. From February 1–15, 2019, the average daily temperature in the Lac La Biche area was lower than −20°C and ten of those days had lows below −30°C (Environment Canada).

3.4. Discussion

Simulation of *in situ* mass attacks of live jack pines by MPB has allowed me to examine the attack dynamics, gallery architecture, and offspring success in jack pine in a way that is more representative of natural infestations than introductions of MPB to cut trees. The attack threshold density for MPB to successfully mass attack a lodgepole pine is ~ 40 attacks/m^2 (Raffa and Berryman 1983a). Jack pine trees in this study with attack densities between 20 and 40 attacks/m^2 produced at least two offspring per female, so it appears that the threshold for mass attack in jack pine is much lower than it is in lodgepole pine. This agrees with my prediction and lends support to the hypothesis that jack pine trees are more susceptible to attack potentially due to a lack of co-evolutionary history with MPB. Jack pine trees induce lower quantities of toxic monoterpenes compared to lodgepole pine when challenged with inoculation by the pathogenic fungal mutualist of MPB, *Grosmannia clavigera* (Arango-Velez et al. 2016, Lusebrink et al. 2016, Erbilgin et al. 2017b) or wounding (Clark et al. 2014). The living jack pine trees in this study were unable

to sufficiently induce resin defenses to pitch out and kill even very low densities of attacking MPB. In lodgepole pine, the optimal attack density is ~60 attacks/m^2, but above this density, the number of pupae per female is reduced due to intraspecific competition for finite resources under the bark (Raffa and Berryman 1983a). In jack pine, the number of adult offspring per female increased linearly with attack density but there was a trend towards a positive effect of phloem thickness. Jack pine trees with thick phloem produced more offspring per female than those with thin phloem at a similar attack density and although this trend is not statistically significant at α = 0.05, it's possible that this is still a biologically relevant result. This effect of phloem thickness increased variation in offspring success across attack densities but there is no evidence of a reduction in the number of offspring per female at high attack densities. In lodgepole pine, the number of brood produced by MPB increases linearly with increasing phloem thickness when food is limited (Amman 1972). It is possible that we did not have a significant (α = 0.05) effect of phloem thickness because few trees in our sample had thick phloem. When jack pine trees were pooled into groups with similar densities, the mean number of adult offspring per female produced was generally lower than the number of pupae per female at similar attack densities reported by Raffa and Berryman (1983a) except for one data point at ~147 attacks/m^2. The number of pupal chambers accurately predicts the number of adult offspring that will emerge from a tree as there is very little mortality between these two stages (Cole 1975) so it's not likely that the pupal chamber counts in Raffa and Berryman (1983a) were an overestimate of the adult offspring that emerged. When MPB attack densities were around the lodgepole pine optimal density, 60 attacks/m^2, in jack pine, an individual tree with thick phloem produced the most adult offspring per female and was the closest to the mean pupae per female produced reported by Raffa and Berryman (1983a) at an equivalent density.

Unlike the number of adult offspring per female, the number of larval and pupal galleries per female were not related to attack density but only to phloem thickness. Mountain pine beetle larval and pupal galleries had a notably different structure in mass-attacked jack pine trees compared to lodgepole pine. Rather than the typically distinct horizontal galleries and oval pupal chambers that are found in attacked lodgepole pine trees (Wood 1963, Safranyik and Carroll 2006), larval galleries appeared wider, longer, and were often fused in jack pine phloem. This made the quantification of pupal chambers particularly difficult in trees that had high attack densities as most were fused with multiple larval galleries as well as other pupal chambers. Trees that had high attack densities most often underestimated the actual number of adult offspring that emerged based on the number of

'pupal chambers' counted. Mortality between the pupal stage and adult emergence in lodgepole pine is typically low (Cole 1975), so while the number of pupal chambers is a reliable measure of brood production in lodgepole pine, it likely is not in jack pine.

The characteristic galleries of MPB feeding in jack pine can be partly explained by jack pine's phloem thickness. Among trees of comparable size, jack pine phloem is thinner on average (1.06–2.06 mm in this study) compared to lodgepole pine phloem (Cole 1973a, Lusebrink et al. 2016, Rosenberger et al. 2017). To consume the same volume of phloem, larvae mining in the thin phloem of jack pine need to mine longer and/or wider galleries, potentially causing indistinct larval galleries and pupal chambers. Larvae developing in thin phloem feed at a faster rate than larvae in thick phloem and this behaviour results in larvae mining further away from the parental gallery, potentially as an adaptation to reduce competition (Cole 1973b, Amman and Cole 1983). If larvae encounter one another while mining in phloem, they can tunnel across/under the other larval mines, double back towards the parental gallery or feed in a way that increases gallery width (Cole 1973b). If larvae mining in thin phloem encounter conspecifics, and they cannot mine above or below other galleries, the only options are to mine wider galleries or double back to the parental gallery which would increase the area under the bark that is consumed. Jack pine trees with thick phloem may simply have a greater count of larval galleries because they are more distinct and easier to count than in trees with thin phloem.

Although there is no evidence of an intraspecific competitive effect with increased attack densities in jack pine, there is some evidence that phloem thickness could be affecting offspring production per individual by increasing crowding. Since I suspected pupal chambers could not be quantified reliably, I also collected data on the area of phloem consumed under the bark. The percentage of area under the bark consumed increased exponentially with attack density and the actual number of offspring that emerged from that area increased with the percent area consumed in a log-log relationship. However, the number of offspring per female produced increased with the log percent area consumed. The number of offspring per female increased quickly until ~10% of the phloem area was consumed, after which, the relationship approaches a saturation point likely somewhere between 7 and 8 offspring per female. If MPB larvae in thin phloem are more likely to run into each other and will then change their behaviour in a way that results in a greater area of phloem consumed, this could limit offspring production from the tree. If two larvae feeding in phloem encounter each other, this can result in death of one or both larvae through cannibalism or simply physical contact (Cole 1973b). Trees in which there is a large

area under the bark is consumed, the incidence of larval contact could have been quite high. In lodgepole pine, MPB lay eggs more densely along the parental gallery when phloem is thick and decrease the density of eggs they lay when they are less than 2.5 cm away from another parental gallery (Amman 1972, Amman and Pace 1976). Changing the density of eggs along the parental gallery in response to phloem thickness and proximity to conspecifics would reduce the likelihood of crowding effects on offspring (Amman and Cole 1983). The population of MPB that entered north-central Alberta primarily has an evolutionary history with lodgepole and ponderosa pine which both have thicker phloem on average compared to jack pine (Rosenberger et al. 2017b) and so adaptations for egg spacing might not have reached the lower limit of phloem thickness that exists in jack pine. The fact that these bolts were from trees that were attacked at biologically relevant densities is also important. When pairs of MPB are introduced to jack pine bolts 3–5 cm apart, the resulting brood production per cm of parental gallery before winter is similar to that in lodgepole pine, despite the thinner phloem in jack pine (Rosenberger et al. 2018). Egg laying density is not changed when the next nearest parental gallery is >2.5 cm away. If the relatively thin phloem of jack pine is limiting how much space there is for MPB that attack at high densities, then there could be selection over time to either avoid trees with thin phloem or avoid trees with densities that would result in lower phloem availability for offspring.

Jack pine phloem has three times more α-pinene than lodgepole pine phloem (Clark et al. 2014). α-Pinene is the precursor to the female aggregation pheromone *trans*-verbenol and beetles that colonize jack pine bolts produce more *trans*-verbenol than those that colonize lodgepole pine (Erbilgin et al. 2014, Taft et al. 2015a). Foraging MPB in the field presented with a choice to infest bolts of different pine species most densely attack jack pine bolts (Cerezke 1995) or bolts with the highest relative α-pinene content (Burke and Carroll 2016). It is likely that the higher release rate of female aggregation pheromone from beetles that infest these bolts (Erbilgin et al. 2014, Taft et al. 2015a) makes it easier to locate and concentrate aggregation. Aggregation in a tree above the optimal attack density results in diminished returns for any MPB females that join the aggregation (Raffa and Berryman 1983a). As a result, MPB has evolved the capacity to signal, using anti-aggregation pheromones, to repel conspecifics in a density dependent fashion (Safranyik and Carroll 2006). The female produced anti-aggregation pheromone verbenone is converted from *trans*-verbenol (Hunt et al. 1989, Hunt and Borden 1990, Cale et al. 2019) but verbenone production does not increase like *trans*-verbenol does in beetles that are actively colonizing jack pine bolts (Erbilgin et al. 2014). Local adaptation of MPB to jack pine

could include a change in beetle response to verbenone. There would be a fitness benefit to females that are repelled by lower concentrations of verbenone in jack pine forests, as they would avoid colonizing a tree that is too densely populated to support their offspring with the poor phloem resources.

The body condition of beetles reared in jack pine was not related to attack density but was positively influenced by phloem thickness. Phloem thickness and nutritional quality both positively affect the size and fat content of MPB when reared in lodgepole pine (Amman and Pace 1976, Graf et al. 2012) and ponderosa pine (Amman and Pasek 1986). I found no effect of attack density on body size or fat content of beetles reared in jack pine, but high attack densities negatively affect MPB size in both lodgepole and ponderosa pine (Amman and Pace 1976, Amman and Pasek 1986). The beetles that emerged from the jack pine bolts in this study were larger on average than beetles that emerge from lodgepole pine (Amman and Pace 1976) despite coming from trees with thinner phloem. Beetles that emerge from ponderosa pine are also larger than those that emerge from lodgepole pine but at equivalent phloem thicknesses which suggests ponderosa pine is more nutrient rich (Amman 1982, Amman and Pasek 1986) or the chemical defenses of lodgepole pine cause sublethal effects that reduce offspring size. Jack pine phloem contains similar amounts of carbon but less nitrogen than lodgepole pine phloem (Lusebrink et al. 2016) so from a nutritional standpoint, jack pine phloem is likely less nutritious than lodgepole pine phloem. The range of body lengths in this study fall within the range of body lengths of beetles that develop in ponderosa pine (Amman and Pasek 1986), even though jack pine phloem is much thinner.

An alternative explanation for the relatively large body size despite the poor host quality of thin phloem and low nitrogen content of jack pine (Lusebrink et al. 2016) is that only large-bodied beetles survive the poor habitat provided by thin phloem and strong larval competition in jack pine. Pine processionary moths are undergoing a range expansion and switching hosts to a pine species with poor nutritional quality and tough needles compared to high-quality host species with soft needles (Zovi et al. 2008). Offspring of the moths that develop on the novel, low-quality host are larger than those that develop on a high-quality host as larger mandibles are required to process the tough plant material (Zovi et al. 2008). If body size is heritable in MPB, and offspring with large body sizes are better competitors and able to survive in thin phloem, then this selective pressure could lead to local adaptation that affects the average body size of MPB in jack pine forests. Graf et al. (2012) examined body size and lipid content of MPB that emerged from lodgepole pine trees that

were different sizes. Body size and fat content were closely correlated, and both increased with host tree diameter (Graf et al. 2012). The beetles that emerged from my experiment were larger on average than what Graf et al. (2012) reported but had lower average relative fat content compared to the beetles in Graf et al. (2012). This difference suggests that while there is an increase in body size of MPB that emerge from mass attacked jack pine in this study compared to lodgepole pine (Graf et al. 2012), there is not a proportional increase in fat content. This is consistent with lab studies as both sexes of MPB that emerge from jack pine bolts infested in the lab have a worse residual body condition than beetles from lodgepole pine (Wijerathna et al. 2019). Lipid content is especially important for females, as it is the main fuel used for flight and could influence dispersal capacity of females that emerge from jack pine (Evenden et al. 2014). Female MPB that emerged from jack pine bolts infested in the lab burn more fat during flight than females from lodgepole pine bolts (Erbilgin et al. 2014) which could also limit MPB success during dispersal through a trade-off between flight and subsequent reproduction (Wijerathna et al. 2019).

My findings illustrate the attack dynamics and offspring performance of MPB that mass attack live jack pine trees. The data obtained from this experiment, however, was based only on beetle development in trees that were felled before winter. In the subset of jack pine left in the field over the winter, no offspring survived. The main reason for lack of survival was that beetles had not developed to the most cold tolerant third–fourth larval instar stages prior to the onset of winter. Simulated mass attack of jack pine trees occurred from the middle to end of a typical flight period of MPB in Alberta (Bleiker and Van Hezewijk 2016). An unusually early fall and extreme winter delayed beetle development in the experimental trees as well as in naturally attacked lodgepole pine trees in Western Alberta (personal observation). Temperatures over the 2018–2019 winter season were predicted to cause 99–100% mortality in overwintering MPB larvae at even the most cold-tolerant stages (Safranyik and Linton 1991, 1998, MacQuarrie et al. 2019), so it is not surprising that we saw 100% mortality of the less cold-tolerant juvenile stages that were present under the bark in our experimental trees over the winter.

Mass attack behaviour only occurs during the epidemic population phase, but the vast majority of the time MPB is in the endemic phase. During the endemic phase, MPB competes with pulse-driven bark beetles and wood boring beetles (Coleoptera: Cerambycidae and Buprestidae) for access to defensively compromised or suppressed hosts that are suitable for reproduction at low densities (Carroll et al. 2006a, Boone et al. 2011, Howe et al. 2022). Jack pine forests have fewer typical endemic hosts on the landscape

which are often also occupied by woodboring beetles that not only consume most of the available phloem but attract woodpeckers which then consume MPB larvae or destroy their habitat (Pokorny 2021). Jack pines infected with dwarf mistletoe present a potential endemic niche for MPB, however, they are often colonized by woodborers as well (Klutsch et al. 2016). The trees harvested in October for this experiment had no woodpecker damage and few wood borers present. The subset of trees left to overwinter that were harvested the following May had many woodborers under the bark even though the trees remained wrapped in mesh until harvest. Since none of the offspring of the parents that mass attacked jack pines survived the winter, it is impossible to determine if interspecific competition and less extreme winter temperatures would have impacted MPB success in jack pines in the field.

Living jack pine present a suitable niche for MPB that enter the boreal forest. The similarities between lodgepole and jack pine mean that jack pine provides the food and habitat that MPB needs. A reduced defensive capacity means that low MPB densities successfully mass attack jack pine, but most trees with thin phloem which resulted in crowding and has the potential to reduce offspring production. It's likely there are just as many barriers as corridors for MPB in jack pine forests. Mountain pine beetle population dynamics are rife with uncertainties, small changes in climate or host susceptibility can result in major shifts in the likelihood and frequencies of outbreaks (Cooke and Carroll 2017). Jack pine is more susceptible to mass attack compared to lodgepole pine which suggests there could be a quick shift between endemic and epidemic behaviour in jack pine forests (Carroll et al. 2006a, Boone et al. 2011, Burke and Carroll 2017). The poor offspring quality of MPB that emerge from jack pine with thin phloem could slow the spread of MPB over the landscape, as dispersal is also density and offspring condition dependent (Evenden et al. 2014, Powell and Bentz 2014). Further studies will need to examine how MPB adapt to these new host conditions. The MPB I released under experimental trees were from mass attacked lodgepole pine. Mountain pine beetles that successfully complete their development within jack pine could be selected for success in colonizing jack pine (Hopkins 1916). There can be an initial cost to switching hosts, but those costs can be minimized with adaptation (Jones et al. 2015). The invasion of the boreal forest by MPB will be limited by its adaptation to jack pine as a host in both population states. If the adaptations MPB has while being selected in jack pine can result in reproductive isolation from beetles that are reproducing in lodgepole pine, speciation could occur rapidly (Bracewell et al. 2017). The long-term relationship between MPB and jack pine forests in Alberta presents an amazing

opportunity to study local adaptation and evolution in a species undergoing selection for a host-switch.

3.5. Tables and Figures

Table 3.1: Linear model formulae with test statistics, degrees of freedom (df), and p-values, as well as the figure number that shows the relationships visually. Adjusted r^2 are reported for linear models and marginal r^2 for linear mixed effects. Number (#) of life stages (larva, pupae, adult offspring) are the total number counted per jack pine (*Pinus banksiana*) bolt. Number (#) of life stages (larvae, pupae, adult offspring) per female (/F) were calculated by dividing the total count by the number of parental galleries on the bolt. Attack density (density) determined by dividing the total number of parental galleries in two 50-cm bolts from each tree by the surface area of the two bolts. Phloem thickness (phloem) was measured from four phloem samples on the lower bole and averaged per tree. The percent (%) area consumed is the total under bark area that was consumed by MPB in one bolt divided by the surface area of that bolt. Transformations of variables and whether they were designated random effects, are denoted in brackets.

Formula	Test statistic, p-value	Figure
# larva ~ density + phloem	Whole model: $F_{2,15} = 21.04$, $p < 0.001$, adjusted $r^2 = 0.70$	3.4a
	Density: $F_{1,15} = 35.67$, $p < 0.001$	
	Phloem: $F_{1,15} = 0.76$, $p = 0.40$	
# larva/F ~ density + phloem	Whole model: $F_{2,15} = 5.02$, $p = 0.02$, adjusted $r^2 = 0.32$	3.4b
	Density: $F_{1,15} = 1.24$, $p = 0.28$	
	Phloem: $F_{1,15} = 6.60$, $p = 0.02$	
# pupae ~ density + phloem	Whole model: $F_{2,15} = 7.47$, $p = 0.006$ adjusted $r^2 = 0.43$	3.4c
	Density: $F_{1,15} = 10.02$, $p = 0.006$	
	Phloem: $F_{1,15} = 1.72$, $p = 0.21$	
# pupae/F ~ density + phloem	Whole model: $F_{2,15} = 3.68$, $p = 0.05$ adjusted $r^2 = 0.24$	3.4d
	Density: $F_{1,15} = 0.38$, $p = 0.55$	
	Phloem: $F_{1,15} = 7.36$, $p = 0.02$	
# adult offspring (log + 1) ~ density + phloem	Whole model: $F_{2,15} = 15.84$, $p < 0.001$ adjusted $r^2 = 0.64$	3.4e
	Density: $F_{1,15} = 24.35$, $p < 0.001$	
	Phloem: $F_{1,15} = 1.73$, $p = 0.21$	

# adult offspring/F ~ density + phloem	Whole model: $F_{2,15} = 6.04$, p = 0.01 adjusted $r^2 = 0.37$	3.4f
	Density: $F_{1,15} = 5.10$, p = 0.04	
	Phloem: $F_{1,15} = 3.83$, p = 0.07	
% area consumed (log + 1) ~ density + phloem	Whole model: $F_{2,15} = 8.97$, p = 0.003, adjusted $r^2 = 0.48$	3.6a
	Density: $F_{1,15} = 13.65$, p = 0.002	
	Phloem: $F_{1,15} = 1.06$, p = 0.32	
# adult offspring (log + 1) ~ % area consumed (log + 1) + phloem	Whole model: $F_{2,15} = 25.68$, p < 0.001, adjusted $r^2 = 0.74$	3.6b
	% area consumed: $F_{1,15} = 40.94$, p < 0.001	
	Phloem: $F_{1,15} = 1.06$, p = 0.32	
# adult offspring/F ~ % area consumed (log +1) + phloem	Whole model: $F_{2,15} = 10.78$, p = 0.001, adjusted $r^2 = 0.54$	3.6c
	% area consumed: $F_{1,15} = 12.13$, p = 0.003	
	Phloem: $F_{1,15} = 2.58$, p = 0.13	
Adult offspring body size ~ phloem + sex + bolt (random)	Marginal $r^2 = 0.71$	3.7a
	Phloem: $\chi^2 = 10.43$, df = 1, p = 0.001	
	Sex: $\chi^2 = 102.06$, df = 1, p < 0.001	
Adult offspring fat content ~ phloem + sex + bolt (random)	Marginal $r^2 = 0.52$	3.7b
	Phloem: $\chi^2 = 6.61$, df = 1, p = 0.01	
	Sex: $\chi^2 = 32.06$, df = 1, p < 0.001	
Adult offspring relative fat content ~ phloem + sex + bolt (random)	Marginal $r^2 = 0.33$	3.7c
	Phloem: $\chi^2 = 3.03$, df = 1, p = 0.08	
	Sex: $\chi^2 = 16.73$, df = 1, p < 0.001	
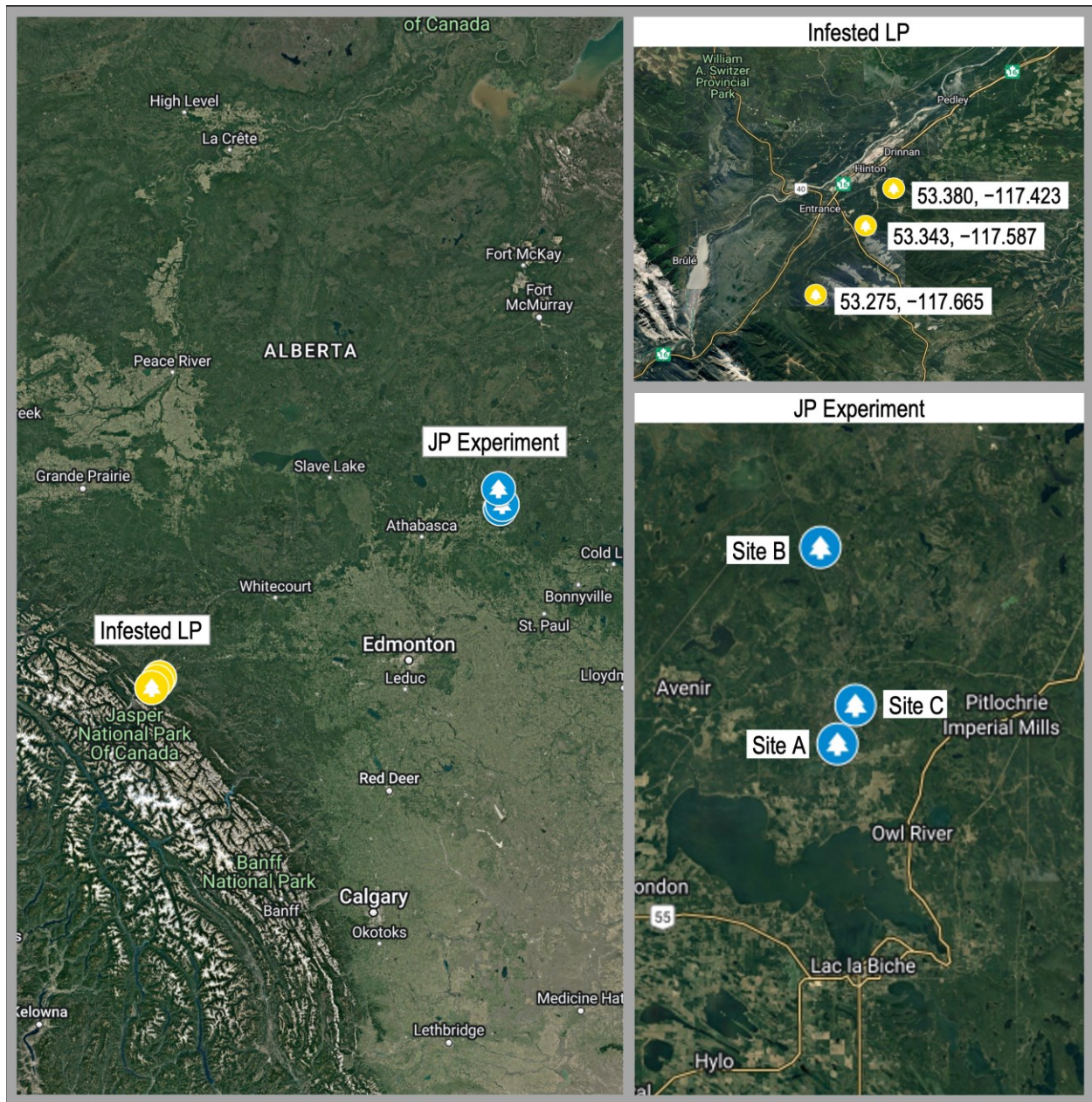
Figure 3.1: Locations where infested lodgepole pine (LP; *Pinus contorta*) were collected (yellow points, left and top right) and locations where jack pine (JP; *Pinus banksiana*) mass attack manipulation experiments (blue, left and bottom right) were performed.
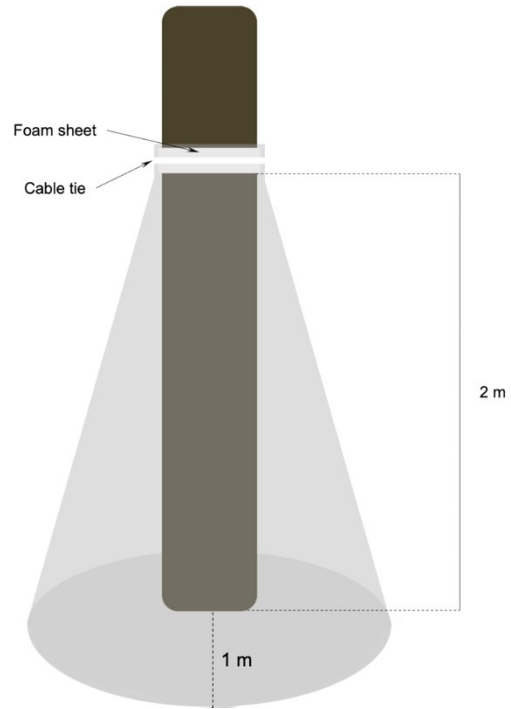
Figure 3.2: Conical wire mesh cage surrounding mature jack pine (*Pinus banksiana*) tree under which MPB were released. A layer of packing foam was placed between the cage top and the bark and cages were secured just above 2 m on the tree bole with 5 staples into the bark and a plastic cable tie, leaving 2 m of the tree exposed under the cage. The base of the cage was buried in the sand ~1 m away from the bole surrounding the tree and completely sealed after beetles were placed within
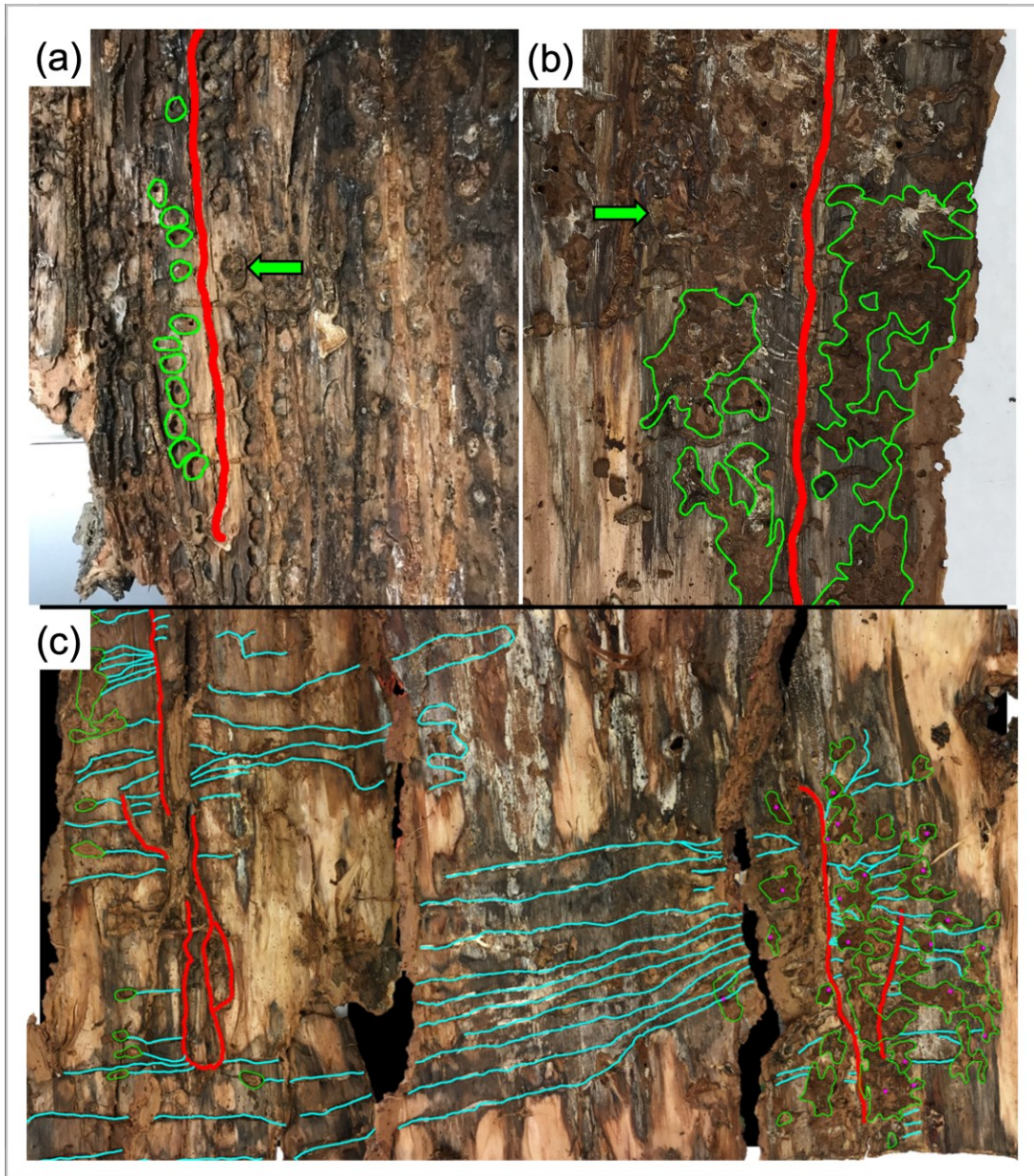
Figure 3.3: Examples of mountain pine beetle (*Dendroctonus ponderosae*) galleries on inner bark of lodgepole pine (a; *Pinus contorta*) and jack pine (b; *Pinus controta*). Lodgepole pine bark in (a) was collected by Antonia Musso from an infested tree 4 km south-east of Hinton AB (53.342, -117.587) in 2018. Parental galleries emphasized with thicker red lines; pupal chambers emphasized in thinner light green lines. Pupal chambers in lodgepole pine (a) have distinct oval shape (see p. 67 of Wood 1963). Pupal chambers in jack pine (b) do not have distinct distal oval shape as late instar larval galleries and pupal chambers are joined together in large areas of consumed phloem. Green arrows in (a) and (b) point to pupal chambers that are not outlined. (c) A low attack density jack pine bolt in which larvae

