

**CONSEQUENCES OF CLIMATE-INDUCED RANGE EXPANSION OF A NATIVE
INVASIVE HERBIVORE IN WESTERN CANADA.**

by

Jordan Lewis Burke

B.Sc., The University of Georgia, 2009

M.Sc., The University of Georgia, 2011

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Abstract

Global climate change is affecting species from all taxonomic groups. Their response to warming and precipitation trends is highly variable, and will likely lead to changes in ecosystem composition that may affect resilience and stability. Eruptive forest insects compete directly with humans for forest resources, and the distribution and magnitude of epidemics is increasing. Here, I describe a series of manipulative experiments using the mountain pine beetle (*Dendroctonus ponderosae*) system to examine biological and life history traits of both the beetle and hosts as they pertain to eruptive population dynamics and range expansion in a warming environment. Mountain pine beetle exhibits population phase-dependent host selection behavior, which I demonstrated is informed directly by monoterpene volatiles in host resin, and reinforced by context-dependent maternal effects arising from parental experience. Recently, mountain pine beetles have experienced dramatic range expansion into novel montane and boreal forests of western Canada. Depressed defensive capability of trees in novel forests may increase generation survival of beetle populations, potentially exacerbating outbreaks in novel systems, and enhance positive feedbacks associated with epidemic phases. Of particular concern is the tendency for elevated levels of α -pinene, an aggregation pheromone precursor, in the defensive resin of trees in novel habitats. I demonstrated that the qualitative content of monoterpenes, specifically the relative concentration of (+)- and (-)- α -pinene, influences the ability of the beetle to aggregate and mass-attack healthy hosts, and may exacerbate outbreaks on a landscape scale, thereby potentially increasing the rate of spread of beetles in novel forests. Finally, I demonstrated that the close association of mountain pine beetle with the defensive expression of hosts has led to selection in native forests for enhanced defenses, and a lack of coevolution in novel forests has likely led to the increased susceptibility to mortality. The present study has advanced our knowledge of eruptive insect dynamics and the response of these economically important species to climate change. This thesis contributes to the body of knowledge pertaining to ecological theory of population dynamics and invasion biology, and identifies areas for further study and effort to mitigate the biological consequences of anthropogenic modification to the environment.

Preface

The following work was conducted in collaboration with my supervisor, Dr. Allan Carroll, who provided guidance on the research problems and concepts in chapters 2-4, and contributed to experimental design and manuscript editing. I developed the methods, collected, prepared and analyzed all data and completed the full write up on this dissertation and all current and forthcoming publications from it. Valuable contributions were made by Drs. Joerg Bohlmann and Murray Isman in both experimentation and manuscript preparation, as members of my advisory committee. Lina Madilao provided guidance and assistance with all chemical analyses. Dr. Natalie Sopinka, April Burke, Garrett Burke, and Tonya Ramey provided editorial support during preparation of the manuscript. Chapter 3 has been published in *Forest Ecology and Management* (<http://www.journals.elsevier.com/forest-ecology-and-management>) in collaboration with Dr. Allan Carroll (Burke and Carroll, 2016).

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1 General introduction

1.1 Forest insects in a changing climate

Climate change due to anthropogenic modification of the environment has caused widespread impact on diverse ecosystems (Easterling, 2000; Walther et al., 2002), including terrestrial (Parmesan et al., 2000; Pounds, 2001) and marine environments (Hoegh-Guldberg, 1999; Smith et al., 1999). There is now a large body of evidence indicating that all ecosystems across the globe are being affected by climate warming and shifting precipitation patterns (Parmesan, 2006; Parmesan and Yohe, 2003). Increasing temperatures often lead to range shifts in terrestrial plants (Aitken et al., 2008; Chen et al., 2011; Davis and Shaw, 2001; Kelly and Goulden, 2008) and terrestrial and marine animals (Carroll et al., 2004; Hickling, 2006; Parmesan et al., 1999; Perry et al., 2005). Warming will not affect all taxa in the same way (Parmesan, 2006), and asymmetrical distributional changes may affect biodiversity, ecosystem resilience, and stability (Cottingham and Carpenter, 1994; Larsen, 1995).