(light blue) mined exceptionally long galleries horizontally away from the parental galleries (red); Pupal areas shown in green and pink dots are exit holes.
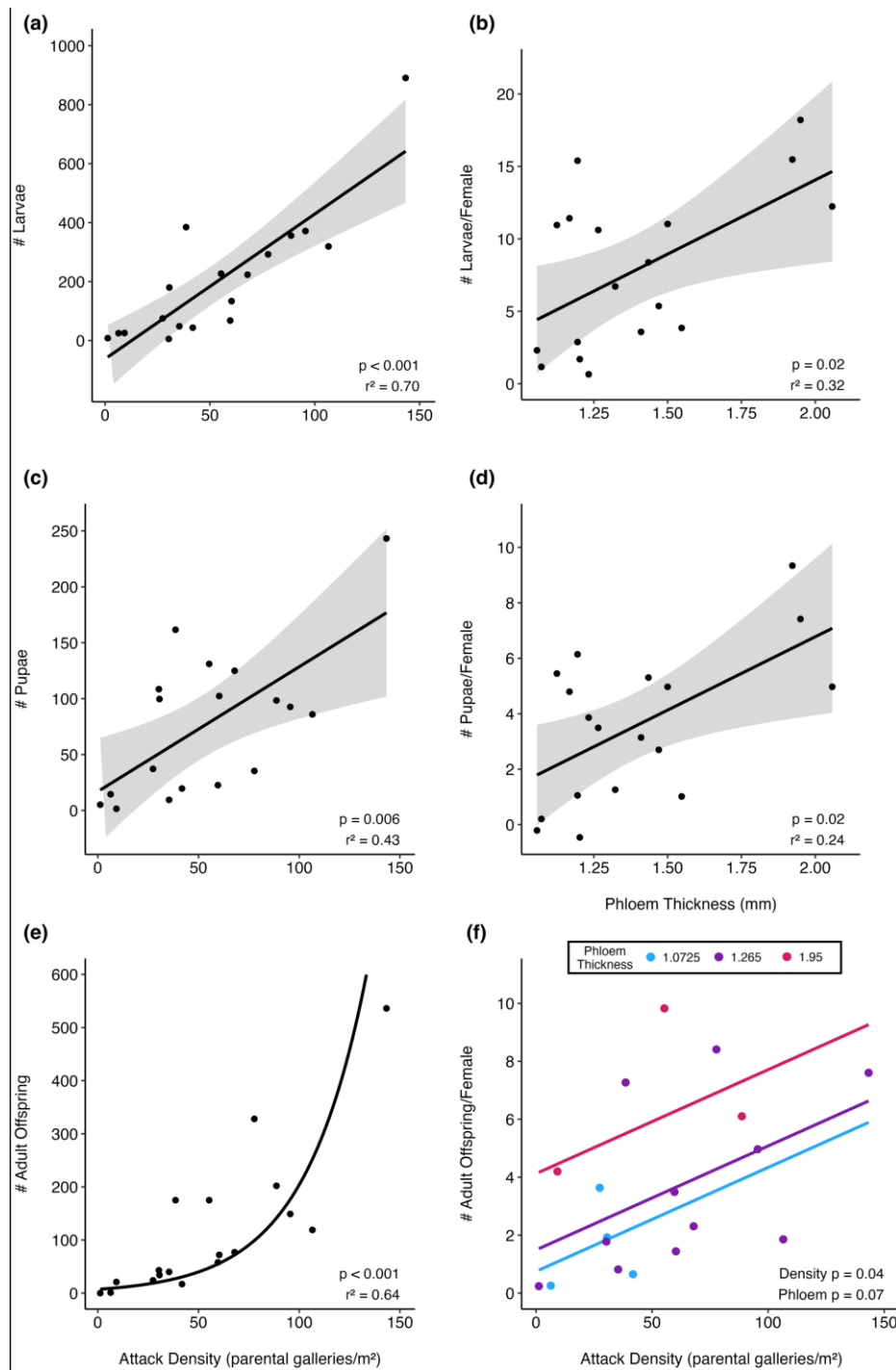
Figure 3.4: Relationships between attack density and total number of larval galleries per jack pine (*Pinus banksiana*) bolt (a), total number of pupae per bolt (c), and total number of adult offspring per bolt (e). The relationships between phloem thickness and number of larvae per female (b) and number of pupae per female (d). The relationship between number of adult offspring per female and both attack density and phloem thickness (f). Formulae for linear models in Table 3.1, p-values are for the independent variable on the x

unless specified otherwise. Fit lines are the predicted values of the linear model on the response (back-transformed) scale. In (f) the predicted values based on phloem thickness are divided into 10, 50, and 90% quantiles.
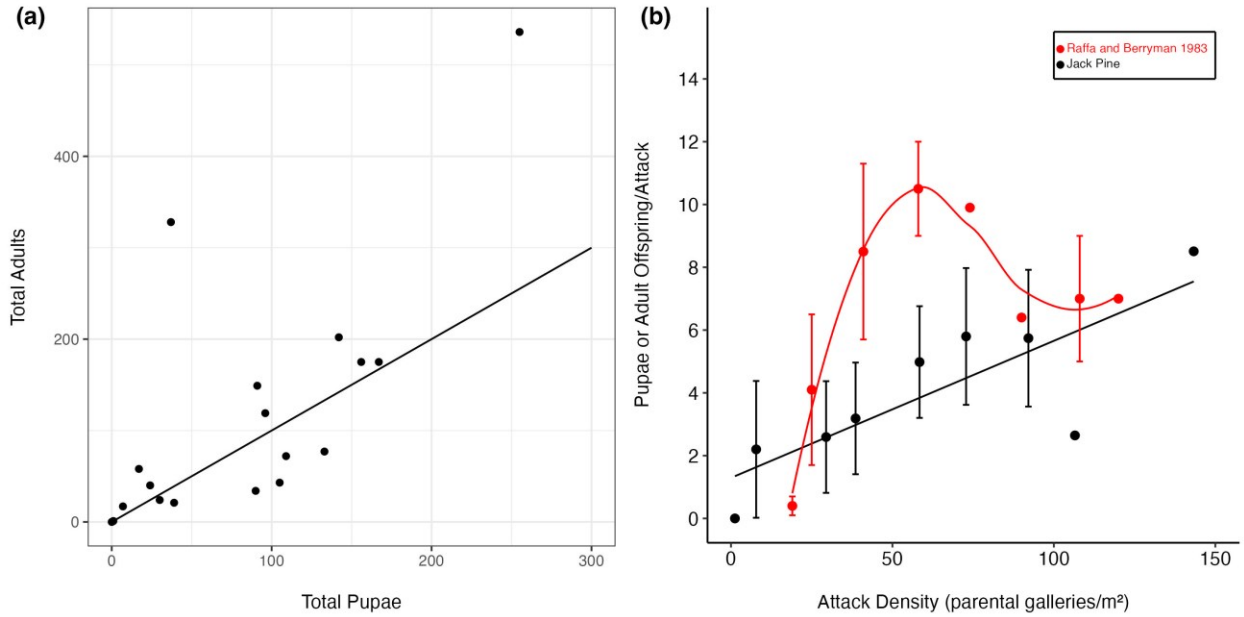
Figure 3.5: (a) Estimated total pupae per bolt and the total number of adults that emerged from the bolt in jack pine (*Pinus banksiana*), with a 1:1 line. (b) Data from jack pine in black and data from Raffa and Berryman (1983) in red. Points are means and error bars are standard errors of the means. Fit line for jack pine is the predicted values of the linear model of adult attack density and adult offspring per female and the curve for Raffa and Berryman (1983) is a local polynomial regression (loess) of the relationship between attack density and mean pupae per female.
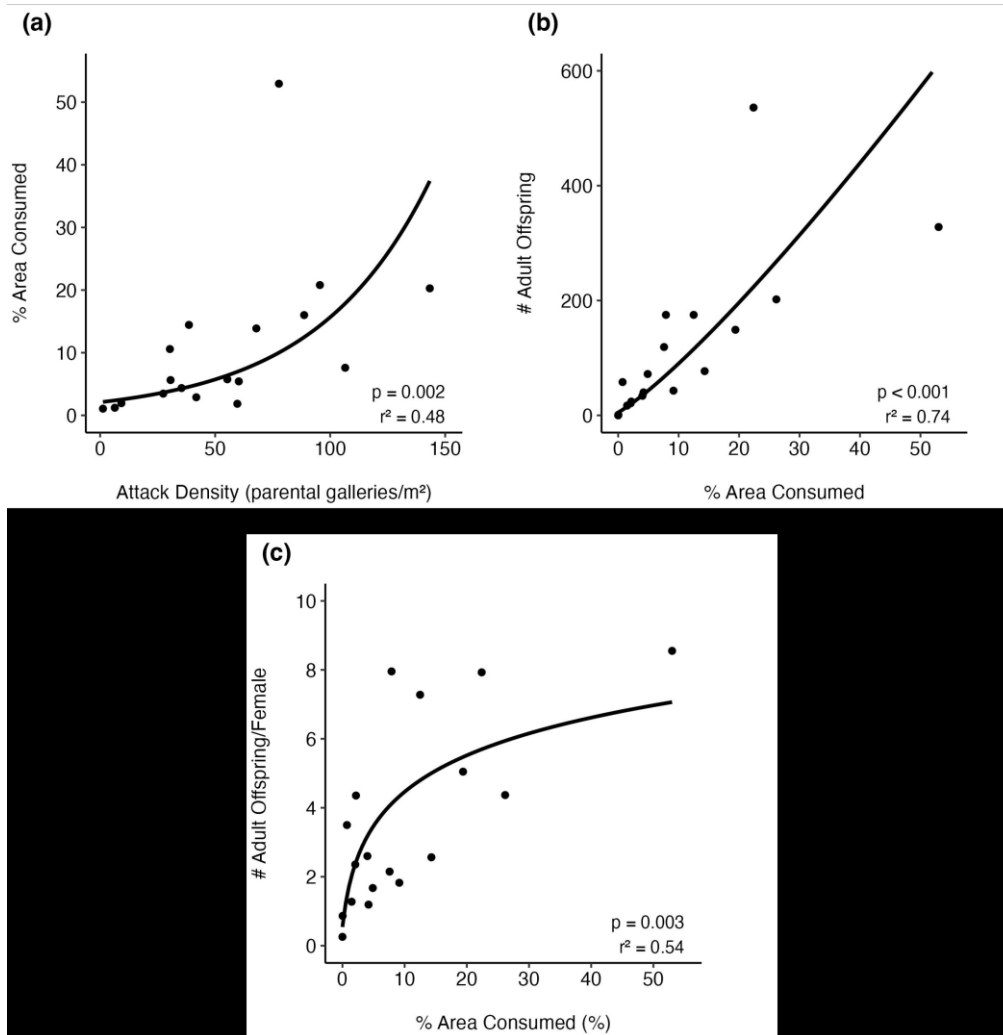
Figure 3.6: (a) Relationship between attack density and percent of under bark area consumed per jack pine (*Pinus banksiana*) bolt (log + 1) transformed. The relationships between percent of under bark area consumed per bolt (log + 1 transformed) and the number of adult mountain pine beetle (*Dendroctonus ponderosae*) offspring that emerged per bolt (log +1) transformed (b); and the number of adult offspring per female (c). Fit lines, p-values, and adjusted r^2 in are from linear models with the variable on the x-axis as the dependent variable and the variable on the y-axis as the independent variable. Fit lines and raw data values are on the response (back-transformed) scale.
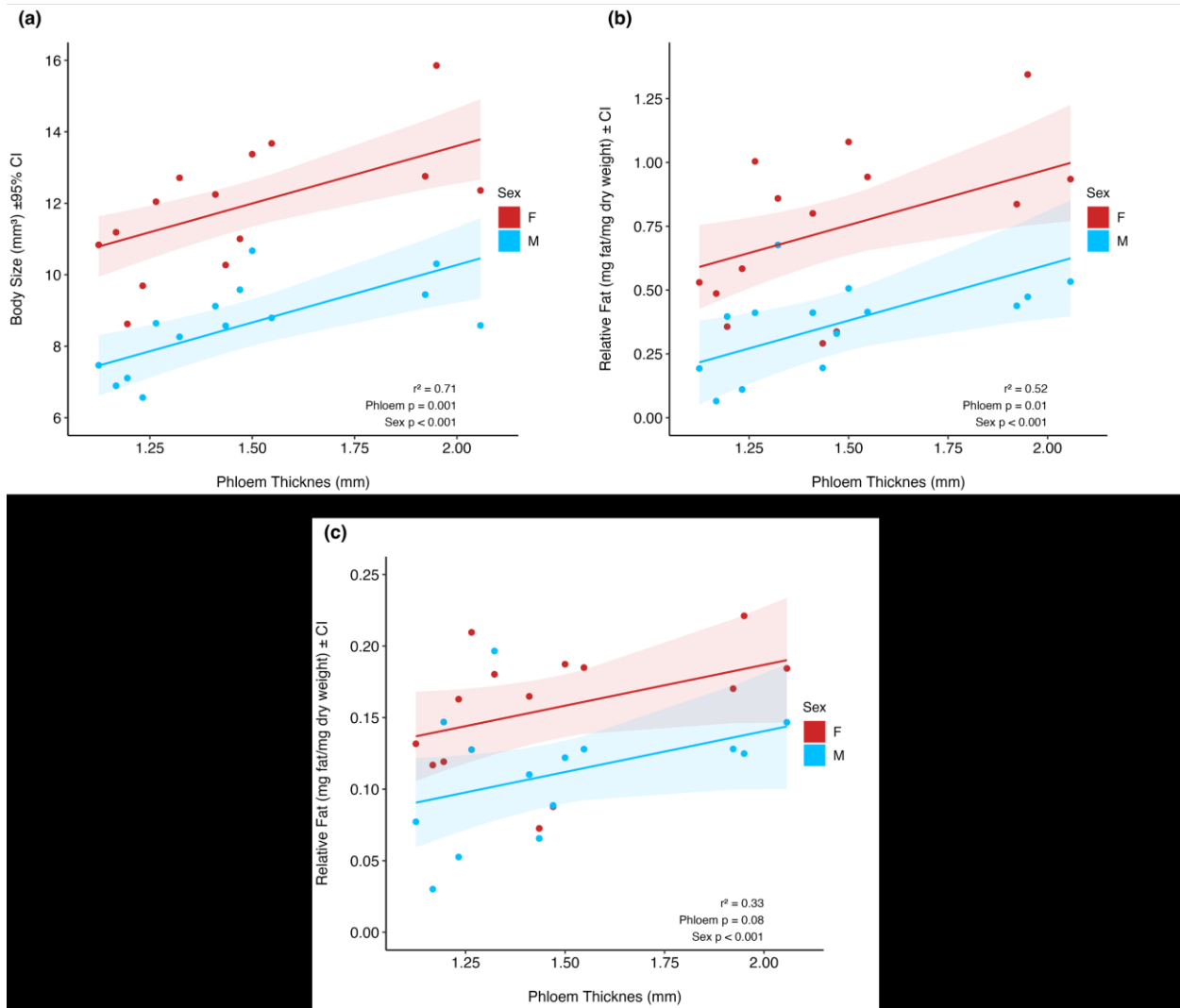
Figure 3.5: The relationship between mean values per bolt of: body size (a), total fat content (b), and relative fat content (c) of male and female adult offspring and phloem thickness per jack pine bolt (N = 14). Regression lines of fitted values, p-values, and adjusted r^2 are from the respective linear model which included both sex and phloem thickness as independent variables.

Chapter 4. Naïve pine terpene response to the mountain pine beetle (*Dendroctonus ponderosae*) through the seasons

Musso A. E., Fortier C., Huber D. P. W., Carroll A. L., and Evenden M. L.

4.1. Introduction:

Herbivorous insects that attack living plants must contend with constitutive and induced physical and chemical defenses (Franceschi et al. 2005, Howe and Jander 2008). Invasive insect herbivores can be particularly successful when they enter defense-free space and encounter plants that are not adapted to resist or tolerate their herbivory (Gandhi and Herms 2010). The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins, is an irruptive bark beetle (Coleoptera: Curculionidae, Scolytinae) that feeds on the phloem of pines (Pinales: Pinaceae), and at epidemic population densities, attacks and kills live trees (Safranyik and Carroll 2006). Mountain pine beetle populations in Canada have undergone a climate change-facilitated range expansion into forests that were not previously suitable for their reproduction and survival (Carroll et al. 2006b, Safranyik et al. 2010, Cullingham et al. 2011). Lodgepole pine, *Pinus contorta* var. *latifolia*, is the most common historic host of MPB, but its range extends further north and east than the historic distribution of MPB prior to its range expansion (Safranyik et al. 2010). Lodgepole pine in the northern part of their range are evolutionarily naïve to MPB attack (Clark et al. 2010, Cudmore et al. 2010). Naïve lodgepole pines have fewer constitutive and induced terpene defenses (Clark et al. 2010, Burke et al. 2017) which is associated with higher reproductive success by MPB when compared with similarly attacked lodgepole pine from the beetle's historic range (Cudmore et al. 2010). The lower defensive capacity of naïve lodgepole pine against MPB has been attributed to selection against the production of energetically costly defenses in populations of trees growing where climatic conditions have precluded the establishment of the beetle (Burke et al. 2017). Recent range expansion by MPB has resulted in its establishment in the western boreal forest (de la Giroday et al. 2012), where they have encountered a novel pine host, jack pine, *Pinus banksiana* (Cullingham et al. 2011).

At epidemic population densities, MPB along with their fungal associates colonize and kill large, vigorously defended pine trees using semiochemical-mediated mass attack. After landing on a potential host tree, pioneer females bore into the bark and release *trans*-verbenol to which both males and females respond (Pitman and Vité 1969, Pureswaran et al. 2000). Responsive males release *exo*-brevicomin to further attract mainly females to the aggregation (Rudinsky et al. 1974, Conn et al. 1983). This semiochemical-mediated mass attack permits aggregation of MPB on a host in only a few days, which overwhelms the constitutive and induced defenses of attacked trees (Raffa and Berryman 1983a). During host colonization, beetles introduce pathogenic blue stain fungal mutualists which grow into the phloem and provide nutrients for developing larvae (Six and Paine 1998, Bleiker and Six 2007). The tree response to MPB mass attack includes both constitutive and induced chemical defenses (Keeling and Bohlmann 2006) including resin that can physically block beetles from entering the phloem tissue, or confine and kill beetles and their associated microorganisms in a lesion of dead tissue (Raffa and Berryman 1983a). Monoterpenes comprise a major component of pine resin and play a pivotal role in beetle-tree interactions. Monoterpenes can be toxic to MPB at high concentrations, but can also act as pheromone precursors, pheromone synergists, and/or kairomones that facilitate MPB host colonization (Seybold et al. 2006, Chiu and Bohlmann 2022).

The major monoterpenes present in both lodgepole pine and jack pine phloem are α- and β-pinene, β-phellandrene, Δ-3-carene, myrcene, limonene, and terpinolene (Clark et al. 2014, Rosenberger et al. 2017b). Although lodgepole pine and jack pine have similar monoterpenes present in their phloem, the proportion and quantity of monoterpenes varies significantly between and within each species (Clark et al. 2010, Taft et al. 2015a, 2015b, Rosenberger et al. 2017b). Monoterpene content in lodgepole pine phloem mostly consists of β-phellandrene which is only present in small quantities in jack pine. By contrast, α-pinene is the most prominent monoterpene jack pine. Mountain pine beetle adults are attracted to volatiles released by lodgepole pine in the absence of pheromones (Moeck and Simmons 1991), and all major lodgepole pine monoterpenes can be sensed by the antennae of male and female beetles (Pureswaran et al. 2004). $(-)$-α-Pinene is a precursor to the female aggregation pheromone $(-)$-*trans*-verbenol and is obtained during feeding by larvae (Chiu et al. 2018) and attacking adults (Vité and Pitman 1968, Hughes 1973). Jack pine phloem has three times more α-pinene than that of lodgepole pine, which translates into a greater production of *trans*-verbenol by females during colonization (Erbilgin et al. 2014, Taft et al. 2015a) and higher rates of mass attacks (Burke and Carroll 2016). Other prominent terpenes such as β-phellandrene, Δ-3-carene, myrcene, and terpinolene are

further exploited by the beetle as synergists that increase MPB attraction to their aggregation pheromone (Miller and Borden 2000, Borden et al. 2008, Klutsch et al. 2017, 2020). The major monoterpenes in jack and lodgepole pine vary in toxicity to adults MPBs with limonene being the most toxic (Reid and Purcell 2011, Chiu et al. 2017, Reid et al. 2017).

Lodgepole pines in the historic range of MPB induce production of β-pinene, Δ-3-carene, myrcene, limonene, and β-phellandrene in response to natural mass attack, wounding, and/or inoculation with fungal mutualists of MPB (Boone et al. 2011, Clark et al. 2014, Burke et al. 2017). Limonene appears to be the most important induced defense, as trees that resist colonization by MPB have higher concentrations of limonene in phloem post-attack (Raffa and Berryman 1983a, Erbilgin et al. 2017a). The amount of all major monoterpenes increases significantly in lodgepole pine trees two months after a successful mass attack (Clark et al. 2012). Lodgepole pine trees that escape or survive attack do not have significant increases in the major phloem monoterpenes two months after the flight period (Clark et al. 2012). The success of a mass attack is dependent on the density of beetles that attack the tree, and the process typically takes place in a period of less than a week (Raffa and Berryman 1983a). Lodgepole pine that are successfully colonized by MPB continue to synthesize and concentrate monoterpenes even after they are otherwise overwhelmed (Clark et al. 2012). Soderberg et al. (2022) found there was no significant increase of monoterpenes four days after inoculation of foxtail pine (*Pinus aristata*) and limber pine (*Pinus flexilis*) with a MPB fungal mutualist, but concentrations increased at least 19-fold 30 days after inoculation. This delayed induced response would likely not affect the process of mass attack but could potentially increase mortality of MPB eggs and larvae that are present at this time point. It is not clear exactly how long after mass attack a tree continues to synthesize and concentrate monoterpenes, but MPB larvae increase expression of genes related to monoterpene detoxification through the autumn into November (Robert et al. 2017).

Accumulating evidence indicates that lodgepole pine and jack pine trees in the expanded range are evolutionarily naïve to MPB attack. The induced chemical defenses of jack pine and naïve lodgepole pine have been examined in seedlings and mature trees using wounding or inoculation with fungal associates of MPB (Lusebrink et al. 2011, 2016; Clark et al. 2014; Arango-Velez et al. 2016; Erbilgin et al. 2017b). Lodgepole pine increase monoterpene concentration after inoculation with *Grosmannia clavigera*, the most pathogenic symbiotic fungus of MPB, but jack pine seedlings do not (Lusebrink et al. 2011;

Arango-Velez et al. 2016). Similarly, lodgepole pine trees in the historic range of MPB significantly increase limonene concentrations after wounding but lodgepole pine outside the historic MPB range and jack pine trees do not (Clark et al. 2014). Outside the historic range of MPB, mature lodgepole pine induces more monoterpenes compared to jack pine (Erbilgin et al. 2017b), so while lodgepole pine outside the historic range are less defensively capable than those in the historic range, they are still more capable than jack pine. However, the monoterpene content of fungal lesions surrounding sites of inoculation in mature jack pine trees is 70 times greater than in phloem tissue away from lesions, but only 10 times greater in the lesion than surrounding phloem in lodgepole pine (Lusebrink et al. 2016), suggesting that while jack pine may seem less able to mount a general response to MPB fungal associates, they may be proficient at local induction.

Although previous studies have examined the terpene response of naïve pines to mechanical wounding and inoculation with MPB fungal associates, they have not considered changes in phloem terpenes in mature trees after mass attack by MPB. To examine how naïve lodgepole pine and jack pine respond to mass attack by MPB, we performed mass attack density manipulation experiments in mature, lodgepole pine and jack pine trees in Alberta, Canada. We collected phloem samples for monoterpene analysis just prior to attack, just after attack in the same season, and the following spring to additionally investigate the long-term terpene response of pines attacked by MPB and test three hypotheses. (i) We hypothesized that since jack pine is a novel host and responds less aggressively to fungal associates of MPB (Lusebrink et al. 2011; 2016; Clark et al. 2014; Arango-Velez et al. 2016; Erbilgin et al. 2017b), that lodgepole pine will induce greater quantities of terpene defenses in response to mass attack than jack pine. From this, we predicted that lodgepole pine will have a greater change in phloem terpene quantities between the samples before mass attack and at the end of the flight period compared to jack pine. (ii) Naïve trees can have a delayed response to colonization by the fungal symbionts of MPB (Soderberg et al. 2022), and MPB larvae upregulate genes related to terpene detoxification over the fall (Robert et al. 2017), so we hypothesized that terpene content would be greatest at the end versus the beginning of larval development. We predicted that phloem terpene samples taken in the spring, following mass attack, would have the highest terpene quantities in both species. (iii) Unlike lodgepole pine that are mass attacked and killed, lodgepole pine that are attacked at low densities and survive mass attack do not substantially increase terpene quantities six weeks after attack (Clark et al. 2012). Therefore, we also hypothesized that attack density would influence induced phloem terpenes in lodgepole pine. We predicted that jack pine that are attacked at a low density

and resist beetle colonization will not have increased quantities of phloem terpenes at the end of the flight season compared to trees that are attacked at densities that result in successful beetle colonization and death of the tree. The results from these experiments will provide insight on how naïve hosts of MPB respond to mass attack in a way that is more representative of a natural mass attack than inoculation with symbiotic fungi.

4.2. Methods:

Lodgepole pine attack density manipulation and phloem sample collection:

In June 2017, we selected a site south of Grande Prairie, Alberta at the leading edge of the MPB outbreak (54.487 N, −118.562 W, Appendix A Fig. A1). The stand was surveyed for lodgepole pine trees that were apparently healthy by visually inspecting dominant trees in the stand for disease-causing organisms. Fifteen lodgepole pine trees with ≥22 cm diameter at 1.3 m and an average phloem thickness ≥1.5 mm were selected. To determine phloem thickness, three samples of bark and phloem were taken with a 10 mm diameter punch from each tree at ~50 cm height at the north, south-east, and south-west aspect of the bole, the phloem layer was separated from the bark and measured with digital calipers to the nearest 0.01 mm. All trees that were initially selected met the average phloem thickness criteria, so no trees were discarded.

Phloem discs for quantification of constitutive monoterpenes were obtained from each of the selected trees on 21 July 2017, just prior to MPB emergence and dispersal. The pre-attack phloem sample was taken using a 10 mm diameter leather punch at 1.3 m on the north aspect of the bole. Phloem and bark from the punches were immediately placed into individually labelled paper coin envelopes and buried in dry ice for transport back to the lab where they were stored at −70°C until analysis. Tools used in sampling phloem were cleaned with 70% ethanol between trees. Phloem sampling location and methods were similar to that described in Clark et al. 2012. To induce beetle attack, each tree was then baited with a commercially available MPB tree bait that released the aggregation pheromones trans-verbenol and exo-brevicomin (Contech Enterprises Inc, Delta BC, Canada).

To achieve various levels of MPB attack densities on baited trees, we monitored trees every two to three days until initiation of attack and then every day during peak flight. Trees were randomly assigned to one of three treatments, low attack density (10

attacks/m^2), medium density (40 attacks/m^2) and high density (80 attacks/m^2). The number of attacks on the lower three metres of the tree bole were counted and divided by the approximate surface area of the tree which was calculated using DBH. Once the desired attack densities had been reached on baited trees, the lower 3 m of each tree was caged in standard aluminum wire mesh insect screening (18×16 mesh count; PHIFER Inc. Alabama, USA) to prevent further attacks.

On 10 September 2017 after MPB flight had ceased, all mesh was removed from experimental trees and post-attack samples were taken ~5 cm to the left of the pre-attack samples using the method described above. If the first sample contained MPB galleries, a second sample was taken immediately to the left until the sample was clear of beetle galleries. Post-overwintering samples were collected on each tree on 13 May 2018, prior to emergence of the brood, in the same manner as the post-attack samples. Since the second and third phloem samples were taken relative to the first sample, attack density likely influenced the proximity of these samples to beetle galleries and/or fungal lesions. Trees were harvested in late May and the attack densities for each tree were confirmed by counting all parental galleries under the bark of the 1 m section of the bole that was 1 m above the ground. Attack densities were grouped as below the mass attack threshold <40 attacks/m^2 or above the mass attack threshold >40 attacks/m2 (Raffa and Berryman 1983a).

Jack pine attack density manipulation and phloem sample collection:

Three jack pine sites north of Lac La Biche, Alberta (site 1: 54.984 N, −112.009 W, site 2: 55.170 N, −112.038 W, site 3: 55.021 N, −111.979 W Appendix A Fig. A1a,b) were selected. At each site, apparently healthy dominant trees that were ≥22 cm diameter at 1.3 m and an average phloem thickness ≥1.0 mm were selected. Average phloem thickness was measured as described above, but the average phloem thickness criteria had to be reduced as jack pine phloem was much thinner on average than lodgepole pine phloem. Nine trees at each site were selected for a total of 27 experimental trees. During experimental tree selection, five trees did not meet the minimum criteria for phloem thickness, two at site 1, two at site 2, and one at site 3, and so new trees were selected. *Dendroctonus ponderosae* populations in jack pine forests were too small to ensure consistent mass attacks on experimental trees following baiting. Therefore, MPB were collected from bolts of infested lodgepole pine trees and released under cages around the experimental jack pine trees, as described below.

To source MPB beetles for introductions to jack pine, 80 lodgepole pine bolts (~50 cm long) were harvested from the lower bole of 20 trees naturally infested by MPBs across three sites near Hinton, Alberta – 53.380 N, −117.543 W, 53.343 N, −117.587 W, and 53.275 N, −117.665 W) in mid-June 2018. Bolts were transported to the University of Alberta and the cut ends were sealed with hot paraffin wax to reduce the rate of desiccation. Twenty bolts were immediately placed in emergence bins (114 L Rubbermaid Hinged Top Tote, fitted with a glass jar), and the remaining bolts were stored at 5°C and removed in batches of 20 over the following three weeks to extend the emergence period. Beetle emergence was checked daily, beetles were counted and placed in vented 500 mL plastic containers filled with jack pine wood chips and kept at 5°C until use in the field (21 July to 13 August 2018).

Experimental trees at each site were fitted with conical cages constructed from standard aluminum mesh insect screening (18×16 mesh size; PHIFER Inc. Alabama, USA). A sheet of packing foam was placed between the top of the cages (2 m above the ground) and the tree bole and was secured with staples into the bark and a plastic cable tie (Appendix A Fig. A2). The base of the cage was buried in the soil ~1 m away from the bole around the tree so that the lower 2 m of the tree was exposed to the released beetles (Appendix A Fig. A2). Pre-attack phloem samples were taken on 26 July 2018 after all trees were caged. For each tree at each site, one 4 L paint bucket half-filled with jack pine wood chips that contained one thousand 1-to-10-day-old MPB was placed between the edge of the cage and the tree bole and the cages were sealed. Beetle release occurred on 1, 8, and 13 August 2018 for sites 1, 2, and 3, respectively, but only eight of the nine trees at site 3 were manipulated so the total number of experimental trees was 26. To vary attack densities, buckets containing beetles were removed from three randomly selected trees at each site 24 h after placement and added to a different set of three randomly selected trees. At each site there were three trees that had a bucket of beetles within the cage for 24 h which was removed, three trees that had an additional bucket of beetles added after 24 h, and three trees (two at site 3) which had no removals or additions.

Post-attack phloem samples were taken on 26, 27, and 28 August 2018 for sites 1, 2, and 3, respectively. Eighteen of the experimental trees (six per site) were harvested in October 2018, leaving one randomly selected tree from each of the attack density treatments (site 3 had two trees remaining, one that had a bucket addition and one with no removals or additions). Post-overwintering phloem samples were taken from the eight remaining jack pine trees on 22 May 2019 before the trees were harvested. Attack densities

per tree were determined by counting all parental galleries under the bark on 1 m of the tree bole that was 1 m above the ground. Attack densities were grouped as <40 attacks/m2 and >40 attacks/m2 but due to the reduction in sample size between post-attack and post-winter samples, the interaction between attack density and time point was only compared with the pre- and post-attack samples in jack pine.

Phloem terpene analysis:

Samples in dry ice were shipped to the Analytical Chemistry Services Laboratory of the BC Ministry of Environment and Climate Change Strategy in Victoria, BC, Canada where they were analyzed using gas chromatography−flame ionization detection and identified with comparison to authenticated standards. Phloem samples were processed and analyzed using the methods outlined in Clark et al (2010). Briefly, frozen samples were weighed and sliced into small pieces using a razor blade and extracted in 4 mL hexane with 250 ppm pentadecane as an internal standard. Samples were inverted and allowed to settle for 24 h before 0.5 mL of solution was transferred to an autosampler vial for gas chromatographic analysis using either a PerkinElmer Clarus 500 or PerkinElmer AutoSystem (PerkinElmer, Waltham Massachusetts), with an INNOwax column (J&W, 25m×0.2 mm id.d, 0.4 μL film). The injection was split (35 mL/min, approximately 39:1; injector temperature 200°C) with helium as the carrier gas (0.90 mL/min at 60°C). The oven temperature was held at 60°C for 1 min, increased at a rate of 3.0°C/min to 85°C, then increased at a rate of 8.0°C/min to 170°C, then increased by 20°/min to 250°C and held for 7 min. The extracted phloem and bark in the remaining solvent were evaporated in a fume hood and then oven-dried at 70°C overnight to remove all residual moisture and weighed. Terpenes were reported as μg/g relative to the dry weight of the sample with a detection limit of 10 μg/g.

Statistical analyses:

All statistical analyses were performed in R (version 4.0.3; R Core Team 2022) within R Studio (version 1.2.5001; RStudio Team 2022). Differences in the quantities of total and individual terpenes in phloem from lodgepole pine trees sampled at the different time points during the attack process and post-overwintering were analyzed by linear mixed models using the lme4 package (Bates et al. 2015) with a log transformation and a term for tree identity fit as a random effect. Linear mixed model fit was assessed by plotting the scaled residuals, examining Levene's test for homogeneity of variance, and Kolmogorov-Smirnov test for overdispersion, using simulateResiduals in the DHARMa package (Hartig 2022).

ANOVA tables to examine the effects of the linear predictors were generated using the car package (Fox and Weisberg 2019) and post-hoc tests on the marginal means were compared using emmeans (Lenth 2022) and multcomp (Hothorn et al. 2008) packages. Adequate model fit could not be found for some terpenes, likely due to many zeros in the dataset, so a Friedman test was performed instead with a post-hoc Nemenyi test using PMCMRplus (Pohlert 2022). For jack pine samples, a Friedman test could not be performed for individual terpenes that did not fit linear mixed effects models due to the uneven sample design, so the 26 trees that were sampled at both pre- and post-attack time points were analyzed using a Wilcoxon rank sum test (wilcox.test; R Core Team 2022) and the eight trees sampled at all three time points were analyzed using linear mixed effects models as described above, or a Friedman test. The effect of attack density and its interaction with sample time point was examined using linear mixed effects models as described above.

To determine if the chemical composition of samples (the presence and abundance of different compounds in each sample) were similar at the different sampling points within each species, non-metric multidimensional scaling (NMDS) was performed using the vegan package (Oksanen et al. 2022). A Bray-Curtis distance matrix was generated (vegdist, method = 'bray') and scores for the NMDS (metaMDS) were plotted. We chose iterative NMDS of a Bray-Curtis dissimilarities as the analysis is robust to datasets with many zeros (Brückner and Heethoff 2017). The dissimilarity among the chemical profile of samples at the three sampled time points was tested statistically using ANOSIM with 999 permutations.

4.3. Results:

A summary of the measured tree characteristics (diameter at 1.3 m and average phloem thickness) for each site can be found in Appendix A Table A1. Fourteen of the 15 baited lodgepole pine trees were attacked by MPB and had parental galleries longer than 5 cm but five of these trees resisted MPB colonization and contained few or no larval brood. Of the eight experimentally attacked jack pine trees that were allowed to over-winter, all had parental galleries longer than 5 cm. Brood in jack pine entered the winter as eggs or very young larvae and none survived to the spring sample, therefore all trees were alive the following year and had lots of healthy phloem.

Terpenes at the three sampled time points:

In lodgepole pine phloem, total terpenes and several individual monoterpene levels increased in phloem samples between the pre-attack samples and the post-winter samples, as predicted (Table 4.1). Contrary to our prediction though, total terpenes in lodgepole pine phloem samples did not increase between pre- and post-attack but were only higher post-overwintering ($\chi2$ = 12.89, df = 2, p = 0.002; Fig 4.1a). There was a large amount of variation among individual trees, however, and total phloem terpene content of some trees decreased post-attack and others increased between pre- and post-attack time points (Fig 4.1c). When compared across time points individually, levels of α-pinene, β-pinene, β-phellandrene, and 2-carene followed a similar pattern to total terpenes, in which quantities were similar pre- and post-attack but significantly increased post-winter (Table 4.1). Levels of β-phellandrene, Δ-3-carene, myrcene, limonene, and terpinolene in lodgepole pine increased significantly in post-winter samples compared to pre-attack samples, but post-attack samples were not different from either pre-attack, or post-winter samples (Table 4.1).

Total terpene levels also increased post-winter in jack pine phloem compared to pre- and post-attack (χ^2 = 11.79, df = 2, p = 0.002, Fig. 4.1b) and there was substantial variation among trees (Fig 4.1d). The differences between pre- and post-attack samples were compared with samples from all 26 trees and the only compound that increased significantly post-attack was β-pinene (Table 4.2). For the eight jack pine trees that were sampled at all three time points, α-pinene, Δ-3-carene, and sabinene were found at significantly greater levels post-winter compared to pre- and post-attack. β-Pinene and terpinolene levels increased significantly post-winter compared to pre-attack and post-attack samples were not different from either pre-attack, or post-winter samples (Table 4.3). Contrary to our prediction, there was no significant effect of attack density or an interaction with sample time point on total terpene content in the phloem of either lodgepole pine (density: χ^2 = 1.85, df = 1, p = 0.17; interaction: χ^2 = 0.04, df = 2, p = 0.98; Fig. 4.2a) or jack pine (density: χ^2 = 0.61, df = 1, p = 0.43; interaction: χ^2 = 0.33, df = 1, p = 0.56; Fig. 4.2b).

A total of 23 individual terpenes, 22 monoterpenes and 1 sesquiterpene ($\square$-caryophyllene) were detected across all phloem samples with 22 found in lodgepole pine phloem and 21 in jack pine phloem (Appendix A Table A2). Camphor and ocimene were detected in lodgepole pine but not jack pine, and pulegone was detected in jack pine but not

in lodgepole pine. Ocimene and pulegone were only detected in one lodgepole pine and one jack pine, respectively. Δ-3-Carene, α-pinene, β-pinene, and β-phellandrene were detected in all lodgepole pine samples across time points; however, only α-pinene was detected in all jack pine trees at all time points. In lodgepole pine, camphene was detected in all trees pre- and post-attack but was absent from one tree post-winter. The most prevalent compounds in jack pine phloem after α-pinene, were β-pinene, myrcene, and limonene. α-Pinene was present in 100% of jack pine trees post-winter and 73% and 69% of jack pine trees pre- and post-attack, respectively. In lodgepole pine phloem, β-phellandrene comprised the highest percentage of all monoterpenes at all time points, followed by α-pinene and Δ-3-carene (Appendix A Table A3). The relative proportion of individual monoterpenes did not change significantly between sample time points. In jack pine, α-pinene was the most prominent compound comprising as much as 68% of the sample on average (Appendix A Table A4). In jack pine phloem, there was no change in percent representation for individual terpenes except for β-pinene which comprised a significantly higher percentage of the post-winter sample compared to pre-attack ($p = 0.04$; Table S4.4).

Chemical profiles:

There were no significant changes in the overall chemical profile of lodgepole pine phloem sampled at the two time points during MPB attack and post-winter ($R = 0.01$, $p = 0.62$; Fig. 4.3a). The three samples for each lodgepole pine tree were similar ($R = 0.44$, $p = 0.001$) however, some trees had distinct chemical profiles from some trees, but their profiles overlapped with the profiles of others (Fig. 4.3c). In jack pine, there was dissimilarity in the chemical profiles of trees at the two time points during attack post-winter ($R = 0.10$, $p = 0.01$). The post-winter samples separated from the pre- and post-attack samples, but with substantial overlap (Fig. 4.3b). There was also evidence that individual jack pine trees had distinct phloem chemical profiles ($R = 0.16$, $p = 0.01$) but with more overlap than lodgepole pine trees did (Fig. 4.3d).

4.4. Discussion:

The finding that phloem terpene content is highest in both species in the spring after trees overwinter provides new insights into pine defensive chemistry and the terpene environment that larval beetles experience post-winter. In both evolutionarily naïve lodgepole pine and jack pine, increased concentration of phloem terpenes was apparent in the spring following MPB attack, but not immediately following attack in the late summer.

These findings differ from previous research done on lodgepole pine within the historic range of MPB where trees that were sampled six weeks after attack had significantly more total and individual terpenes compared to trees that escaped or survived attack (Clark et al. 2012). At that time point, eggs and early instar larvae would experience a terpene-rich environment, putatively causing beetle mortality. Other than two studies that assessed the impacts of resin volatiles or volatiles of individual terpenes on eggs (Reid and Gates 1970, Raffa and Berryman 1983b), the toxicity of terpenes to immature life stages is unknown (Chiu and Bohlmann 2022). However, the short-term defensive response by naïve populations of lodgepole pine trees is less pronounced than experienced ones (Clark et al. 2010, 2014, Burke et al. 2017) and naïve trees produce more offspring per female than trees with a co-evolutionary history with MPB (Cudmore et al. 2010). Therefore, it is likely that immature beetle life stages are generally susceptible to high concentrations of monoterpenes, and a less monoterpene rich under-bark environment in the weeks following attack could contribute to increased MPB offspring survival in naïve trees in the expanded range. Unlike previous work in lodgepole pine (Clark et al. 2012), MPB in our study were manipulated to select trees that met certain size and vigour criteria, eliminating the element of beetle choice of hosts. A comparable study in the historic range would need to be performed to ensure that the effect seen in this study is indeed due to a lack of coevolutionary history between the tree and the beetle. In post-attack samples in jack pine the only monoterpene that increased significantly was β-pinene. These findings support previous research that show an increase in β-pinene content but no changes to other monoterpenes in the phloem of jack pine and naïve lodgepole pine two days post-wounding (Clark et al. 2014). In contrast, limonene is induced post wounding in lodgepole pine in the historic range of MPB but not in naïve lodgepole pine or jack pine (Clark et al. 2014). Monoterpenes also do not increase in jack pine seedlings in response to either mechanical wounding or *G. clavigera* inoculation after four weeks (Lusebrink et al. 2011, Arango-Velez et al. 2016). Thus, it appears that jack pine trees are not able to induce similar amounts of toxic terpenes such as limonene in response to an experimentally applied MPB attack, and it is therefore likely that jack pine could also allow the production of more MPB brood per female compared to lodgepole pine populations in the historic range of the beetle.

Attack density did not influence total terpene content post-attack or post-overwintering. Therefore, trees attacked at low densities and survive would have high levels of terpenes in the phloem the following year. Strip-attacked trees have successful MPB colonization within a portion of the tree, beetles will complete development within the infested portion, but the rest of the tree remains alive (Safranyik and Carroll 2006). The

lodgepole pine trees with low density attacks in our study had large areas of uncolonized phloem that would be available for beetles the following year, and thus residual levels of defensive chemicals could protect trees from subsequent colonization. In lodgepole pine, individual monoterpenes that are toxic to MPB, such as Δ-3-carene, myrcene, and limonene (Reid and Purcell 2011, Chiu et al. 2017, Reid et al. 2017), were present at higher concentrations in phloem samples taken after winter. This means the live phloem that survived attack may be less habitable for beetles that attempt to subsequently colonize previously lightly infested lodgepole pine. Interestingly, limonene and myrcene, which are particularly toxic to MPB (Chiu et al. 2017), were not increased significantly in jack pine in the post-winter samples. Jack pine trees that survive a low density or strip attack would therefore likely not have increased toxicity to MPB the following year.