The fundamental complexity of natural ecosystems means accurate models intended to predict their response to perturbation are particularly difficult to produce (Aber and Federer, 1992; Berryman, 1992; Berryman et al., 2002), and so require detailed analyses of shifting trophic interactions in response to climate change in order to maintain relevancy. In forested ecosystems, the most important consequences of changing climates have been the expansion and increasing magnitude of biotic disturbances (Ayers and Lombardero, 2000, Dale et al., 2001; Weed et al., 2013). Forest ecosystems provide vital ecological and economic services, and most are being negatively affected due to increasing temperatures and disturbance (Bonan, 2008; McNulty and Aber, 2001). The distribution of tree species is predicted to change dramatically due to shifting temperatures and precipitation patterns (Aitken et al., 2008), and tree mortality in

western North America is increasing due to more severe biotic and abiotic disturbance patterns (H. Adams et al., 2009; Dale et al., 2001; van Mantgem et al., 2009). Abiotic stressors and biotic disturbances can also interact; for example, increasing incidence of drought can increase susceptibility of trees to disease or infestation by bark beetles (Adams et al., 2012; Kolb et al., 2016), and carbon deposition into the atmosphere due to post-mortality decomposition may compound warming through positive feedbacks between the atmosphere and biosphere (Adams et al., 2010, Kurz et al., 2008). Climate change impacts on forest ecosystems have been especially intense due to disturbance by herbivorous insects, which surpass fire and other factors in their influence (Dale et al., 2001; Raffa et al., 2015; Ramsfield et al., 2016).

Temperate coniferous and boreal forests are of particular interest, as they comprise the largest area of forested land on earth, contribute significantly to global net primary productivity (Melillo et al., 1993), and are subject to widespread disturbances that shape species diversity and distribution (Mattson and Addy, 1975). They are also expected to experience more significant temperature change than other forest systems, as warming will be greater at higher latitudes (IPCC, 2007). The coniferous forests of North America have witnessed unprecedented and widespread mortality events in recent years from forest insects, especially bark beetles (Bentz et al., 2010; Logan et al., 1995; Raffa et al., 2008; Ramsfield et al., 2016). Disturbance is a vital process in forest ecosystems, and therefore detailed investigation of the effects of increasing temperatures on the distribution and biology of disturbance agents is critical for the protection of the resource. While it is obvious that insect disturbance patterns are changing, the complexity of forest ecosystems presents a challenge when trying to uncover the proximate causes of change. What aspects of herbivore and host biology are most subject to perturbation, and how might these contribute to the observed increasing distribution and severity of herbivore impact?

1.2 Influence of temperature on forest insects

1.2.1 Direct effects of temperature on metabolism and range

Insects are highly sensitive to changes in temperature due to their high mobility and reproductive potential (Bernardo et al., 2007; Calosi et al., 2008), and increasing temperatures due to global warming trends have affected the physiology and distribution of many species (Frazier et al., 2006; Logan et al., 2003; Musolin, 2007). Insects are ectothermic, and may benefit from warming due to increased metabolic rate and quicker development time, which can enhance their reproductive potential (Bentz et al., 1991; Messenger, 1959). Decreased development time may lead to a shift in voltinism, which may lead to dramatic increases in population size over large areas (Bentz et al., 1991; Hansen et al., 2001; Jönsson et al., 2009). The results of increased temperature are not necessarily beneficial however, and may negatively affect the timing of diapause (Musolin, 2007) or egg production and oviposition (Carroll and Quiring, 1993). Variation in the response of insect species to changing temperatures is likely to lead to changes in species composition, potentially disrupting stable trophic interactions that have evolved over millions of years (Dawkins and Krebs, 1979; Deutsch et al., 2008).