Our data show that low density and strip-attacked trees may be primed to resist subsequent attack with increased phloem terpenes in the following year. Perplexingly, strip- and low density-attacked trees are preferred by early flying MPB (Rasmussen 1974) and are more susceptible to mass attack the following year (Safranyik and Carroll 2006). *Dendroctonus ponderosae* has evolved to use many terpene defenses to its advantage and while toxic, many of the individual monoterpenes that increased in concentration after winter in both lodgepole pine and jack pine are also used by MPB for host location and pheromone communication (Seybold et al. 2006, Chiu and Bohlmann 2022). Some compounds act as kairomones and MPB are attracted to volatiles released from lodgepole pine bolts (Moeck and Simmons 1991) but β-phellandrene is the only monoterpene that attracts MPB on its own and only in high concentrations (Miller and Borden 1990). β-Phellandrene is the most abundant monoterpene in volatile collections from the boles of mature lodgepole pine trees and α-pinene is the major component from volatile collections of mature jack pine tree boles (Lusebrink et al. 2016). While the major compounds of bole volatile collections from both species match which compounds are the most abundant in the phloem of each species, it is not clear whether the higher emissions are proportional to phloem concentrations. If phloem terpene concentrations do correlate with volatile production from the lower stem of the tree, the high concentrations of β-phellandrene in lodgepole pine the year following an unsuccessful mass attack may make these trees more apparent and could explain why they are preferred by beetles that emerge early in the flight period (Rasmussen 1974, Safranyik and Carroll 2006). *Pinus banksiana* that survive attack may not have this increased apparency the following flight season as β-phellandrene remains a minor component of phloem terpene contents. High terpene levels are also indicative of healthy trees that have thick phloem which are preferred by MPB in the

epidemic population state (Boone et al 2011). *Dendroctonus ponderosae* that emerge from mass attacked trees prefer to colonize phloem with high concentrations of monoterpenes in the lab but beetles from an endemic state prefer low monoterpene phloem (Burke and Carroll 2017). Potentially, rather than using volatile cues to directly locate these trees, MPB pioneering females may use random landing within the stand (Byers 1996) and then assess the host quality with gustatory cues (Raffa and Berryman 1982a) and greater phloem terpene contents would increase the acceptance after landing. Whether MPB would accept a primed tree would likely depend on the population state as beetles that emerge from an endemic condition might avoid the high terpene content phloem of trees that survived a low-density attack or were strip-attacked the previous year (Burke and Carroll 2017). Several compounds that increased substantially in lodgepole pine phloem post-winter – Δ-3-carene, α-pinene, β-pinene, myrcene, and terpinolene – all synergize MPB response to its aggregation pheromone (Miller and Borden 2000, Borden et al. 2008, Klutsch et al. 2017). It is possible that a preference for trees with high levels of monoterpenes might benefit early fliers as the abundance of pheromone synergists may help joining beetles find the tree efficiently, which is particularly important when population densities are low at the start of the emergence period (Klutsch et al. 2020).

While terpene levels in jack pine phloem were high post-winter, the highly concentrated monoterpenes were those with relatively low toxicity that act as strong aggregation pheromone synergists to MPB. A jack pine tree that survives a low-density attack or that is strip-attacked by MPB could be efficiently attacked by beetles the following year, even at low insect population densities. All jack pine trees left to overwinter in our experiment survived attack, as MPB larvae experienced high winter mortality, likely due to low temperatures in the winter of 2018-2019. In early February 2019, there was a one-week period where temperatures in Lac La Biche did not rise above $-30°$C, three days had lows below $-40°$C (Environment Canada). An early fall also contributed as the larvae were only at the first instar stage when temperatures decreased in September 2018. Winter temperatures were cold enough to induce 90-100% mortality of the cold hardy fourth-instar stage (Safranyik and Linton 1991, 1998, MacQuarrie et al. 2019), which resulted in 100% mortality of the early instar larvae in the infested jack pine trees in our experiment. These trees, despite being attacked at sufficient densities for successful colonization, had an abundance of live phloem available since young larvae had not mined far from the parental gallery. A situation like this would present an opportunity for MPB to behave as they would in the epidemic population state within a tree that is more susceptible via the greater abundance of aggregation pheromone synergists.

It is possible that a seasonal change, unrelated to MPB attack, could account for some of the increased phloem terpene concentration after winter in the MPB attacked trees in this study. The attacked trees were sampled 10 months after the initial attack, in May, and baseline samples were taken in July the previous year. Provincial regulations require that MPB infested trees are removed from the landscape by Jun 1, so we are unable to extend the sampling window at this time. Volatile collections from the bole of lodgepole pine trees one year after inoculation with *G. clavigera* in July had similar quantities of monoterpenes to pre-inoculation volatile collections even though the trees had increased volatiles in the period immediately after inoculation (Lusebrink et al. 2016). Phloem terpene content could have been lower in the trees attacked by MPB in this study if samples were taken one whole year after attack, particularly if the damage to the bark caused by mass attack combined with increased temperatures over the season increased volatilization of terpenes from the phloem. Future studies could include terpene samples from trees that are not attacked by MPB at all time points to form a baseline of seasonal changes in phloem terpene content to avoid this issue. The addition of a wounding, but not attacked, treatment would also help distinguish the difference between the insect/symbiont induced response from a general wounding response.

The climate change facilitated range expansion of MPB has allowed us to examine insect-plant interactions in hosts that have not co-evolved with an invasive herbivore. Our results are consistent with the hypothesis that lodgepole pine in the expanded MPB range are evolutionarily naïve to its herbivory, as lodgepole pine do not exhibit an induced terpene response in the first months after an experimentally-applied mass attack. *Dendroctonus ponderosae* likely applies selective pressure to populations of pine species that experience frequent outbreaks, increasing terpene response in these trees. Continued research on the defensive capabilities of novel pine hosts remains important, as MPB spread through the boreal forest via jack pine presents a risk not only to the boreal forest but other pine species in eastern North America (Cooke and Carroll 2017, Rosenberger et al. 2017b).

4.5. Tables and Figures:

Table 4.1 Mean (SE) terpene amount (μg/g of dry weight) at different stages of attack (N = 14 at each stage) in lodgepole pine. Bolded p-values and means followed by different letters within the same row indicate significant differences ($p < 0.05$).

Terpene	Pre-Attack Mean (SE)	Post-Attack Mean (SE)	Post-Winter Mean (SE)	p-value
Total Terpenes	4525.71 (890.30) a	5785.00 (1334.79) a	17685.71 (9525.71) b	**p = 0.002** [1]
β-phellandrene	1972.86 (399.71) a	2508.57 (645.30) ab	8126.43 (5091.20) b	**p = 0.03** [1]
Δ-3-carene	839.79 (332.74) a	939.43 (323.27) ab	3173.36 (1753.85) b	**p = 0.03** [1]
α-pinene	742.86 (186.61) a	923.50 (273.03) a	2338.57 (722.58) b	**p < 0.001** [1]
β-pinene	350.29 (96.28) a	568.21 (209.29) a	2121.43 (1329.23) b	**p < 0.001** [1]
myrcene	84.07 (18.02) a	110.79 (26.66) ab	369.57 (226.67) b	**p = 0.01** [2]
limonene	103.00 (19.00) a	133.21 (26.55) ab	359.14 (189.01) b	**p = 0.003** [2]
terpinolene	55.57 (16.21) a	100.29 (33.07) ab	320.07 (201.41) b	**p = 0.05** [2]
sabinene	33.86 (6.77)	58.93 (17.68)	233.21 (145.75)	p = 0.19 [2]
α-phellandrene	57.29 (36.20) a	60.36 (28.84) a	227.21 (128.32) b	**p < 0.001** [2]
p-cymene	117.57 (76.16)	119.71 (90.98)	183.29 (91.92)	p = 0.78 [2]
camphene	52.36 (8.92)	57.93 (11.57)	126.29 (54.14)	p = 0.10 [2]
α-caryophyllene	23.71 (16.90)	15.50 (12.80)	103.57 (72.08)	p = 0.09 [2]
γ-terpinene	3.93 (2.15)	6.00 (3.50)	37.64 (23.29)	p = 0.21 [2]
2-carene	0.00 (0.00) a	0.00 (0.00) a	30.64 (6.58) b	**p < 0.001** [2]
bornyl acetate	33.79 (16.46)	33.29 (11.26)	29.43 (10.00)	p = 0.49 [2]
α-terpinene	10.00 (10.00) ab	5.50 (5.50) a	27.86 (18.00) b	**p = 0.02** [2]
borneol	1.50 (1.50)	5.07 (3.28)	19.50 (11.98)	p = 0.14 [2]
α-terpineol	16.57 (5.77)	19.43 (6.00)	19.36 (8.26)	p = 0.97 [2]
linalool	0.93 (0.93) a	3.64 (2.00) ab	15.93 (9.93) b	**p = 0.04** [2]
α-thujone	4.57 (3.43)	6.64 (6.64)	10.43 (7.11)	p = 0.90 [2]
camphor	9.57 (3.98)	7.86 (4.11)	8.93 (3.37)	p = 0.99 [2]
ocimene	0	0	1.43 (1.43)	p = 0.37 [2]

[1] p-values from ANOVA of linear mixed effects model and letters from estimated marginal means with Tukey adjustment.
[2] Differences between stages determined by Friedman Test followed by *post hoc* Nemenyi test.

Table 4.2 Mean (SE) terpene amount (μg/g of dry weight) of all trees at the first two stages of attack in jack pine (N = 26 at each stage). p-values were determined using a Wilcoxon rank sum test, bolded values are significant at α = 0.05.

Terpene	Pre-Attack Mean (SE)	Post-Attack Mean (SE)	p-value
Total Terpenes	2313.85 (390.67)	10811.92 (5133.13)	p = 0.38
α-pinene	1348.08 (221.37)	7999.62 (3771.65)	p = 0.21
Δ-3-carene	218.65 (80.57)	935.85 (763.68)	p = 0.77
β-pinene	121.85 (36.68)	1039.31 (540.89)	**p = 0.04**
terpinolene	23.42 (10.63)	96.92 (72.81)	p = 0.64
myrcene	94.73 (22.80)	151.00 (61.74)	p = 0.99
sabinene	6.04 (3.35)	70.12 (61.37)	p = 0.55
limonene	344.85 (138.29)	245.77 (77.04)	p = 0.58
β-phellandrene	55.96 (18.65)	113.04 (38.22)	p = 0.26
camphene	35.65 (21.17)	84.73 (38.23)	p = 0.36
α-caryophyllene	5.77 (5.77)	0.00 (0.00)	p = 1.00
α-terpineol	2.50 (1.80)	5.12 (3.63)	p = 0.81
γ-terpinene	0.00 (0.00)	6.15 (6.15)	p = 1.00
linalool	6.54 (3.40)	9.08 (4.87)	p = 0.69
α-phellandrene	3.46 (3.46)	2.12 (2.12)	p = 1.00
α-terpinene	1.08 (0.76)	2.81 (1.95)	p = 0.63
bornyl acetate	8.85 (5.49)	8.65 (4.64)	p = 0.94
borneol	0.00 (0.00)	2.27 (2.27)	p = 1.00
2-carene	2.27 (2.27)	3.31 (2.30)	p = 1.00
α-thujone	27.08 (25.73)	0.00 (0.00)	p = 0.25
pulegone	0.46 (0.46)	0.00 (0.00)	p = 1.00

Table 4.3 Mean (SE) terpene amount (μg/g) and standard error of trees that were present at all three stages of attack in jack pine (N = 8). Bolded p-values and means followed by different letters within the same row indicate significant differences (p < 0.05).

Terpene	Pre-Attack Mean (SE)	Post-Attack Mean (SE)	Post-Winter Mean (SE)	P-value
Total Terpenes	2337.5 (641.13) a	6398.75 (3127.69) a	52637.5 (22049.43) b	**p = 0.003** [1]
α-pinene	1306.25 (340.18) a	5068.75 (2709.80) a	31875 (12536.81) b	**p = 0.002** [1]
Δ-3-carene	394.63 (239.29) a	212.5 (84.24) a	10369.63 (6853.29) b	**p = 0.002** [1]
β-pinene	128.38 (84.56) a	549 (321.65) ab	5742.5 (2460.14) b	**p = 0.001** [1]
terpinolene	48.75 (32.04) a	40.13 (29.34) ab	1303.63 (833.57) b	**p = 0.02** [1]
Myrcene	112.13 (48.77)	102.25 (28.61)	911 (414.09)	p = 0.30 [2]
sabinene	6.88 (6.88) a	10.38 (5.47) a	792.5 (538.71) b	**p = 0.002** [1]
Limonene	169.88 (75.64)	148 (56.24)	682.5 (207.59)	p = 0.30 [2]
β-phellandrene	60.88 (26.30)	75.88 (27.67)	472.63 (190.14)	p = 0.14 [2]
camphene	77.63 (63.38)	73.63 (32.92)	381.75 (151.03)	p = 0.31 [1]
α-caryophyllene	0 (0)	0 (0)	171.88 (147.55)	p = 0.06 [2]
α-terpineol	3.00 (3.00)	3.38 (3.38)	133.5 (74.49)	p = 0.09 [2]
γ-terpinene	0 (0)	0 (0)	115.63 (73.64)	p = 0.06 [2]
Linalool	6.13 (6.13)	0 (0)	28 (26.06)	p = 0.36 [2]
α-phellandrene	0 (0)	0 (0)	23.25 (15.56)	p = 0.13 [2]
α-terpinene	2.13 (2.13)	0 (0)	23.25 (15.24)	p = 0.37 [2]
Bornyl acetate	4.5 (2.95)	10.5 (10.5)	20.75 (14.77)	p = 0.23 [2]
p-cymene	0 (0)	0 (0)	20.63 (18.86)	p = 0.14 [2]
Borneol	0 (0)	7.38 (7.38)	9.63 (6.32)	p = 0.37 [2]
2-carene	7.38 (7.38)	5.75 (5.75)	3.88 (3.88)	p = 1.00 [2]
α-thujone	2.5 (2.5)	0 (0)	1.5 (1.5)	p = 0.60 [2]
pulegone	1.5 (1.5)	0 (0)	0 (0)	p = 0.37 [2]

[1] Differences between stages determined by linear mixed effects model followed by ANOVA and post hoc Tukey HSD.
[2] Differences between stages determined by Friedman Test followed by *post hoc* Nemenyi test.
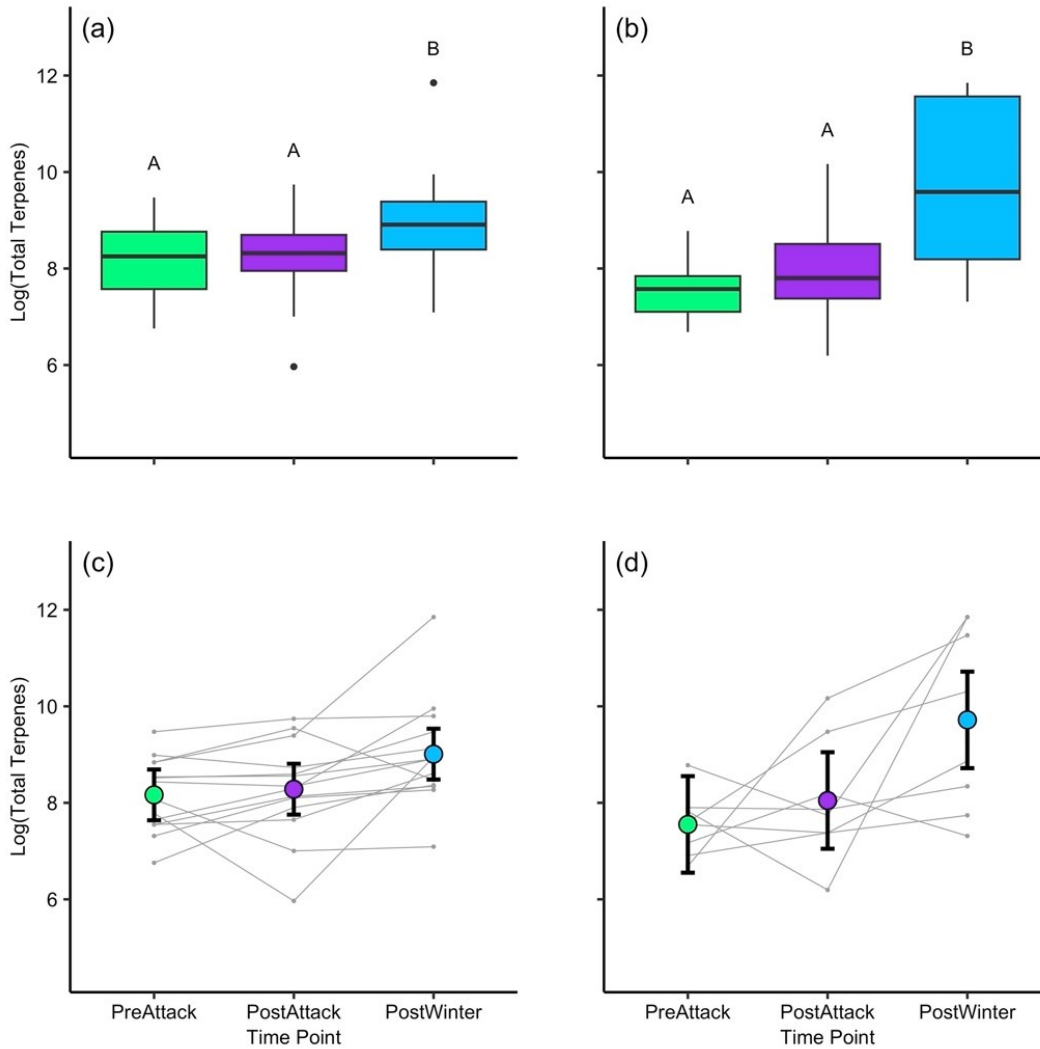
Figure 4.1 (a) and (b): Box-and-whisker plots of total terpenes (μg/g, log transformed) at three sampled time points in lodgepole pine (N = 14) and jack pine (N = 8). Top and bottom of the boxes denote data falling within the first and third quartiles, respectively, midline indicates the median, and whiskers indicate the maximum and minimum value, or 1.5 times the interquartile range, whichever is smaller. Box-and-whisker plots with different letters above them indicate significant difference in terpene content by attack stage (Tukey HSD). (c) and (d): Total terpenes (μg/g, log transformed) at the three sampled time points in lodgepole pine and jack pine individual trees (light grey lines) and the estimated marginal mean ± 95% confidence intervals in the respective time point colours.
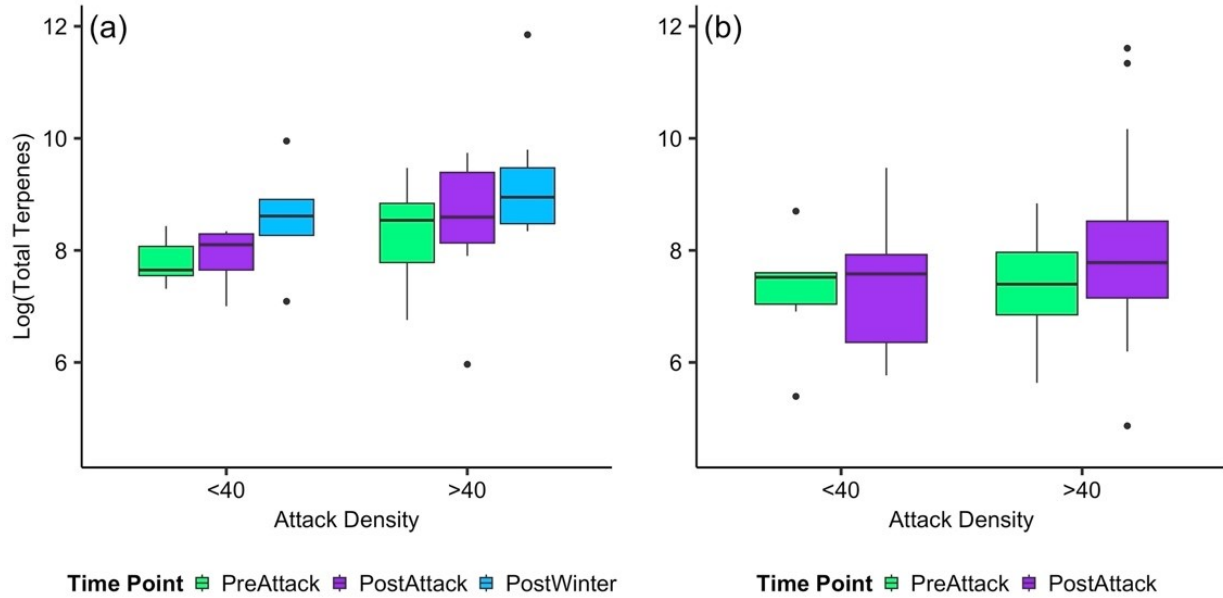
Figure 4.2 Box-and-whisker plots of total terpenes (μg/g, log transformed) at sampled time points, separated by attack density (attacks/m^2) for lodgepole pine (a) and jack pine (b). Top and bottom of the boxes denote data falling within the first and third quartiles, respectively, midline indicates the median, and whiskers indicate the maximum and minimum value, or 1.5 times the interquartile range, whichever is smaller.
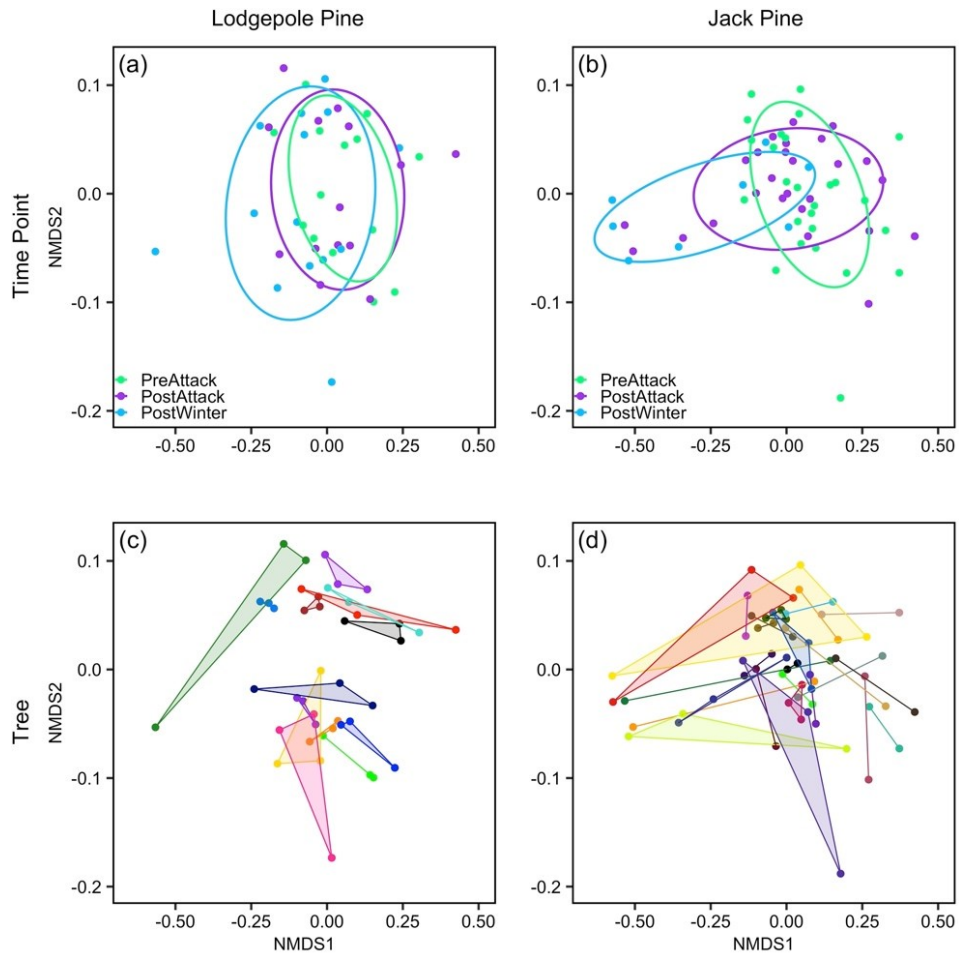
Figure 4.3 Plots of non-metric multidimensional scaling (NMDS) ordinations for terpenes in lodgepole pine (a and c) and jack pine (b and d) pine by attack stage (a and b) and individual tree (c and d). In (a) and (b) points are coloured by attack stage and ellipses drawn using ordellipse (*vegan*; 60% confidence interval). In C and D, points are coloured by individual trees with trees with three points connected and filled and trees with two points connected by a single line.

Chapter 5. Performance and preference of the mountain pine beetle, *Dendroctonus ponderosae* Hopk., in lodgepole and jack pine bolts

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5.1. Introduction

Herbivorous insects must evolve adaptations to overcome evolved plant defenses against herbivory, such as phytochemicals, in an evolutionary arms race (Mello and Silva-Filho 2002). Insects vary in their host breadth from monophagous specialists that exploit a few plant species within a genus to polyphagous insects that feed on members of several different plant families (Cates 1980). Specialist herbivores tend to have specific adaptations to tolerate severe plant defenses, whereas generalists tend to trade off the ability to combat specific defenses for increased host breadth (Cornell and Hawkins 2003). Most herbivorous insects are specialist feeders and must quickly and efficiently detect and orient to suitable hosts (Forister et al. 2015). Insects will locate hosts using multimodal signals including olfactory cues (Bruce et al. 2005), visual cues (Blake et al. 2019, Van Der Kooi et al. 2021), tactile cues (Rojas et al. 2003), and gustatory cues (Agnihotri et al. 2016). The combination of these cues forms a host 'gestalt' that specialist insect herbivores are adapted to use in host detection and acceptance behaviours (Courtney and Kibota 1990). Specific cues may only be important at certain stages of host selection and acceptance (Silva and Clarke 2020). For example, some cues are responsible for long-range attraction towards the host and other cues are required on contact with the host to confirm suitability (Saint-Germain et al. 2007). The appropriate/inappropriate landings theory suggests that insects will land on many plants within a patch but will only accept the most suitable host plant (Finch and Collier 2000).

Insect herbivores that can reproduce on more than one plant species do not necessarily prefer all potential host species equally. Rather than accept the first suitable host they encounter, they may use ranked cues to select the best host species amongst the plants available to them (Silva and Clarke 2020). There are several theories that postulate why insect herbivores prefer certain plant hosts over others (Refsnider and Janzen 2010). The preference-performance hypothesis, which suggests that females will prefer to oviposit on hosts that maximize the fitness of their offspring, has been tested repeatedly in

herbivorous insect systems (Gripenberg et al. 2010). Optimal foraging theory, when integrated with the preference-performance hypothesis, balances the needs of the adult by considering realized fecundity as well as offspring fitness in the host selection process (Scheirs and De Bruyn 2002). Natal host experience and learning can also lead to preferences for certain host species (Szentesi and Jermy 1990, Davis and Stamps 2004). Hopkins' host selection principle (HHSP) proposes that herbivorous insects will prefer the host species that matches their natal host and has been applied to many phytophagous insects as well as parasitoids, with mixed results (Barron 2001). Hopkins' host selection principle was conceived during observations of the mountain pine beetle (MPB), *Dendroctonus ponderosae* (Coleoptera: Curculionidae), that preferentially attacked lodgepole pine (*Pinus contorta*) trees in forests where it was mixed with ponderosa pine (*Pinus ponderosa*; Hopkins 1916). The founding population of MPB that Hopkins observed came from a neighboring lodgepole pine forest and picked out the lodgepole pines from the mixed stand (Hopkins 1916). Hopkins hypothesized insects would exhibit natal host fidelity because, "... a species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted" (Hopkins 1916). Adaptation to the natal host as described by Hopkins is vague but has mostly been interpreted as preference for host that matches the larval host in holometabolous insects (Barron 2001). The mechanism for how natal host preference is acquired and maintained over metamorphosis has been speculated to be related to pre-imaginal conditioning in the larval stage, imaginal conditioning as early adult experience, maternal effects, and learning (Barron 2001, Anderson and Anton 2014).

Mountain pine beetles colonize, feed on, and reproduce within host trees which causes the eventual death of all or part of the host (Safranyik and Carroll 2006). Mountain pine beetle is an irruptive herbivore, and the host selection process is dependent on population state and density (Raffa et al. 2008, Howe et al. 2022). In the endemic population state, at low population densities, MPB will preferentially colonize stressed trees with low defensive capacity (Carroll et al. 2006a). In response to density-dependent (Boone et al. 2011) or context-dependent maternal effects (Burke and Carroll 2017), MPB will shift host preference to well defended trees that have more resources for reproduction. Positive feedback of this behaviour can result in development of large-scale MPB outbreaks called epidemics (Safranyik and Carroll 2006). Female MPB are the host pioneers and so are the only sex that make host choices. Males respond to aggregation pheromone, and when population densities are high, more pairs are recruited to mass attack large, well defended trees. Pioneer females orient towards preferred host trees by primary attraction to long-range olfactory cues (Moeck and Simmons 1991) and the combination of visual and

olfactory cues (Campbell and Borden 2006b). There is evidence, however, that females simply land at random within a tree stand (Byers 1996), assess the identity and quality of the potential host with contact chemical and gustatory cues (Raffa and Berryman 1982a, Wood 1982), and either accept the host by initiating colonization or fly again to randomly land on another tree. The spatial scale at which the beetle responds is important to determine whether there is directed orientation to host cues. At a large spatial scale, MPB exhibits primary attraction but at a small spatial scale (i.e., within a stand, between individual trees), beetles land on hosts at random (Saint-Germain et al. 2007). This behaviour agrees with the appropriate/inappropriate landings theory (Finch and Collier 2000), and is adaptive in MPB because mistakes in host selection at any population density can result in serious fitness costs (Raffa et al. 2016). Most pine species in MPBs current range are susceptible to mass attack by MPB, but the most common hosts are lodgepole pine, ponderosa pine, and western white pine (*Pinus monticola*; Safranyik and Carroll 2006). The host breadth of MPB extends beyond these common hosts as they can reproduce in as many as 22 different pine species and two spruce (*Picea*) species (Wood 1963, Furniss and Schenk 1969, Huber et al. 2009). Outbreaks in arboreta that contain pine and spruce trees that typically grow outside the historic range of MPB, further exemplify the breadth of MPB herbivory within the genus *Pinus*, and the ability of MPB to opportunistically colonize spruce (Furniss and Schenk 1969, Smith et al. 1981, Cook and Martinez 2018b).

While Hopkins' host selection principle was described with MPB in mind, it does not apply consistently to MPB that have access to more than one pine species, or indeed even between the two species that Hopkins initially observed. Mountain pine beetles that emerge from lodgepole pines and are introduced to cut logs (bolts) of lodgepole or ponderosa pine will accept ponderosa pine phloem faster and produce more fertile parental galleries with eggs in ponderosa pine bolts (Rosenberger et al. 2017b). Offspring of MPB that are introduced to ponderosa pine bolts develop faster and are larger on average than offspring of MPB that are introduced to lodgepole pine bolts (Amman 1982). When given the choice between cut material of both species, beetles that emerge from lodgepole and ponderosa pine both prefer to colonize ponderosa pine (West et al. 2016). Mountain pine beetle host selection when presented with a choice of cut material from lodgepole and ponderosa pines is therefore more in alignment with the preference-performance hypothesis than Hopkins' host selection principle since the host that results in the highest offspring quality is preferred. However, preference for one species of cut bolts in the field or lab may not reflect behaviour of MPB foraging for live trees in the field. In areas where high-elevation lodgepole

pine forests are replaced by low-elevation ponderosa pine forests, both species are attacked equally when beetle populations are at high densities (West et al. 2014).

Climate change and large population outbreaks of MPB have resulted in range expansion that has allowed MPB access to pine species at higher latitudes and elevations (Cooke and Carroll 2017, Sambaraju and Goodsman 2021, Bentz et al. 2022). Host species in the expanded range can be considered "evolutionarily naïve" to MPB attack because they lack defensive adaptations present in populations that have historically experienced frequent MPB herbivory (Clark et al. 2010, Cudmore et al. 2010, Raffa et al. 2013, Burke et al. 2017). Naïve hosts may be preferred by female MPB that are optimally foraging to maximize their realized fecundity through reduced mortality risk from colonizing a less well defended tree (Raffa and Berryman 1983a). Within the historic range of MPB, high elevation pine species, such as limber pine (*Pinus flexilis*) and whitebark pine (*Pinus albicaulis*), have only sporadically experienced MPB herbivory over the last ten thousand years, but attacks have become more frequent with climate change (Gibson et al. 2008, Bentz et al. 2011). Whitebark pine has fewer chemical and physical defenses to protect itself against MPB colonization than lodgepole pine (Raffa et al. 2013). After mass attack by MPB, a greater proportion of whitebark pine trees have successful offspring production compared to lodgepole pine, and both hosts produce similar numbers of offspring per female (Bentz et al. 2015) so there is no cost to fecundity in switching hosts. Optimal foraging theory and the preference-performance hypothesis would predict that female MPB will preferentially colonize whitebark pine as there is less risk in a colonization attempt without a cost to fecundity, but in the field, lodgepole pine is the preferred host during outbreaks in mixed forests (Raffa et al. 2013, Bentz et al. 2015). The preference for the common historic host over a naïve one, despite the naïve host reduced defensive capacity, could be evidence for Hopkins' host selection principle. Preference for an historic host could indicate that there is adaptation or learning by the beetle to specifically detect the historic host, or that the naïve host lacks a cue that promotes acceptance.

Facilitated by climate change, MPB has established populations east of the Rocky Mountains in Alberta and successfully reproduced within natural stands of jack pine (*Pinus banksiana*) in Canada's boreal forest (Cullingham et al. 2011, de la Giroday et al. 2012). Jack pine is a suitable host of MPB reproduction (Cerezke 1995, Cullingham et al. 2011, Erbilgin et al. 2014, Rosenberger et al. 2017b, 2018), has fewer chemical defenses (Clark et al. 2014, Arango-Velez et al. 2016, Lusebrink et al. 2016), and is more susceptible to mass attack (Chapter 3) than lodgepole pine. Whether the natal host of MPB affects preference

for lodgepole or jack pine as a reproductive host has not been studied to date. Using no-choice and choice experiments, I examined both the preference and performance of MPB in lodgepole and jack pine bolts in the lab with beetles that emerged from mass attacked lodgepole or jack pine trees. Based on field observations made while attempting to induce mass attack of standing jack pine trees using infested lodgepole pine bolts (Chapter 3), I hypothesize that MPB females will preferentially choose their natal host for reproduction, as postulated in Hopkins' host selection principle. In the field, female MPB preferred to re-enter remaining green phloem of lodgepole pine bolts that were attacked the previous season instead of accessing live jack pines (Chapter 3). Since MPB coevolved with lodgepole pine (Clark et al. 2010, Raffa et al. 2013, Burke et al. 2017), this could mean they are adapted to prefer lodgepole pine over jack pine. I predict that MPB will behave in agreement with Hopkins' host selection principle and females that emerge from lodgepole pine will prefer lodgepole pine cues in choice experiments. If the observed preference for lodgepole pine in the field is due to a shared evolutionary history, then females that emerge from a single generation in jack pine will maintain the preference for lodgepole pine and so also prefer lodgepole. Alternatively, if host preference is driven by larval or early adult experience, MPB females that emerge from mass attacked jack pine will prefer cues from jack pine over lodgepole pine as natal host fidelity is gained during development.

To examine MPB performance in lodgepole and jack pine bolts, I performed no-choice experiments in the lab by introducing beetles that emerged from bolts of mass attacked lodgepole or jack pine trees to uninfested bolts of either lodgepole or jack pine. I hypothesized that MPB would perform differently in the two pine hosts and performance would depend on the natal host species. If MPB females choose hosts in agreement with both Hopkins' host selection principle and the preference-performance hypothesis, then offspring number and quality will be greatest in the natal host species that is preferred. The area where lodgepole and jack pine forests overlap is a mosaic of pure species and hybrids (Cullingham et al. 2012, Burns et al. 2019), so while mixed stands of lodgepole and jack pine do not occur, there could be patches of each species within the dispersal distance of MPB. Populations of MPB that enter jack pine forests from outbreaks in west central Alberta will likely originate from lodgepole pine forests, so understanding host preference, acceptance, and performance of MPB in these two species could help in our understanding of MPB movement in this novel habitat.

5.2. Methods

Insects

These experiments were conducted twice, once with beetles that attacked trees in 2018 and again with beetles that attacked trees in 2019. To obtain adult MPB to inoculate bolts in the lab for no-choice experiments, naturally mass attacked lodgepole pine bolts were collected near Hinton, Alberta in 2018 and 2019, and from jack pine mass attack manipulations in Lac La Biche, Alberta in 2018 (Fig. 5.1). Mass attacked trees from both species were felled and two, 50-cm length bolts were cut from the lower bole of each tree. In 2019, mass attack manipulations in live jack pine trees were not feasible due to the MPB population crash over the winter of 2018-19. Instead, five to seven pairs of MPB that had emerged from lodgepole pine were introduced to 50-cm length bolts from 10 different healthy jack pine trees that were harvested from the Lac La Biche area in fall 2019 (Fig. 5.1). Parents were allowed to colonize the jack pine bolts for five weeks before they were transferred to a 5°C cooler for a minimum of two months to provide an artificial "winter" period. Bolts that were infested naturally or in the lab were stored in a cooler at 5°C until use. Bolts were removed from the cooler as needed and placed in emergence bins (Rubbermaid Hinged Top Tote, 114 L, with a hole cut at the front fitted with a glass jar) to collect emergent beetles which were used in experiments within 10 days of emergence.

Plant materials

Healthy lodgepole and jack pine trees were harvested for choice and no-choice experiments in the fall of 2018 and 2019 (Fig. 5.1). In 2018, 20 healthy lodgepole and jack pines across three sites near Swam Hills and Lac La Biche Alberta, respectively, were felled and two 50-cm bolts were harvested per tree for use in both choice and no choice experiments. In 2019, 10 lodgepole pines from two sites, one near Hinton and one near Edson Alberta, and 10 jack pines from two sites, both near Lac La Biche Alberta, were selected and four 50-cm bolts were harvested and used in no-choice experiments or infested with MPB pairs in the lab to produce MPB for olfactometer experiments. The cut ends of the bolts were sealed with hot paraffin wax, transported to the University of Alberta. Bolts were stored at 5°C until use within 3 months of collection in both years for no-choice experiments and within 6 months for choice experiments performed in 2018. The increased length of storage for bolts that were used in choice assays in 2018 was allowed because

beetles in these choice assays only entered, and didn't reproduce within, the bolts they were responding to in the assay.

Phloem and bark tissue were harvested from both lodgepole and jack pines during one week in August 2019 for use as odour sources in olfactometer bioassays. Tissue was harvested from 20 lodgepole pines across two sites, one near Hinton and one near Grande Cache, Alberta, and 20 jack pine across two sites, one in Slave Lake and one in Lac La Biche, Alberta. Four 7×7 cm tissue samples were excised with a chisel and mallet from each tree at 1.3 m above the ground at each of the cardinal directions on the bowl. Samples were immediately placed in labelled paper bags and buried in dry ice until they could be placed in a −70°C freezer.

Data management, visualization, and model diagnostics

All analyses for data collected in the following experiments were performed in R (version 4.0.3; R Core Team 2022) run within RStudio (version 1.2.5001; RStudio Team 2022). Data wrangling and visualization was performed with packages within *tidyverse* (Wickham and Grolemund 2016, Wickham et al. 2019), and final figures made with *ggplot2* (Wickham 2016), *patchwork* (Pederson 2022), and *ggpubr* (Kassambara 2022). Statistical model diagnostics were performed with by plotting scaled residuals with *DHARMa* (Hartig 2022) and testing the normality of the residuals of the model with Shapiro-Wilk, if necessary. *DHARMa* plots of scaled residuals display a q-q plot; Kolmogorov-Smirnov, dispersion, and outlier tests; and plots the scaled residuals vs. the predicted values. Only models that met assumptions and showed no significant issues in *DHARMa* plots are reported.

No-choice experiment

Mountain pine beetles that emerged from infested lodgepole or jack pine bolts were introduced to uninfested bolts of either lodgepole or jack pine in a 2x2 full factorial design. The combinations were as follows: 1) adult MPB that emerged from lodgepole pine inoculated into lodgepole pine bolts for reproduction; 2) adult MPB that emerged from lodgepole pine inoculated into jack pine bolts for reproduction; 3) adult MPB that emerged from jack pine inoculated into jack pine bolts for reproduction; and 4) adult MPB that emerged from jack pine inoculated into lodgepole pine bolts for reproduction. These

combinations can also be described as beetles from lodgepole that did or did not host switch and beetles from jack pine that did or did not host switch.

To perform introductions, uninfested lodgepole pine and jack pine bolts were removed from cold storage and kept at 22°C for 24 hours to allow the bolts to warm to room temperature. In order to collect offspring from each individual pair, and not from the entire bolt at once, the bark of each bolt was scored vertically using a Dremel with a 1.2 cm carbide cutting wheel (EZ544; Robert Bosch Tool Corp, Racine WI USA) to divide the bolts into five (2018) and two (2019) separate sections that were a minimum of 10 cm wide (Fig. 5.2a). Score lines prevented the beetles that were introduced to the bolts from crossing into other sections of the bolts, so offspring from different pairs did not comingle. Score lines were formed with two vertical cuts ~1.5 cm apart, the bark and phloem were peeled away from the wood between two vertical cuts and were filled with hot paraffin wax to slow phloem desiccation (Fig. 5.2a). The number of sections was reduced in 2019 to accommodate the larger bark area consumed by MPB in jack pine which was observed in Chapter 3 to reduce any potential competition between larvae.

One female-male pair of beetles was introduced to the centre of each section ~5 cm from the base of the bolt through a 5 mm entry point in the bark created with a cork borer (Fig. 5.2b). Females were introduced first in a 1.5 mL microcentrifuge tube glued to the bark at the entry point to access the phloem and xylem layers (Fig 5.2b). If after 24 h, female inoculation was successful as indicated by the presence of boring dust, a single male was introduced to the tube. Females were replaced if they did not initiate boring for 48 h and males were replaced if they did not enter the inoculation point within 24 h. In 2018, inoculated bolts were housed at 22°C for four weeks and then transferred to 14°C for three weeks to synchronize offspring development. Bolts were then placed at 5°C for two to three months as an artificial winter period. Because the 14°C treatment did not synchronize development as expected, bolts in 2019 were held at 22°C for five weeks and then moved immediately to 5°C for two to three months. Inoculated bolts were removed from cold storage and placed at 22°C for the completion of offspring development. The offspring from each beetle pair were collected in individual cages of aluminum wire insect screen (18´16 mesh size: PHIFER Inc. Alabama USA) positioned over each section of each bolt (Fig. 5.2c,d). Bolts were checked daily for adult offspring emergence and beetles were collected from each caged section at the base of the bolt. Offspring beetles were separated by sex using the stridulation trait of male beetles (Rosenberger et al. 2016) and placed individually in 1.5 mL microcentrifuge tubes at −20°C until analysis. Once no adult MPB offspring had

emerged from the bolts for over 30 days, bolts were autoclaved, debarked, and the presence of parental galleries ≥3 cm was recorded.

The body size (volume) of each offspring beetle was determined by measuring the pronotum width and body length with a stereomicroscope with ocular micrometer at 1.6x. Pronotum width and body length were used to calculate body size with the equation for the volume of an ellipsoid (Reid and Elkin 2005), a = half the body length, b = half the pronotum width, and c = half the pronotum width:

$$V = \frac{4}{3}\pi abc$$

The lipid content of each beetle was quantified with a Soxhlet extraction method (Atkins 1969). Beetles were dried in an oven at 60°C for 24 h, weighed to the nearest 0.01 mg, and then placed individually in perforated 0.2 mL microcentrifuge tubes. Tubes with beetles were placed in a Soxhlet apparatus and washed every 20 min with warm petroleum ether (Sigma-Aldrich) for 8 h. Beetles were then dried again for 24 h at 60°C before they were weighed. Pre-extraction dry weight was subtracted from post-extraction dry weight to determine lipid content. Using the body volume and lipid content measurements, a body condition residual index was created by fitting a linear model of body size as a function of lipid content and the residual of each beetle from the model represented its relative condition (Reid and Elkin 2005). Beetles with a positive residual condition value had a better body condition than predicted by the linear model and those with a negative value had a poorer condition than predicted by the linear model (Fig. 5.3).

Data for the effect of natal host species and reproductive host species bolt on the probability of parental gallery construction, larval offspring production, adult offspring production, production of larvae after constructing a parental gallery, and production of adult offspring after successful larval production, were analyzed with generalized linear mixed effects models with a binomial family and a term for bolt fit as a random effect in lme4 (Bates et al. 2015). *emmeans* (Lenth 2022) and *broom* (Robinson et al. 2022) were used to generate tables of estimated marginal means, upper and lower confidence levels, and p-values for the comparison between the estimated probability of each measure against the null probability of 0.5. The mean number of offspring per female produced in the reproductive host was analyzed only for the pairs that constructed a parental gallery and the effect of natal host, reproductive host, and their interaction on the number of offspring produced per female was analyzed with a generalized linear mixed effects model with a

zero-inflated Poisson family, and a term for bolt fit as a random effect using *glmmTMB* (Brooks et al. 2017). Tables of estimated marginal means and standard errors were also generated with *emmeans* and broom. The plot for the number of offspring per female produced by natal-reproductive host combination was constructed using *ggplot2* (Wickham 2016), and *ggbreak* (Xu et al. 2021). There was no effect of the year of no-choice experiment or an interaction between year and natal host or reproductive host in any of the probability or offspring per female models tested, so data from both years was pooled.

In the data for offspring performance, year had a significant effect in all models used to examine the effect of natal and reproductive host on offspring performance so the body condition measurements of offspring from bolts manipulated in 2018 and 2019 were analyzed separately. In the 2018 dataset, two outliers for offspring lipid content were identified by the functions *testOutliers*() and *outliers*() from *DHARMa* and so were removed. The effects of sex, natal host, reproductive host, and the interaction between natal and reproductive host, on body size, lipid content, relative lipid content (mg of lipid per mg dry mass), and residual body condition were compared using linear mixed effects models (*lme4*; Bates et al. 2015) with a term for the introduced pair nested within bolt fit as a random effect. ANOVA tables of test statistics and p-values for the fixed effects in the linear mixed effects models were generated using *car* (Fox and Weisberg 2019) and pairwise means comparisons were performed using *lmerTest* (Kuznetsova et al. 2017)

Static air choice arena trials

To test the hypothesis that offspring beetles have a colonization preference for either lodgepole or jack pine, in 2018, female MPB were given the choice between four bolts in a static air arena experiment. Two lodgepole pine bolts and two jack pine bolts (each bolt from a different tree) were placed in each of the four corners of an arena (Fig. 5.4a; 1.7 $\times$ 0.85 $\times$ 0.9 m). One hundred female MPB that emerged from multiple bolts of either mass attacked lodgepole, or jack pine were released in the centre of the arena and allowed to move freely. After 24 h, the number of beetles that had bored into each bolt was counted. Beetles that did not enter bolts were recorded as on/under each bolt or elsewhere in the arena. Bolts were then peeled and the number of beetles that entered the phloem tissue was recorded. Data from the static air bioassay were analyzed using 2-way ANOVA with the species of bolt responders chose, the natal host of the responders, and the interaction between natal host and species choice, as fixed effects.

Olfactometer bioassay

In 2019, I performed olfactometer bioassays to test the hypothesis that female offspring prefer olfactory cues released by either lodgepole or jack pine. This approach allowed the isolation of olfactory from visual and gustatory cues as and eliminated the issue of pheromone production that could have occurred in the static air arena trials in which beetles were allowed to enter bolts. As there was no source of naturally mass attacked jack pine trees in 2019, uninfested lodgepole and jack pine bolts were infested in the lab to provide beetles for this bioassay. Ten bolts from each species had five to six pairs of beetles introduced around the bottom of the bolt as described previously. After five weeks at 22°C, bolts were transferred to 5°C for two to three months before they were placed in emergence bins at 22°C, as described above.

Olfactometer bioassays were conducted using a modified six-way olfactometer (Analytical Research Systems Inc., Gainesville, FL) attached to an airflow system (Model OLFM-6C-ADS+VAC, Analytical Research Systems Inc., Gainesville, FL) connected to building air. Two arms of the olfactometer were blocked, two arms supplied clean air, and two arms contained host plant phloem tissue, one with tissue from jack pine and one with tissue from lodgepole pine (Fig. 5.4b). Host tissue samples consisted of two ~1.5 cm×1.5 cm squares of phloem and bark, each from a different tree. Air flowed through the olfactometer at 1.0 L per min and a central vacuum removed air from the olfactometer arena at 3.0 L per min. The olfactometer was illuminated with four 15W LED light bulbs at angles that reduced glare on the plexiglass lid and prevented shadows within the olfactometer arena. Bioassays were recorded from above with a USB webcam (Akyta-N5-Webcam, China) on a tripod. A single female MPB <10 d post emergence from either lodgepole or jack pine bolts that were infested in the lab, was released in the centre of the olfactometer arena, and its movements were recorded for 60 min. To quantify the time spent in the different quadrants of the olfactometer, the arena was divided into six sections and the number of seconds spent by each female MPB in each section was recorded. Beetles were not able to enter the arms of the olfactometer, so only the time the beetles spent visible was included in the analysis. The proportion of time spent near species of phloem in the olfactometer assays was analyzed with generalized linear models with a beta error family using *glmmTMB* (Brooks et al. 2017).

5.3. Results

No-choice experiment.

The total number of bolts that were inoculated with pairs of beetles for each natal-reproductive host combination and the number of pairs that produced a parental gallery, at least one larval gallery, and at least one adult offspring, are reported in Table 5.1. There was no effect or interaction of natal or reproductive host on the probability of parental gallery construction, production of larval galleries, adult offspring production following parental gallery construction (p > 0.05; Table 5.2). Although the mean probability of constructing a parental gallery was not different between groups, beetles that had the same natal and reproductive host (i.e., did not switch hosts) were more likely than random (50%) to construct a parental gallery whereas the probability of parental gallery construction for beetles that did switch hosts could not be differentiated from random. There was a non-significant trend that beetles introduced to jack pine as a reproductive host had a lower probability of producing adult offspring (50% or less) than beetles that were introduced to lodgepole pine (63-64%) (Table 5.3, Fig. 5.5c). There was also no difference in the probability of producing a larval gallery after construction of a parental gallery, but beetles that emerged from jack pine produced larvae from parental galleries more often than random (Table 5.3, Fig. 5.5d). Of beetles that produced larval galleries in the reproductive host, beetles that emerged from jack pine and didn't host switch had the lowest probability of producing adult offspring (Table 5.3, Fig. 5.5e), and while not significant, this was the largest drop in survival from one stage to the next. The number of adult offspring produced per female was also not affected by natal host, reproductive host, or their interaction (Table 5.2) but beetles that had lodgepole pine as a natal host and were introduced to lodgepole pine as a reproductive host had the highest mean adult offspring produced per female (Fig. 5.5f).