Increasing temperatures, especially in temperate forests, seem to have the greatest impact on insects by altering their spatial distribution (Parmesan, 2006). At high elevation, increasing temperature may reduce the potential range of certain insects, as the high-temperature limits of cold-adapted species advance upward, while becoming favorable for more low-temperature limited species. (Wilson et al., 2005). Decreased development time in response to rising temperatures may also result in range contractions by disrupting phenological synchrony (Visser and Holleman, 2001) [Section 1.2.2]. Most often, however, increasing temperature has led to range expansions into previously unsuitable habitats (Carroll et al., 2004; Chen et al., 2011;

Hickling et al., 2006, Parmesan et al., 1999). In Holarctic regions, this often manifests as an expansion north into areas previously too cold for survival (Jönsson et al., 1999; Netherer and Schopf, 2010; Parmesan et al., 1999; Tr  n et al., 2007). Range expansion can lead to altered ecological interactions with host plants (Braschler and Hill, 2007; Cullingham et al., 2011), and even the potential for increased spread of detrimental human diseases by insect vectors (Rochlin et al., 2013).

1.2.2 Indirect effects of temperature through changes in developmental synchrony

Many herbivorous insects rely on developmental synchrony with host-plant phenology for access to specific tissues or tissue-stages, such as young, newly flushed foliage (Crawley and Akhteruzzaman, 1988). Insects are more physiologically sensitive to temperature than trees, and so increasing temperature potentially has greater impact on insect biology than on their hosts (Bale et al., 2002). Herbivores that synchronize their development with foliage production are closely tied to this trait, and locally adapt to variation among individual trees (Crawley and Akhteruzzaman, 1988; van Dongen et al., 1997), and even within individual trees (Carroll and Quiring, 1994). Warming can then result in asynchronous development with the specific host trait to which they have adapted (Powell and Logan, 2005). For example, warming temperatures in spring may advance egg hatch timing of folivorous Lepidoptera enough that they hatch well before budburst of their host, resulting in reduced food availability (Visser and Holleman, 2001). Warming may also lead to range shifts to colder climates where synchrony can be maintained, or a shift in the potential range in which a species can outbreak (Bale et al., 2002; Marciniak, 2015). This can affect other species that utilize the same host; for example, the northward advancement of outbreaks of the folivorous western spruce budworm (*Choristoneura occidentalis*) has the

potential to influence activity by the stem-feeding Douglas-fir beetle (*Dendroctonus pseudotsugae*), that takes advantage of the depressed defensive capacity of defoliated hosts (Marciniak, 2015).

Insects may also display synchrony of emergence to facilitate cooperative behaviors. Periodic cicadas (*Magicicada* spp.) are a dramatic example of synchronized life histories, and their mass emergence every 13 or 17 years aids in mate location and overwhelms predators (Koenig and Liebhold, 2005). Many species of tree-killing bark beetles (Kausrud et al., 2011) employ cooperative, pheromone-mediated mass-attack, and will synchronize development and emergence to maintain adaptive seasonality (i.e. coincidence with summer drought) and the potential for widespread cooperative behavior (Aukema et al., 2006; Berg et al., 2006; Økland and Bjørnstad, 2003). In Holarctic forests, rising temperatures are predicted to lead to northward advance in the area in which synchrony can be achieved, and may lead to northward advance in potential outbreak range for tree-killing bark beetles (Bentz et al., 2001; Bentz et al., 2010; Logan et al., 1995; Raffa et al., 2015; Safranyik et al., 2010). Tree-killing eruptive bark beetles' ability to synchronize and mass-attack trees is a critical aspect of their life history, and it is important to investigate how changing climates and shifting range will affect their ability to aggregate.