The effect of natal host and reproductive host on certain offspring condition measurements was different depending on year and so the years were analyzed separately. Adult offspring body size was not affected by natal host, reproductive host, or their interaction in either 2018 or 2019 (Table 5.2) but females were significantly larger than males in both years (2018 p = 0.002, 2019 p = 0.001; Fig. 5.6a,b). Lipid content of adult offspring had a trend similar to that of body size in both years, and natal host, reproductive host, and their interaction had no effect (Table 5.2) but females had significantly more fat than males in 2018 (χ^2 = 7.56, df = 1, p = 0.006; Fig. 5.6c) but not in 2019 (χ^2 = 3.33, df

= 1, p = 0.07; Fig. 5.6d). Adult offspring relative lipid content and residual condition were not significantly influenced by offspring sex in both years, and inclusion of sex in the models resulted in violations of model assumptions and so was removed from the models. The relative lipid content of adult offspring was not significantly affected by natal host, reproductive host, or their interaction in 2018 (Table 5.2, Fig. 5.7a), but there was a trend that offspring of beetles that had lodgepole pine as both the natal host and reproductive host had slightly greater relative lipid content. In 2019, there was effect of natal host, reproductive host, or their interaction on relative lipid content of adult offspring (Table 5.2, Fig. 5.7b). Reproductive host had a significant effect on mean residual condition of adult offspring in 2018 (χ^2 = 4.29, df = 1, p = 0.04), offspring from combinations that had lodgepole pine as a reproductive host were in better relative condition compared to offspring from combinations with jack pine as the reproductive host (Fig. 5.7c). In 2019, however, residual condition of adult offspring was not affected by natal host, reproductive host, or their interaction (Table 5.2, Fig. 5.7d).

Choice experiments

Choice bioassays with bolts in a static air arena were largely unsuccessful, as few beetles met the criteria as responders. Of the 100 beetles that were released an average of 6 beetles (range: 3–22) bored into the bark and entered the bolts. Most beetles were found on top of or underneath the bolts in the arena, these counts were added to the number of beetles that entered the bolt and compared as a response category of on, under, or entered the bolt. Beetles that emerged from either pine species had no orientation preference for the bolts of either species ($F_{1,14}$ = 0.41, p = 0.53; Fig. 5.8a) but twice the number of beetles that emerged from lodgepole pine were found under, on top of, or within any bolt after the bioassay ($F_{1,14}$ = 16.45, p = 0.001; Fig. 5.8a). There was a non-significant trend that beetles that emerged from lodgepole pine entered bolts by the end of the bioassay more often ($F_{1,14}$ = 4.00, p = 0.07; Fig. 5.8b) but there was no preference for bolts of either pine species ($F_{1,14}$ = 0.37, p = 0.55; Fig. 5.8b).

In olfactometer experiments, beetles that emerged from lodgepole pine spent equal proportions of time in all sections of the olfactometer (χ^2 = 3.10, df = 3, p = 0.38; Fig. 5.9a). Beetles that emerged from jack pine spent the most time in sections containing clean air in the olfactometer bioassay, next most time in sections that were blocked, and the least amount of time near both species' pine phloem (χ^2 = 54.54, df = 3, p < 0.001; Fig. 5.9b). As the proportion of time spent in the sections that contained phloem from both species did

not differ, sections that contained phloem were classified as phloem in general. Female beetles that emerged from lodgepole pine spent more time in the sections that contained phloem and there was no difference in the amount of time they spent in sections with clean air or were blocked (χ^2 = 26.28, df = 2, p < 0.001; Fig. 5.9c). Females from jack pine still spent the most time in sections that contained clean air but did not spend different proportions of time in sections that contained phloem or were blocked (χ^2 = 12.20, df = 2, p = 0.002; Fig. 5.9d).

5.4. Discussion

There was no evidence that the natal or reproductive host affected the likelihood that MPB would construct a parental gallery as the variance around the mean probability for each combination all overlapped. In similar studies, Rosenberger et al. (2017b, 2018) introduced beetles that emerged from lodgepole pine to bolts of various eastern pine species. Females were also just as likely to enter jack pine phloem as lodgepole pine phloem, produce fertile parental galleries (Rosenberger et al. 2017b), and produced similar numbers of offspring per female (Rosenberger et al. 2018). Beetles that emerged from lodgepole that entered lodgepole and jack pine bolts in this study also had no difference in the likelihood of constructing fertile parental galleries and produced similar numbers of offspring per female. There is a trend within the data that beetles that were introduced to a reproductive host that was the same species as their natal host had a greater probability of constructing parental galleries. Within the natal-reproductive host combinations, beetles only had an estimated probability of successful parental gallery construction with a confidence interval that did not overlap with 50% when the natal and reproductive host were the same species (no host switch). The estimated probability of parental gallery construction by beetles that did host switch had confidence intervals that overlapped with 50% so there is no evidence that probability of accepting the host is different from random, but true mean could be above 50%. Females that did not successfully construct a parental gallery after entering the reproductive host could have rejected the host and bored out or died after initial gallery construction. The number and sex of any beetles in the initial parental gallery could not be determined as the unsuccessful parental galleries were filled with saprophytic fungi that made old adult beetles brittle and when the bolts were autoclaved and debarked, any remaining parent beetles were destroyed. Some pairs had single exit holes near the entry point but whether it was made by the female or male could not be determined. If females entered, then rejected and bored out when they were forced to host switch, this could

explain why these combinations were no more likely to have successful parental galleries than random, on average. If females did reject the reproductive host more frequently when it didn't match their natal host, this could provide evidence for Hopkins' host selection principle in MPB emerging from lodgepole and jack pines.

When MPB attack jack pine bolts in the field and are then reared in the lab so their offspring complete their life cycle with no exposure to winter, they produce fewer offspring per female than MPB that attack lodgepole pine bolts, likely due to high attack densities (Cerezke 1995). Colonization of novel hosts can result in similar numbers of larvae after mass attack but the quality of offspring and the number of adult offspring that emerge after exposure to natural winter conditions is lower in novel than historic hosts (Rosenberger et al. 2017a, 2018). The probability of adult offspring production was slightly lower in jack pine reproductive hosts regardless of the natal host, but not statistically significant. This trend agrees with previous evidence of reduced MPB survival in bolts of novel hosts. To emulate natural conditions and synchronize development, I treated bolts with "fall" and "winter" conditions prior to offspring collection. Mountain pine beetle brood exposed to natural temperature variation synchronize development for overwintering and synchronous emergence in the spring (Bentz et al. 1991, 2001). As temperatures decrease, older larvae slow development while younger larvae maintain development rate to catch up to the older larvae before winter (Bentz et al. 1991). Three weeks under simulated fall conditions was not sufficient to slow older larval development and allow younger larvae to develop to the cold tolerant stage. As a result, the subsequent emergence period from bolts in this study was long, with some adults emerging immediately after the winter treatment but others taking much longer to develop and emerge.

The average adult offspring produced per female did not differ in the various natal-reproductive host combinations, but overall offspring production from all bolts was low, and averaged half that of similar studies in which bolts experienced natural winter temperature variation (Rosenberger et al. 2018). At the 5°C winter temperature used in this study, MPB development ceased, but saprophytic fungal growth continued in the phloem and could have led to decreased offspring survival. Beetles in the Rosenberger et al. (2017a, 2017b, 2018) studies were introduced to bolts of different species within three days of bolt harvest. This experiment was done over fall–winter and with inconsistent emergence from the bolts that were sources of the natal host beetles, females were introduced to bolts over a period of up to 3 months after bolt harvest. The length of bolt storage did not influence reproductive host acceptance or offspring production, but it could have increased the variation in these

data making it difficult to detect differences between the groups. It is possible that a combination of delayed introduction of beetles to cut bolts and a lack of natural fall/winter temperatures that prevented saprophytic fungal growth, resulted in low reproductive output of the beetle pairs in this study. These data show interesting trends but with the amount of variation observed, more data is needed to draw definitive conclusions about successful colonization and reproduction in these host switching scenarios.

Although there was no difference in offspring production, there were differences in offspring condition based on reproductive host in the experiment conducted in 2018. While the body size and lipid content of adult offspring did not differ with the natal-reproductive host groups tested, relative lipid content was marginally different and residual condition was significantly different. Offspring of beetles that had jack pine as a reproductive host had lower relative fat content and residual body condition than beetles that had lodgepole pine as a reproductive host. Jack pine seems to be a less suitable host for MPB since offspring reared in jack pine bolts were lower quality compared to those reared in lodgepole pine bolts in this study. Mountain pine beetles that emerge from lodgepole pine and choose to colonize a jack pine over lodgepole pine would have a cost in their offspring condition. Jack pine has thinner phloem on average than lodgepole pine (Lusebrink et al. 2016, Rosenberger et al. 2017b) and so has fewer resources for developing MPB offspring. There was, however, a trend that beetles that emerged from jack pine and colonized jack pine did not have as much of a reduction in relative lipid content. If jack pine phloem in bolts was the only reason for offspring condition reduction, then beetles that emerged from jack pine and colonized jack pine as a reproductive host should have offspring with lower relative fat content that is proportional to the reduction seen in beetles that emerged from lodgepole pine and colonized jack pine. There may be some adaptation to jack pine phloem that occurred in the single generation so that there is less cost in colonizing the poorer nutrition host species. Body size, total lipid, and relative lipid contents of beetles from the 2018 experiment did not differ significantly by natal or reproductive host with an $\alpha = 0.05$, but residual condition did differ. A residual condition index that includes body volume as a measure of size rather than mass (Reid and Elkin 2005) and is a good estimation of relative offspring condition when fresh weight of the beetle is not measured.

The costs of host switching varied with experiment year in this study. There was no evidence that host switching for beetles that emerged from lodgepole pine was costly in the 2019 experiment as none of the adult offspring measurements differed depending on natal or reproductive host. The difference between 2018 and 2019 experiments could be related

to the low reproductive success of the parents that entered the bolts in 2019. The parent beetles that were used to colonize the bolts were from lodgepole pine that experienced extreme winter conditions in February 2019 that resulted in widespread MPB mortality (MacQuarrie et al. 2019). The surviving beetles used as parents in the 2019 experiment may have suffered sublethal effects that limited their reproductive capacity (Marshall and Sinclair 2012). Another difference between the 2018 and 2019 experiments was that fewer pairs of parental beetles were introduced to the bolts in the no-choice experiments in 2019 than in 2018. Offspring reared in jack pine consume large areas of thin phloem, so the reduction in the number of pairs in 2019 gave the offspring ample space to mine large galleries and reduce any competition. Females from a single parental gallery reared in jack pine bolts colonized by only one pair of beetles have greater fat content than those reared in lodgepole pine under similar conditions (Lusebrink et al. 2016) which suggests that at low density condition, MPB offspring in jack pine may have an advantage. However, in live jack pine that are mass attacked at biologically relevant densities, phloem thickness and attack density both positively influence offspring production (Chapter 3). Mass attacked jack pine trees with thick phloem also produce offspring that are larger and have more fat content than mass attacked jack pine trees with thin phloem, regardless of attack density (Chapter 3). Since there is no evidence that high attack densities in live jack pines reduces offspring production per female, the reduction in number of pairs and increase in space available to offspring developing in jack pine was likely not necessary.

The experiments designed to test host preference did not provide any evidence for a preference by MPB that emerge from lodgepole or jack pine for bolts of lodgepole and jack pine, or their phloem volatiles. The static air arena was unsuccessful as most beetles took shelter underneath, instead of colonizing the bolts. Beetles that emerged from jack pine did respond less to bolts in general and only 20% of the released beetles were found on, under, or within the bolt. It is possible that 24 h was not long enough for them to respond to bolts, as energy use increases acceptance of host material (Jones et al. 2020). Although, host choice of MPB reared in ponderosa or lodgepole pine measured immediately post emergence, resulted in preferential colonization of ponderosa pine (West et al. 2016). Allowing multiple MPB to choose between lodgepole and jack pine bolts over a period longer than 24 h could result in most beetles choosing jack pine due to increased aggregation pheromone production (Cerezke 1995, Erbilgin et al. 2014). Jack pine has three times more α-pinene content than lodgepole pine (Clark et al. 2014), which is the precursor to the female-produced aggregation pheromone *trans*-verbenol. Beetles that colonize jack pine produce more pheromone than those that colonize lodgepole pine (Erbilgin et al. 2014)

which leads to greater attack densities (Burke and Carroll 2016). Indeed, when natural populations of MPB are given lodgepole and jack pine bolts to colonize, jack pine bolts are attacked at the highest density (Cerezke 1995). Future lab studies on host preference could be conducted in a wind tunnel in which both visual and olfactory cues can be presented in an arena with more realistic air flow. Wind tunnel bioassays with bark beetles are notoriously difficult (Choudhury and Kennedy 1980, Akers and Wood 1989) and would also not prevent the influence of pheromone production on subsequent responders after beetles begin to colonize bolts.

In the olfactometer bioassays, a single beetle was presented with cues from both host species, which emulates the host response of pioneer beetles, as there is no additional pheromone cue presented. The MPB tested in the olfactometer bioassays also did not exhibit a host preference for either lodgepole or jack pine phloem volatiles but females from lodgepole pine responded more to phloem volatiles in general. Subsequent olfactometer assays in our lab (Petro et al. unpublished data) have revealed that beetles with reduced fat stores after flight exercise respond mor strongly to host volatiles. The beetles in Petro et al. emerged from naturally mass attacked bolts collected in the field. Although the response beetles in the olfactometer bioassay in this study did not experience an exercise period, yet strongly responded to phloem volatiles, they emerged from bolts that were artificially infested in the lab. In a different experiment (data not reported in this thesis), I found MPB that emerged from lodgepole pine bolts that were infested in the lab weighed less than MPB that were collected from bolts of naturally mass attacked lodgepole pine. Fresh weight correlates with fat content (Evenden et al. 2014) so it is possible that the beetles from lab-infested bolts in this study had lower relative fat content and so were responsive to host volatiles without exercise. Data on the lipid content of the beetles after the olfactometer assay could be included in a future model for time spent near host volatiles to see if it affects, or has an interaction with, which sections of the arena the beetles spend time in.

Although the no-choice experiments showed that beetles that the offspring of beetles that emerged from lodgepole pine and were introduced to jack pine had a lower residual condition compared to beetles from lodgepole pine that did not switch hosts, beetles that emerged from jack pine bolts in this study appeared to be repelled by host volatiles and avoided the air plumes with lodgepole and jack pine phloem volatiles. Since beetles that emerge from lodgepole pine respond more strongly to host volatiles when they have a lower residual condition due to reduced fat stores, this demonstrates a marked difference in behavioural response to host cues by beetles that emerge from jack pine. The difference in

response of beetles that emerged from lodgepole, and jack pines could be explained by the difference in host quality which can affect MPB response to host cues. Mountain pine beetles in the endemic population state in lodgepole pine forests prefer to colonize small diameter, suppressed or defensively compromised trees that often have thin phloem (Carroll et al. 2006a, Boone et al. 2011). When MPB are introduced to bolts that simulate endemic population density condition, their offspring display strong natal host effects and behave differently than offspring of MPB that emerge from an epidemic population density condition (Burke and Carroll 2017). The offspring of a single generation of females that are introduced to bolts in an endemic condition avoid high concentrations of pine defense chemicals whereas beetles that emerge from mass attacked bolts (epidemic condition) readily accept high defense chemical environments (Burke and Carroll 2017). Since jack pine has fewer chemical defenses (Clark et al. 2014, Arango-Velez et al. 2016, Lusebrink et al. 2016) and thinner phloem on average than lodgepole pine (Lusebrink et al. 2016, Rosenberger et al. 2017b), it could represent a rearing condition that is similar to a typical endemic lodgepole pine host. The phloem present in the olfactometer may have produced a signal that was representative of a well-defended host and MPB that emerged from jack pine ignored sections with host volatiles due to natal host effects. Testing the response of beetles that are reared in jack pine to different concentrations of host defense compounds in the olfactometer would help determine if the observed lack of attraction is the result of beetle-perceived host defense capacity.

The data from the static air arena bioassay could supply further support that the difference in response to host cues between beetles that emerge from lodgepole, and jack pine is due to a shift in behaviour caused by natal host quality. All bolts in the static air arena were large-diameter bolts, so while the jack pine bolts likely had lower chemical defenses compared to the lodgepole pine bolts in the arena, the visual cues of large diameter trees may also have repelled beetles if they display behaviour typical of the endemic population state condition. When selecting hosts appropriate for the endemic population state, MPB may use a host 'gestalt' that includes visual, olfactory, and gustatory cues. Performing a similar arena experiment with small and large diameter bolts with MPB that emerge from jack pine, would help determine if visual cues are also important in avoidance of potentially well defended hosts. The effect of poor condition of beetles that emerge from jack pine could also have been observed in the probability of acceptance of beetles that emerged from jack pine and entered jack pine as a reproductive host. Beetles that emerge from jack pine in poor condition may be desperate and enter hosts more readily (Byers 1999, Latty and Reid 2010, Jones et al. 2020). When beetles that emerged

from jack pine were introduced to jack pine reproductive hosts, there were no visual cues, there was no choice to move away from olfactory cues, and gustatory cues were easily accessible. The trend that beetles with jack pine as a natal host more readily accepted jack pine as a reproductive host could indicate there is evidence in this system for natal host effects related to natal host quality. If natal host quality influences MPB host acceptance behaviour (Burke and Carroll 2017), gustatory cues are likely important in assessing the suitability of the reproductive host. If MPB is to adapt and optimize reproduction within jack pine trees in the boreal forest, colonization preference for reproduction in jack pine following larval development in jack pine, could result in reproductive isolation, evolution of a host form (Funk 2012) or even host race (Mopper 1996).

There is a cost to MPB offspring quality in the host switch from lodgepole to jack pine which potentially presents a stumbling block to its spread across the boreal forest (Safranyik 1968). If reproductive host preference is based on offspring performance in MPB, lodgepole pine should be the preferred host of beetles that develop in lodgepole pine but there was no preference for lodgepole pine in either the static air or olfactometer bioassays. If there is a significant fitness cost in reduced offspring quality to MPB that that colonize jack pine (Raffa et al. 2016), there could be selection for preference for lodgepole pine over time in the areas where the trees are within the dispersal distance of MPB. Populations of MPB from lodgepole pine forests that spill over into jack pine forests would have low-quality offspring which could have lower fecundity as there are reductions in the number of offspring produced in beetles that have low energy stores (Wijerathna et al. 2019). These populations could burn out quickly due to low fecundity of low-quality offspring, so MPB need a niche to persist in the low-density endemic population state. Since MPB that emerge from jack pine readily accept jack pine host material they may be primed to prefer hosts that are more typical of the endemic niche (Burke and Carroll 2017). Populations of MPB that enter jack pine forests at low density have limited success in suppressed hosts typical of the endemic niche due to high interspecific competition with other tree-infesting insects and predation by woodpeckers (Pokorny 2021). Preference for suppressed hosts within jack pine could then further limit MPB success in this new forest ecosystem. It appears MPB host selection and preference in lodgepole and jack pine is more complicated than simple natal host species fidelity as how Hopkins' host selection principle has been interpreted or even the preference-performance hypothesis. Experience in the natal host can affect subsequent host selection through learning (Davis and Stamps 2004, Little et al. 2019), and the developmental stage at which natal host conditioning takes place can be important in predicting whether natal host experience affects subsequent host preference (Petit et al.

2017). Natal host fidelity can also be dependent on the nutritional quality of the natal host (Lhomme et al. 2017) and MPB displays natal host quality context-dependent behaviour (Burke and Carroll 2017). This study highlights the likely importance of natal host quality rather than natal host species as the driver of offspring host selection behaviour, but similar experiments that manipulate preference and performance within lodgepole and jack pine hosts of different quality would disentangle species effects from quality. Mountain pine beetle population dynamics can change quickly in response to perturbations in ecosystems and changes in host susceptibility (Cooke and Carroll 2017). Continuing to monitor and investigate MPB populations in its expanded range into the boreal forest in Canada, will help to determine if MPB can adapt to find a niche in the boreal forest

5.5. Tables and Figures

Table 5.1: Number of unique bolts manipulated by natal host – reproductive host combinations, L = lodgepole pine, J = jack pine. *Total Bolts* are the total number of bolts per combination that (*Total Pairs*) were introduced across, with 2–5 pairs per bolt. *N Bolts* are the total number of bolts that had at least one offspring emerge, and *N Pairs* are the number of pairs that were introduced across *N Bolts* per combination. Next columns are the number of pairs across the *N Bolts* that produced at least one offspring that produced a parental gallery, at least one larval gallery, and at least one adult offspring.

Natal – Reproductive	Total Bolts (Total Pairs)	N Bolts (N Pairs)	Pairs across N bolts that produced:		
			A parental gallery	A larval gallery	An adult offspring
L–L	6 (15)	4 (11)	8	7	7
L–J	4 (11)	3 (9)	6	5	4
J–J	4 (14)	4 (14)	13	11	7
J–L	7 (18)	5 (16)	12	12	10

Table 5.2: The response variables, random, and fixed effects for each statistical model reported in this chapter. Test statistics, degrees of freedom, and p-values for each fixed effect are also reported. Interactions between fixed effects are denoted by a colon between the factor names.

Type	Response	Random	Fixed	Test stat	df	p-value
GLM – binomial	Constructed a parental gallery	Bolt	Natal host	$\chi^2 = 0.34$	1	0.56
			Reproductive host	$\chi^2 = 0.06$	1	0.81
			Natal : Reproductive	$\chi^2 = 2.06$	1	0.15
GLM – binomial	Produced at least one larva	Bolt	Natal host	$\chi^2 = 1.54$	1	0.21
			Reproductive host	$\chi^2 = 0.01$	1	0.93
			Natal : Reproductive	$\chi^2 = 0.18$	1	0.67
GLM – binomial	Produced at least one offspring	Bolt	Natal host	$\chi^2 = 0.01$	1	0.92
			Reproductive host	$\chi^2 = 1.05$	1	0.31
			Natal : Reproductive	$\chi^2 = 0.05$	1	0.83
GLM – binomial	Produced larva | parental gallery	Bolt	Natal host	$\chi^2 = 0.01$	1	0.94
			Reproductive host	$\chi^2 = 0.07$	1	0.79
			Natal : Reproductive	$\chi^2 = 0.0$	1	0.98
GLM – binomial	Produced adult offspring | larva	Bolt	Natal host	$\chi^2 = 0.32$	1	0.57
			Reproductive host	$\chi^2 = 0.80$	1	0.37
			Natal : Reproductive	$\chi^2 = 0.0$	1	0.99
GLM – zi poisson	Number of offspring per female	Bolt	Natal host	$\chi^2 = 0.26$	1	0.61
			Reproductive host	$\chi^2 = 1.63$	1	0.20
			Natal : Reproductive	$\chi^2 = 0.41$	1	0.52
LM	Body size (2018)	Pair/Bolt	Natal host	$\chi^2 = 0.02$	1	0.89
			Reproductive host	$\chi^2 = 0.0$	1	0.97
			Natal : Reproductive	$\chi^2 = 0.95$	1	0.95
			Sex	$\chi^2 = 9.90$	1	**0.002**
LM	Body size (2019)	Pair/Bolt	Natal host	$\chi^2 = 0.72$	1	0.40
			Reproductive host	$\chi^2 = 3.74$	1	0.053

			Natal : Reproductive	$\chi^2 = 1.0$	1	0.32
			Sex	$\chi^2 = 11.76$	1	**0.001**
LM	Lipid content (2018)	Pair/Bolt	Natal host	$\chi^2 = 0.14$	1	0.71
			Reproductive host	$\chi^2 = 1.92$	1	0.17
			Natal : Reproductive	$\chi^2 = 1.25$	1	0.26
			Sex	$\chi^2 = 7.56$	1	**0.006**
LM	Lipid content (2019)	Pair/Bolt	Natal host	$\chi^2 = 0.0$	1	0.97
			Reproductive host	$\chi^2 = 1.31$	1	0.25
			Natal : Reproductive	$\chi^2 = 1.15$	1	0.28
			Sex	$\chi^2 = 3.33$	1	0.07
LM	Relative lipid content (2018)	Pair/Bolt	Natal host	$\chi^2 = 1.78$	1	0.27
			Reproductive host	$\chi^2 = 2.88$	1	0.09
			Natal : Reproductive	$\chi^2 = 2.43$	1	0.12
LM	Relative lipid content (2019)	Pair/Bolt	Natal host	$\chi^2 = 0.29$	1	0.60
			Reproductive host	$\chi^2 = 1.05$	1	0.31
			Natal : Reproductive	$\chi^2 = 1.21$	1	0.27
LM	Relative condition (2018)	Pair/Bolt	Natal host	$\chi^2 = 0.55$	1	0.46
			Reproductive host	$\chi^2 = 4.29$	1	**0.04**
			Natal : Reproductive	$\chi^2 = 2.55$	1	0.11
LM	Relative condition (2019)	Pair/Bolt	Natal host	$\chi^2 = 0.49$	1	0.48
			Reproductive host	$\chi^2 = 0.58$	1	0.58
			Natal : Reproductive	$\chi^2 = 1.03$	1	0.31
ANOVA	Number on top of, under, and inside bolt	n/a	Natal host	F = 16.45	1, 14	**0.001**
			Choice	F = 0.41	1, 14	0.53
			Natal host : Choice	F = 0.11	1, 14	0.75
ANOVA	Number entered bolt	n/a	Natal host	F = 4.00	1, 14	0.07
			Choice	F = 0.37	1, 14	0.55
			Natal host : Choice	F = 0.04	1, 14	0.85
GLM – beta family	Prop time spent	n/a	Odour	$\chi^2 = 3.10$	3	0.38

| GLM – beta family | Prop time spent | n/a | Odour | $\chi^2 = 54.54$ | 3 | **< 0.001** |

Table 5.3: Measures of success after pairs of beetles were inoculated into pine bolts in four natal–reproductive host combinations: L = lodgepole pine, J = jack pine, first letter represents the natal host and second letter the reproductive host. Mean probabilities with lower confidence level (LCL) and upper confidence levels (UCL) are presented for each natal host/reproductive host combination of, after boring into the phloem, parental gallery construction, production of at least one larval gallery, and production of at least one adult offspring. The mean conditional probabilities are also presented, the probability of larval gallery production after successful parental gallery production (larval | parent), and the probability of adult offspring production if larval galleries were present (adult | larval). There were no differences in mean probabilities across combination groups. Means with bolded text are significantly greater than 0.5 (α = 0.05).