1.2.3 Expected outcomes of warming temperatures on forest insects

The combined effects of higher metabolic activity and potential shifts in voltinism or potential synchrony due to warming will likely lead to higher rates of herbivory overall in most forest ecosystems (Bentz et al., 2010; Dale et al., 2001; Weed et al., 2013). Fossil evidence suggests that warm periods are accompanied by increased incidence of insect herbivore damage,

as well as an increase in the diversity of damage types, which suggests increased herbivory rates and migration of species into novel habitats was common during warming periods (Currano et al., 2008; Wilf, 2008). It is reasonable then to expect the combination of direct and indirect effects of recent warming on insects to lead to elevated herbivory and invasions of novel ecosystems (Dukes et al., 2009; Ramsfield et al., 2016), where the potential for increased herbivory will be further enhanced by weak or absent co-evolutionary relationships (Herms and McCullough, 2014; Gandhi and Herms, 2010; Raffa et al., 2013). What's more, the asymmetry of effects, such that some insects will be better adapted to expand than others, means that normative trophic interactions even within contiguous populations of hosts are sure to change in the face of warming temperatures (Deutsch et al., 2008; Parmesan, 2006). A better understanding of the complex interacting effects of warming temperatures on forest insects is required, especially for those species that compete directly with humans for resources, such as eruptive tree-killing bark beetles.

1.3 Consequences of warming-induced range expansion by eruptive bark beetles

1.3.1 State-dependent life history strategies of eruptive bark beetles

In eruptive species, the constraints on low-density populations are very different than those on high-density populations (Eveleigh et al., 2007; Raffa et al., 2008). For example, during low-density phases, population growth may be restricted due to difficulties in mate or host location due to Allee effects (Liebhold and Tobin, 2008; Wallin and Raffa, 2004), whereas in high-density phases populations may be limited by resource availability due to intraspecific competition (Krebs, 1978; Guo et al., 1991). Diverse animal taxa exhibit density-dependent behaviors that facilitate survival in varying population phases; for example, individuals may be

more or less aggressive in foraging behaviors when resources are scarce or abundant due to varying conspecific density (Luttbeg and Sih, 2010; Sih et al., 2004a,b). Some insects exhibit dramatic density-dependent physiological changes in development time and even morphology; immatures of the desert locust (*Schistocerca gregaria*) undergo rapid developmental and morphological changes in periods of high density and resource abundance, directly in response to conspecific-associated stimuli, such as physical contact (Despland and Simpson, 2000; Hagele and Simpson, 2000; Roessingh et al, 1998; White, 1976). The effect of density-dependent resource availability may also manifest as a maternal effect, where ovipositional behavior and/or egg size depend on conspecific density and resource abundance, such that the quality of food resources for parentals affects offspring physiology (Ginzburg and Tanneyhill, 1994; Plaistow and Benton, 2009). Changes in biology as a function of density are an ultimate adaptation to varying environmental conditions (Guo et al., 1991; Sokolowski et al., 1997); however the proximate causes for shifts in strategy by highly influential species like eruptive bark beetles is not entirely clear.

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are important agents of change in temperate coniferous forests, especially in recent decades when certain species have experienced enormous population eruptions (Raffa et al., 2015). Of the ca. 7000 known species of bark beetles (Bright and Skidmore, 2002), only a few undergo true population eruptions. The normative state of these eruptive species is in the low-density endemic phase, during which they attack nutritionally suboptimal, vigor-impaired hosts (Coulson, 1979; S. Wood, 1982). Under these conditions, populations are constrained by negative feedbacks associated with interspecific competition (Safranyik and Carroll, 2006; Smith et al., 2011) and host defenses (Raffa and Berryman, 1982a; Raffa and Berryman, 1983a,b; Safranyik et al., 1975). However, periodically

populations will increase in size beyond certain thresholds such that cooperative mass-attack of otherwise healthy trees becomes possible (Berryman, 1972; Berryman et al., 1989). These hosts are abundant and highly nutritious, but are also well defended, and eruptive bark beetles must aggregate and attack quickly and at sufficient densities to overwhelm the defensive capacity of the tree and kill all or part of the stem, which is required for reproduction (Raffa and Berryman, 1983b). They are aided during this population phase by mutualistic fungi and bacteria, which are introduced into stem tissues where they disrupt water conductance and help detoxify defensive chemicals (A. Adams et al., 2009; Boone et al., 2013; Six, 2003; Six and Bentz, 2007).