Natal	Reproductive	Parent gallery	Larval gallery	Adult Offspring	Larval | Parent	Adult | Larval
L	L	**0.82 (0.50–0.95)***	0.64 (0.34–0.86)	0.64 (0.34–0.86)	0.78 (0.42–0.94)	**1.00**
L	J	0.67 (0.33–0.89)	0.56 (0.25–0.82)	0.44 (0.18–0.75)	0.83 (0.37–0.98)	0.80 (0.31–0.97)
J	J	**0.93 (0.63–0.99)****	**0.79 (0.51–0.93)***	0.50 (0.26–0.74)	**0.85 (0.55–0.98)****	0.64 (0.34–0.86)
J	L	0.75 (0.49–9.03)	0.75 (0.49–0.90)	0.62 (0.38–0.82)	**1.00**	**0.83 (0.52–0.97)***

* Estimated probability is different from 0.5 p $\leq$ 0.05
** Estimated probability is different from 0.5 p $\leq$ 0.01
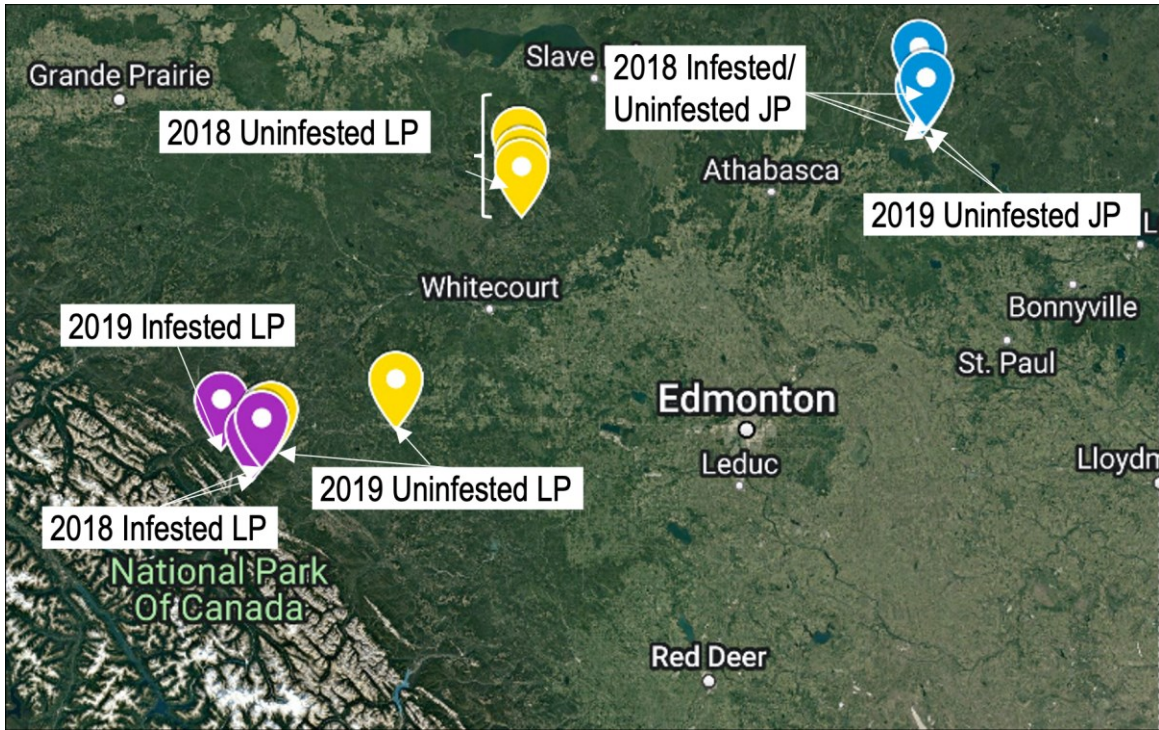
Figure 5.1: Map of the sampling locations of uninfested and infested materials used in Chapter 5 experiments.
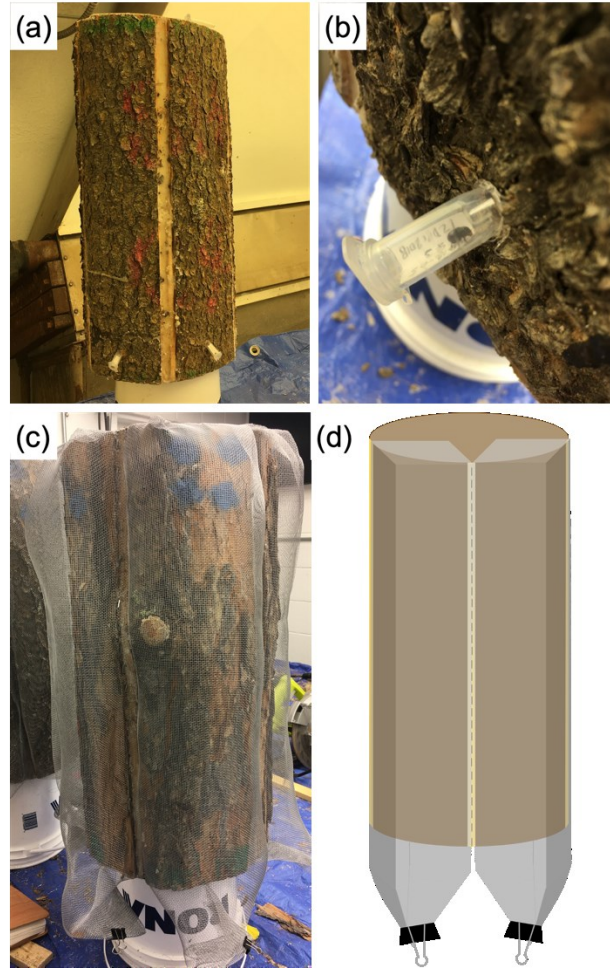
Figure 5.2: Images of methods for no-choice experiments. (a) Vertical scoring on bolts filled with wax to make separate sections. (b) MPB female in introduction tube attached to the base of an inoculation bolt. (c) Wire screening constructed around individual bolt sections. Screening was stapled to the wood down the score lines on either side of the section to form a tube. The top of the tube was folded down to form the top of the cage and stapled into the top of the bolt. The bottom of the tube was seamed along the cut edges of the screening and the opening in the base of the tube was folded over and kept closed with a large binder clip. (d) Line drawing of the caged sections.
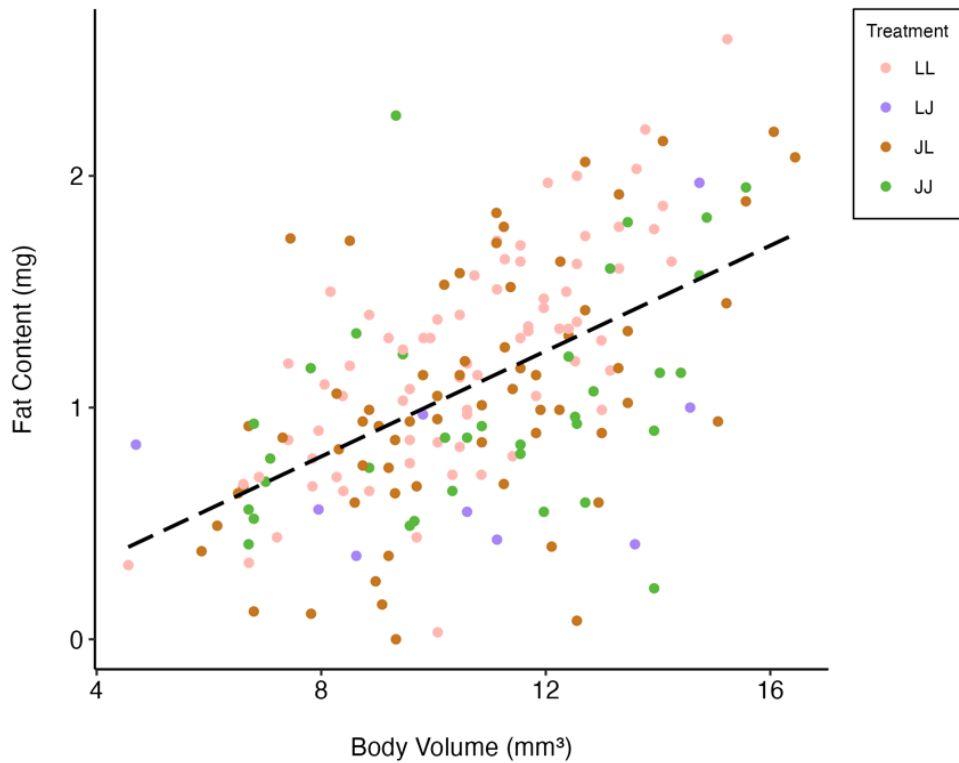
Figure 5.3: Linear regression of body volume and fat content, the residual of each point is the relative condition of that beetle within the dataset and represents the beetle's residual condition. Beetles that fall below the line (negative residuals) represent a worse condition and beetles above the line (positive residuals) represent a better condition than predicted by the linear model.
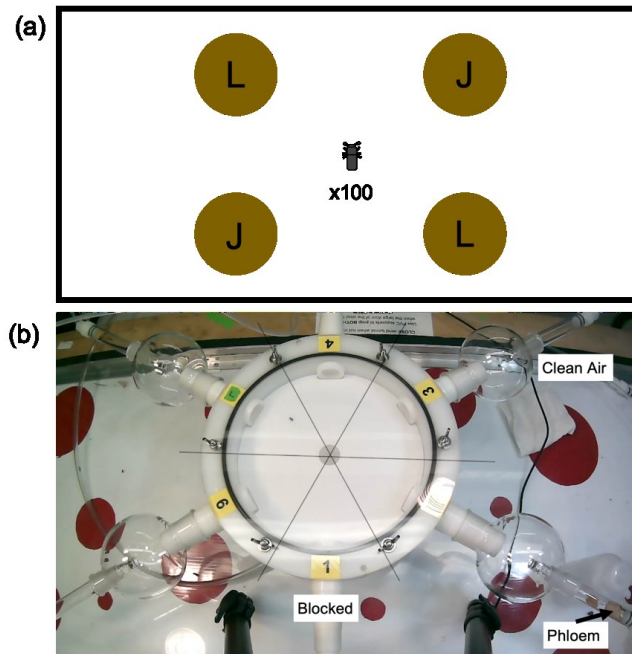
Figure 5.4: Images of methods for choice experiments. (a) Diagram of static air arena bioassay used in 2018. Arena dimensions: 1.7 x 0.85 x 0.9 m. Four bolts were placed in opposite corners of the arena in a square, two from lodgepole pine and two from jack pine. One hundred female MPB that emerged from bolts of either mass attacked lodgepole pine or jack pine. (b) Photo of modified six-way olfactometer used in 2019. Two arms (1 and 4) were blocked, two arms supplied clean air (3 and 6), and two arms contained host plant tissue, one with jack pine phloem (2) and one with lodgepole pine phloem (5).
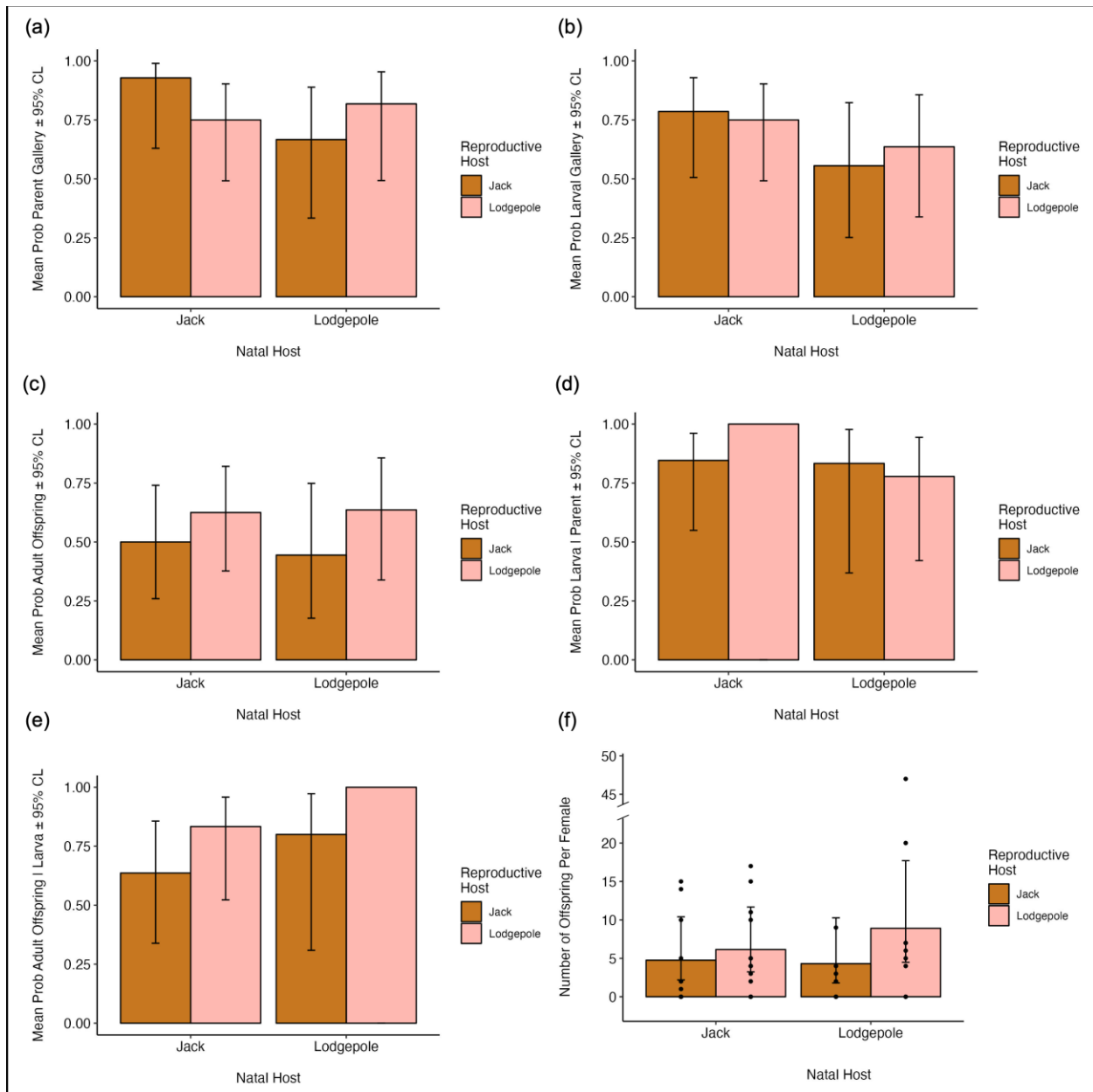
Figure 5.5: Marginal means (bars) and 95% confidence levels (CL) of the probability of (a) constructing a parental gallery after introduction, (b) producing at least one larval gallery, (c) producing at least one offspring, estimated by generalized linear mixed effects models with a binomial family. (d) Number of offspring per female natal and reproductive host combinations; points are raw data, bars represent marginal mean number of offspring per female for each natal-reproductive host group, error bars are 95% confidence levels (CL) of the marginal mean estimated by generalized linear mixed effects model with a zero-inflated Poisson family.
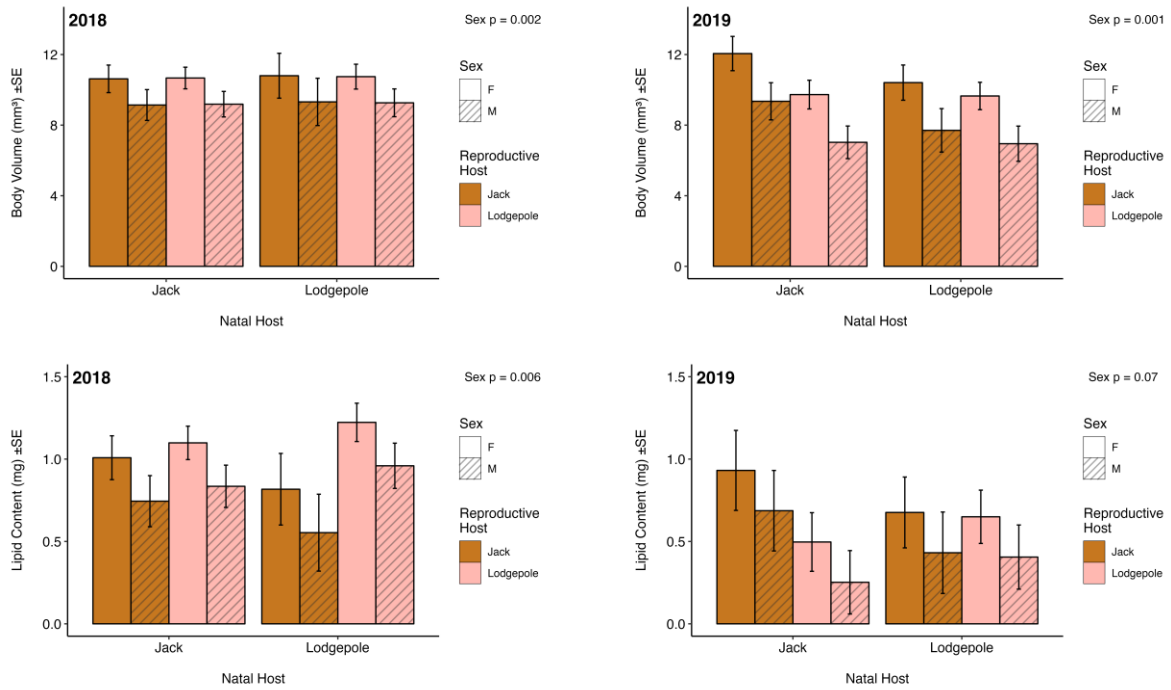
Figure 5.6: Effect of sex, natal and reproductive host species on offspring body size (volume) and lipid content in 2018 and 2019 no choice experiments. Bars representing males within the natal-reproductive host combinations are hatched. Sex was the only statistically significant factor, p-values for sex from ANOVA table of linear mixed effects models with a term for bolt fit as a random effect.
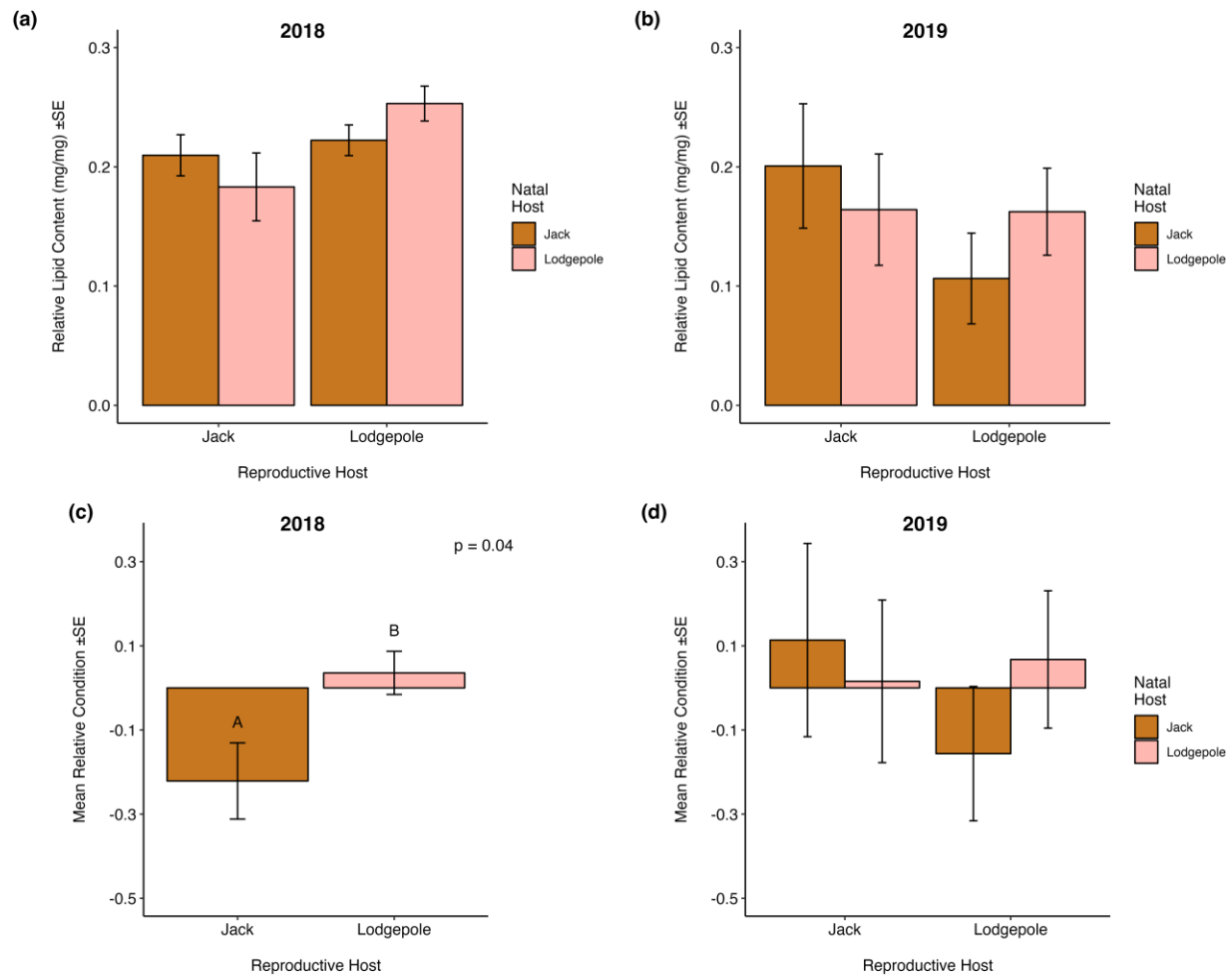
Figure 5.7: Results of natal and reproductive host species on offspring relative fat content (mg of fat per mg of dry mass) and residual condition in 2018 and 2019 no choice experiments. Bars are the estimated marginal mean of the relative fat content or condition of each natal-reproductive host combination and error bars are the standard error of the mean estimated by linear mixed effects models with a term for parent pair nested within bolt fit as a random factor. P-value within the plot is from an ANOVA table of the linear mixed effects model. Different letters above the bars within a plot indicate a significant difference between the means (Satterhwaite's approximation to degrees of freedom, 95% confidence level).

(a)

(b)
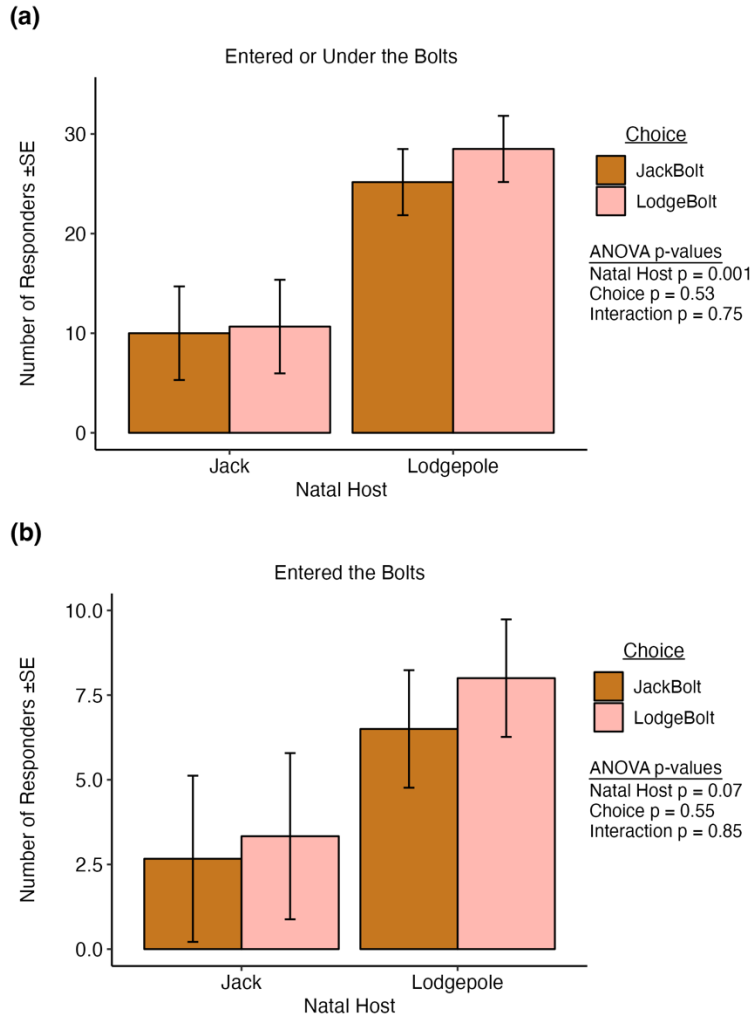
Figure 5.8: Results of static air arena choice experiment trials. Bars represent estimated marginal means of the number of female beetles that responded, and error bars represent the standard error of the mean. (a) The total number of females that were on top of, underneath, or had entered the bolt. (b) Includes only the total number of females that had bored into the bolt.
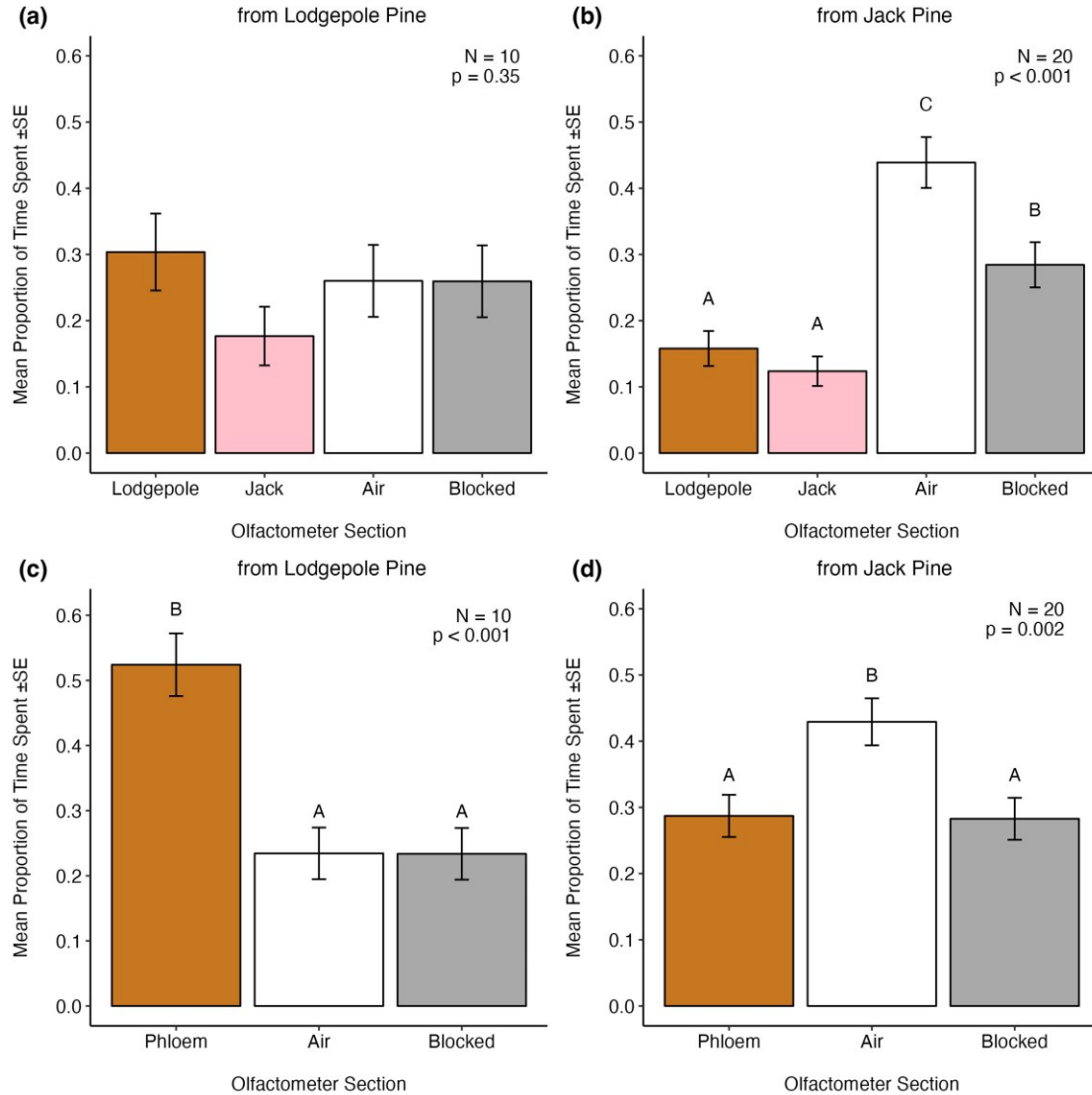
Figure 5.9: Results of olfactometer bioassay. (a) and (b), time spent in the sections that contained lodgepole or jack pine phloem, clean air, and blocked, divided by the total time spent in all four sections. (c) and (d), time spent in the two sections that contained phloem (one lodgepole pine and one jack pine), the two sections that had clean air, and the two blocked sections, divided by the total time spent in all three types of sections. Bars represent estimated marginal means and standard errors (estimated by beta GLM) of single females from lodgepole (a and c) or jack (b and d) pine. Bars with different letters are significantly different with Tukey p-value adjustment ($\alpha = 0.05$).

Chapter 6.

6.1. Conclusions

Mountain pine beetle is now likely a permanent part of Alberta's forests. Pine forests in Alberta differ from those in the historical range and MPB has both advantages and disadvantages in entering this new territory. My initial goals were to determine the mass attack thresholds in lodgepole and jack pine in Alberta. For lodgepole pine, the relationship between offspring production and attack density in 2015 and 2016 had completely opposite trends. Although climate change has increased the suitability of this region much of it is still in the low-moderate suitability range (Carroll et al. 2006b). If environmental conditions become less optimal for MPB, the habitat moves from low to very low suitability so small changes in temperature or precipitation could push them into the suboptimal. This is especially true with jack pine forests which are currently even less suitable than the western portions of the province. I was able to get MPB to mass attack mature jack pine trees and it is clear from the data that there is a lower threshold for mass attack, but none developed to the cold tolerant stage before winter. Mountain pine beetle outbreaks last ~10 years on average (Safranyik and Carroll 2006) and MPB arrived in west-central Alberta in 2006. When I began my experiments in 2016, it was likely at the tail end of the outbreak which could help explain the reduction in success of my mass attack manipulations in the field in lodgepole pine and the second year of the preference performance experiments. However, if the mass attack threshold for lodgepole pine is lower, given the evidence from the first year of the experiment and the estimated attack threshold for jack pine is correct, a lower population density of MPB on the landscape could result in shift from the endemic to incipient epidemic population state (Cooke and Carroll 2017).

Until the next outbreak, MPB will remain in low densities in lodgepole pine forests. The data I collected on phloem terpene content through the seasons after MPB attack showed that trees that are attacked at low densities could be more attractive to foraging beetles the next year, but most importantly, they have great quantities of pheromone synergists and precursors. Beetles foraging at low densities respond more to aggregation pheromone when more synergists are present (Klutsch et al. 2020). In a year where enough beetles attack a tree but don't win the battle, the next year that tree could be efficiently attacked, and since fewer attackers are needed to overcome host defenses, this further

changes the outbreak dynamics and could have resounding changes in outbreak cycles (Cooke and Carroll 2017).

The risk of MPB spread into jack pine forests remains but jack pine hosts as habitat for MPB have some drawbacks even though they are easier to colonize. Jack pine has thin phloem, and this seems to change the behaviour of MPB larvae. Beetles that emerged from the mass attack experiment in Chapter 3 were all like the lodgepole natal host to jack pine reproductive host combinations in the no choice experiment in Chapter 5. In Chapter 3, The lower mean number of offspring per female produced at variable attack densities relative to the historic range could have been because the parents of those beetles performed a host switch. If beetles that all emerged from jack pine could be introduced to living jack pine trees in the same way that allowed them to attack the tree at natural densities, it's possible that the average number of offspring per female across densities would be closer to that reported in the historic range.

Collectively, there is evidence that jack pine is a poor-quality host. Beetles that emerge from jack pine avoided bolts and host volatiles in choice bioassays, behaviour that is similar to beetles that emerge from a simulated endemic population state (Burke and Carroll 2017). If the beetles in endemic simulations are changing their behaviour in response to the phloem thickness of their natal host, then this could be why beetles that emerge from jack pine avoid strong host cues and beetles from lodgepole pine do not. This lends more evidence that the evidence of crowding in the gallery characteristics in jack pine could also indicate a reduction in reproductive output. Larval behaviour is extremely difficult to record but while jack pine has a thin phloem layer, the bark layer is very thick and so perhaps larval behaviour in jack pine could be recorded and quantified to determine if there is larval mortality occurring when galleries fuse.

Insects that can host switch using ecological fitting are more likely to become pests (Petit et al. 2017). Although MPB was found to have successfully mass attacked jack pine in 2011 (Cullingham et al. 2011), MPB has not quickly invaded jack pine forests as was once feared (Nealis and Cooke 2008). Many attributes of MPB, jack and lodgepole pines, made the risk seem very high. Brood production of MPB that attack naïve lodgepole pines is consistently greater than that of pines in the historic range (Cudmore et al. 2010). Like naïve lodgepole pines, jack pine also has fewer constitutive and induced defenses in response to simulated MPB attack, so a greater number of offspring per female across attack densities would be expected. This was not the case; however, it is extremely difficult

to compare one year of data in jack pine with 40 years' worth of experiments in lodgepole pine.

It will be extremely interesting to see how MPB populations continue to behave in Alberta. If there is a decrease in outbreak interval in the expanded range, it could be because of a reduced mass attack threshold in naïve pines, particularly if weather in the years previous have been hot and dry which lower plant defensive capacity. If MPB manage to enter jack pine forests in enough numbers to mass attack a tree, their offspring will behave differently which could either help them establish a niche or could make jack pine a dead-end host. Forests in Alberta are projected to continue to increase in suitability for MPB (Carroll et al. 2006b), so the thresholds that might be limiting MPB now could move in the future. There are so many possibilities and perhaps the only thing we can do is continue to hang out in the forest and count beetles.

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Appendix A. Supplementary Materials for Chapter 3

Table A 1: Summary of tree characteristics by site and year. DBH is diameter at 1.3 m on the tree bole.

Year	Location	Species	Site	N trees	Mean ± sd DBH (cm)	Mean ± sd phloem thickness (mm)
2017	Grande Prairie	Lodgepole	n/a	15	25.13 ± 1.99	1.93 ± 0.26
2018	Lac La Biche	Jack	1	9	29.42 ± 2.39	1.39 ± 0.20
2018	Lac La Biche	Jack	2	9	28.88 ± 3.46	1.34 ± 0.32
2018	Lac La Biche	Jack	3	8	28.59 ± 2.61	1.48 ± 0.35
2018	Lac La Biche	Jack	All	26	28.98 ± 2.77	1.40 ± 0.29

Table A 2: Individual terpenes detected in the number of trees and (percent of trees) at different stages of MPB attack in lodgepole and jack pines. PrA = "pre-attack", PoA = "post-attack", PoW = "post-overwintering".

Terpene	Number and (percent) of lodgepole pine trees			Number and (percent) of jack pine trees		
	PrA N = 14	PoA N = 14	PoW N = 14	PrA N = 26	PoA N = 26	PoW N = 8
2-Carene	0 (0)	0 (0)	12 (85.7)	1 (3.8)	2 (7.7)	1 (12.5)
3-Carene	14 (100)	14 (100)	14 (100)	15 (57.7)	14 (53.8)	7 (87.5)
α-Caryophyllene	5 (35.7)	3 (21.4)	7 (50)	1 (3.8)	0 (0)	3 (37.5)
α-Phellandrene	8 (57.1)	11 (78.6)	13 (92.9)	1 (3.8)	1 (3.8)	2 (25)
α-Pinene	14 (100)	14 (100)	14 (100)	26 (100)	26 (100)	8 (100)
α-Terpinene	1 (7.1)	1 (7.1)	4 (28.6)	2 (7.7)	2 (7.7)	2 (25)
α-Terpineol	8 (57.1)	8 (57.1)	6 (42.9)	2 (7.7)	3 (11.5)	4 (50)
α-Thujone	2 (14.3)	1 (7.1)	2 (14.3)	3 (11.5)	0 (0)	1 (12.5)
β-Pinene	14 (100)	14 (100)	14 (100)	19 (73.1)	18 (69.2)	8 (100)
β-Phellandrene	14 (100)	14 (100)	14 (100)	14 (53.8)	16 (61.5)	7 (87.5)
Borneol	1 (7.1)	3 (21.4)	6 (42.9)	0 (0)	1 (3.8)	2 (25)
Bornyl-acetate	7 (50)	9 (64.3)	6 (42.9)	4 (15.4)	4 (15.4)	3 (37.5)
Camphene	14 (100)	14 (100)	13 (92.9)	9 (34.6)	10 (38.5)	6 (75)
Camphor	5 (35.7)	4 (28.6)	6 (42.9)	0 (0)	0 (0)	0 (0)
γ-Terpinene	3 (21.4)	3 (21.4)	5 (35.7)	0 (0)	1 (3.8)	3 (37.5)
Linalool	1 (7.1)	3 (21.4)	5 (35.7)	4 (15.4)	5 (19.2)	2 (25)
Myrcene	14 (100)	13 (92.9)	14 (100)	19 (73.1)	19 (73.1)	7 (87.5)
Ocimene	0 (0)	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)
p-Cymene	12 (85.7)	12 (85.7)	11 (78.6)	0 (0)	0 (0)	2 (25)
Pulegone	0 (0)	0 (0)	0 (0)	1 (3.8)	0 (0)	0 (0)
Limonene	14 (100)	13 (92.9)	14 (100)	20 (76.9)	17 (65.4)	6 (75)
Sabinene	10 (71.4)	12 (85.7)	12 (85.7)	3 (11.5)	6 (23.1)	5 (62.5)

Terpinolene   11 (78.6)   13 (92.9)   12 (85.7)   7 (26.9)   9 (34.6)   6 (75)

Table A 3: Mean (SE) percent of individual terpenes compared to total terpene content at different stages of attack in lodgepole pine, only terpenes that comprised >1% of total terpene content are shown.

Terpene	PreAttack N = 14	PostAttack N = 14	PostWinter N= 14
3-Carene	14.63 (2.96)	14.05 (2.66)	14.31 (2.66)
α-Pinene	18.38 (3.79)	17.79 (3.60)	20.02 (3.91)
β-Pinene	8.44 (1.66)	9.24 (1.94)	10.81 (2.82)
β-Phellandrene	44.01 (4.62)	45.38 (4.76)	41.70 (5.02)
Bornyl-acetate	1.88 (1.09)	0.91 (0.36)	0.36 (0.19)
Camphene	1.79 (0.54)	1.43 (0.32)	1.03 (0.20)
Myrcene	1.78 (0.11)	1.78 (0.17)	1.85 (0.14)
p-Cymene	2.10 (0.76)	1.52 (0.60)	1.42 (0.54)
Limonene	2.78 (0.53)	2.58 (0.55)	2.57 (0.40)
Sabinene	0.78 (0.21)	0.91 (0.15)	1.10 (0.21)
Terpinolene	1.14 (0.26)	1.52 (0.28)	1.34 (0.24)

Table A 4: Mean (SE) percent of individual terpenes compared to total terpene content at different stages of attack in jack pine, only terpenes that comprised >1% of total terpene content are shown. Means followed by different letters within the same row indicate significant differences (Tukey HSD, $p < 0.05$).

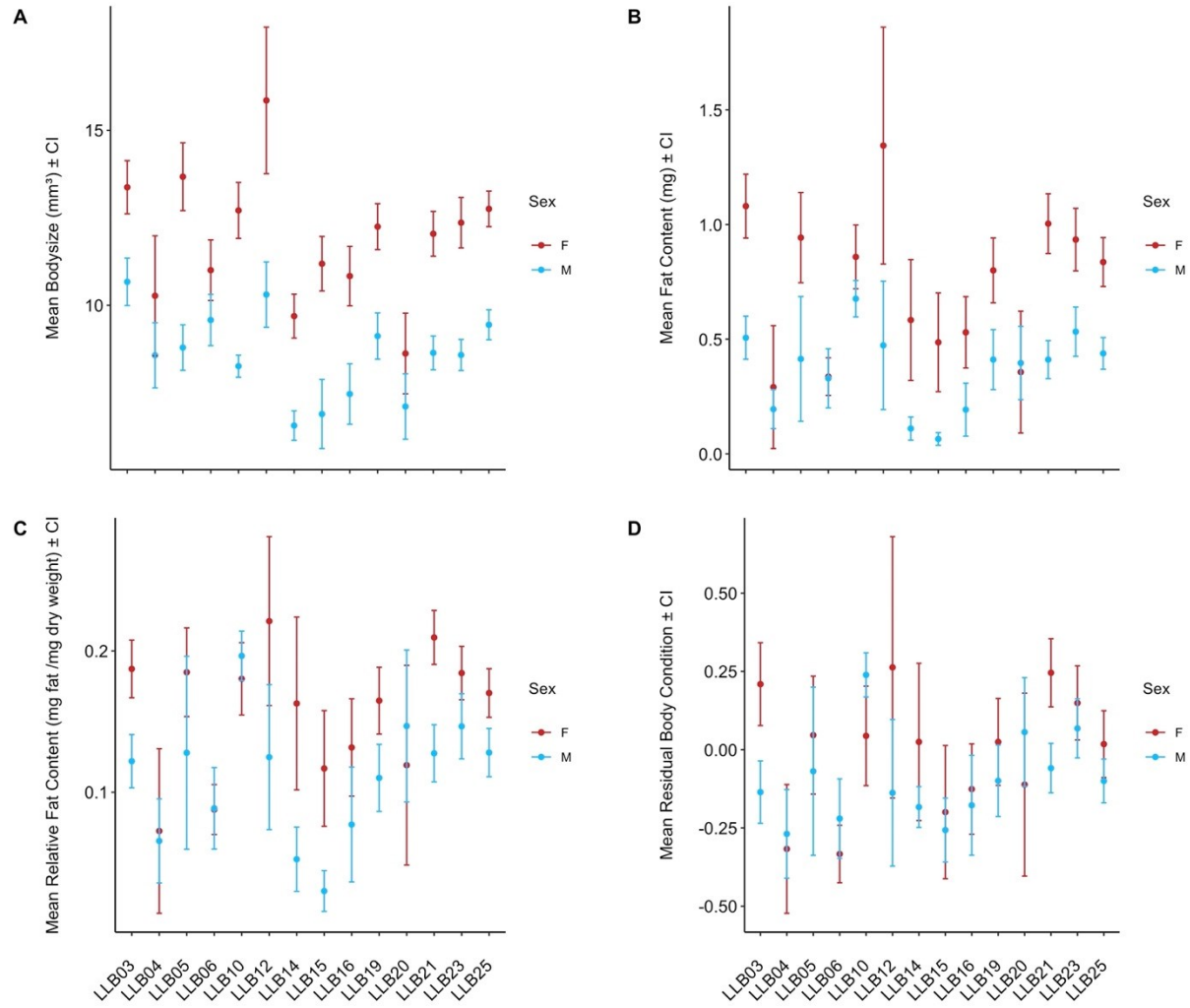| Terpene	PreAttack N = 26	PostAttack N = 26	PostWinter N = 8
3-Carene	8.31 (2.00)	8.88 (2.10)	10.63 (4.65)
α-Pinene	59.61 (3.29)	68.31 (3.32)	66.67 (4.33)
β-Pinene	3.83 (0.70) a	5.91 (1.00) ab	9.24 (1.15) b
β-Phellandrene	5.54 (1.62)	3.39 (1.20)	1.64 (0.75)
Camphene	2.41 (1.99)	0.54 (0.17)	0.59 (0.15)
Linalool	1.39 (0.99)	0.85 (0.76)	0.02 (0.02)
Myrcene	3.43 (0.68)	1.90 (0.35)	2.23 (0.63)
Limonene	11.75 (2.41)	5.17 (1.54)	5.66 (3.35)
Terpinolene	0.70 (0.35)	0.96 (0.39)	1.26 (0.58)

Figure A 1: Means and 95% confidence intervals per bolt for male and female body measurements; (A) body size (volume mm^3), (B) fat content (mg), (C) relative fat content (mg fat /mg dry weight), (D) residual body condition.
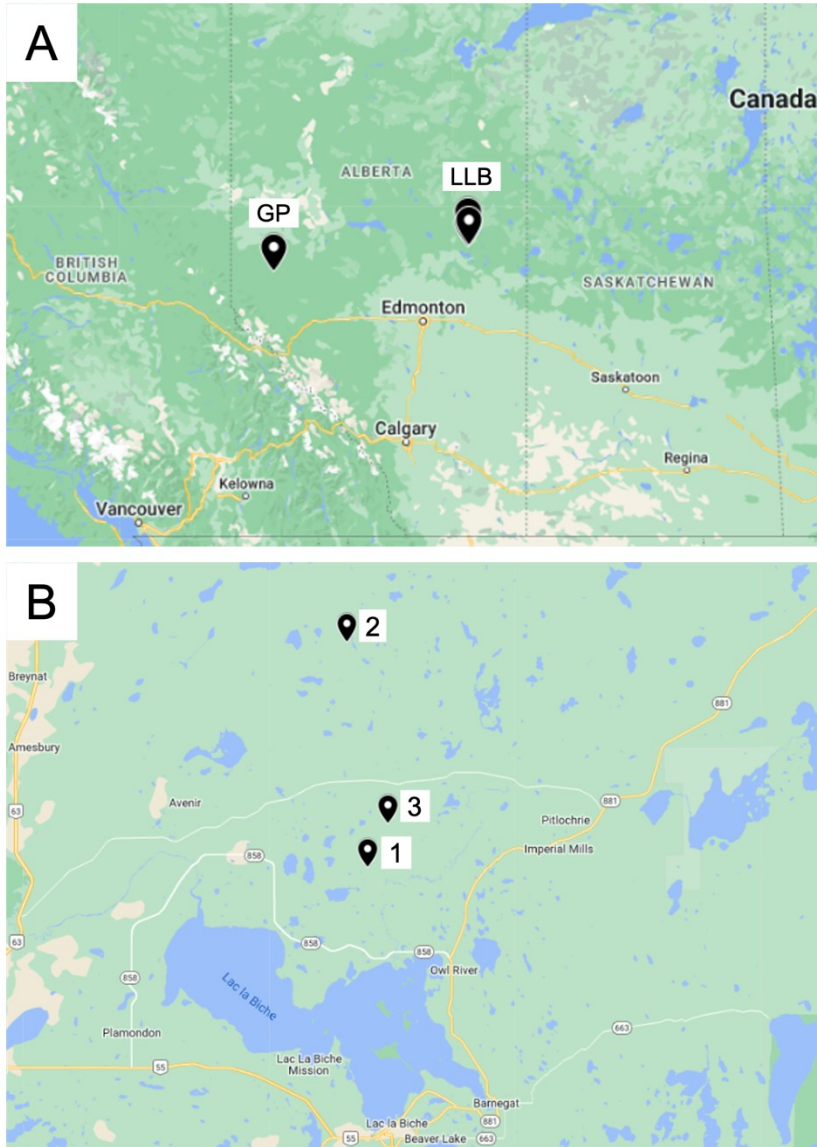
Figure A 2: Map of field study locations. Grande Prairie (GP) experiment was performed in 2017, and Lac La Biche (LLB) study was performed in 2018. B: Three experiment sites north of Lac La Biche selected in 2018, sites are numbered in the order they were manipulated.
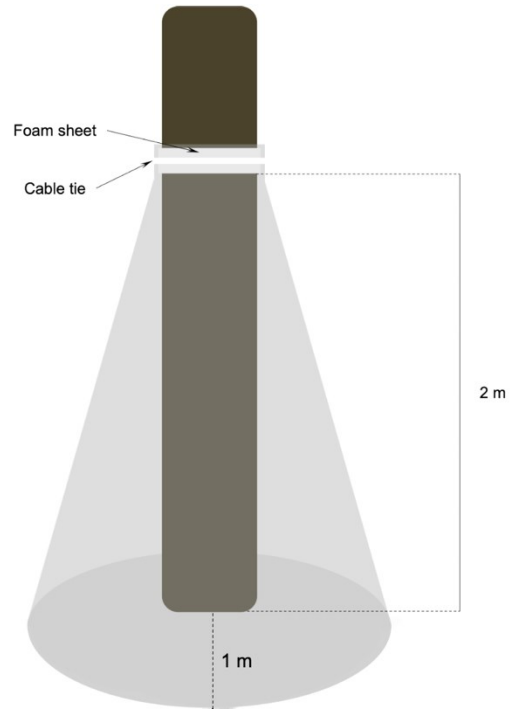
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Figure A 3: Conical wire mesh cage surrounding mature jack pine tree under which MPB were released. A layer of packing foam was placed between the cage top and the bark and cages were secured just above 2 m on the tree bole with 5 staples into the bark and a plastic cable tie, leaving 2 m of the tree exposed under the cage. The base of the cage was buried in the sand ~1 m away from the bole surrounding the tree and completely sealed after beetles were placed within.