Successful inhibition of defenses by micro-organisms is also density-dependent, as high rates of inoculation are required to elicit a systemic effect in the host (Raffa and Berryman, 1983b). If successfully initiated, the epidemic phase is characterized by positive feedbacks. Large, competitor-free trees with higher volume of thicker and more nutritious phloem act as net beetle sources, and subsequent generations are larger, thereby facilitating mass-attack of even more trees, ultimately leading to population eruptions (Amman and Cole, 1983; Amman and Pace, 1976; Berryman, 1982; Coulson, 1979; Raffa et al., 2008; Safranyik and Carroll, 2006).

Therefore, the niche occupied by endemic eruptive bark beetles is very different than that of epidemic beetles, and aspects of their life histories are expected to be population state-dependent (*sensu* McNamara and Houston, 1996). Population-phase transition is an adaptation to overcome constraints to growth that arise when confined to vigor-impaired hosts that are occupied by heterospecific competitors, and provides access to abundant high-quality resources (Raffa et al., 2008). However, the proximate causes that lead to state-dependent shifts in behavior in eruptive bark beetles are not clear, and manipulative experiments (e.g. Hagele and Simpson, 2000; Lester

et al., 2005; Roessingh et al., 1998; Wallin and Raffa, 2002, 2004) are required to tease apart the multiple interacting forces that may drive these transitions.

1.3.2 The influence of host defenses on state-dependent life histories and cooperative behavior of bark beetles

The resistance of a stand of trees to eruptive bark beetles is determined by the ability of trees to defend against attack (Boone et al., 2011; Christiansen et al., 1987; Raffa and Berryman, 1982b). Trees defend against attack by subcortical insects through multiple defensive systems. As beetles penetrate the bark they sever constitutive resin ducts that exude toxic resin that physically and chemically repels invaders (Berryman, 1972; Shrimpton and Whitney, 1968). If the beetles successfully penetrate and enter the phloem tissue, a highly complex induced response is initiated during which toxic secondary resin is produced, eventually leading to a necrotic lesion intended to isolate invaders from healthy tissues (Franceschi et al., 2005; Shrimpton, 1973). When sufficient numbers of beetles attack a tree, the constitutive system fails, and therefore stand-level induced defensive capacity determines the beetle population threshold at which an epidemic can be initiated successfully (Raffa and Berryman, 1982b; Safranyik and Carroll, 2006). When the overall defensive capability of a stand of trees is reduced, for example during drought, outbreaks can readily develop (Thomson and Shrimpton, 1984), and the increasing incidence of drought combined with warming temperatures has led to an enormous increase in the area affected by eruptive bark beetles in North America (Bentz et al., 2010; Kolb et al., 2016; Preisler et al., 2012; Raffa et al., 2015).

Transition in eruptive bark beetles from the endemic to epidemic phase is facilitated by a state-dependent shift in host preference from impaired to vigorous trees (Boone et al., 2011; Wallin and Raffa, 2004). When density is high and aggregation is possible, beetles will shift

host selection to prioritize larger and better defended hosts (Amman and Cole, 1983; Berryman et al., 1989; Raffa and Berryman 1987). Bark beetles discriminate between host and non-host species via gustatory assessment of volatile defensive compounds in phloem resin (Pureswaran and Borden, 2003, 2005; Raffa and Berryman 1982a). There is now substantial evidence that population state-dependent host selection is informed by the defensive characteristics of potential hosts as well; eruptive bark beetles in low density seem to seek and attack trees with weakly concentrated resin, and in high density seek and attack trees with strongly concentrated resin (Boone et al., 2011; Wallin and Raffa, 2004). However, it is not entirely clear whether selection is dependent on volatile concentrations directly, or whether this association is an artifact of other search criteria. Controlled, manipulative experiments are required to isolate the role of resin volatiles.

Epidemic-phase colonization and aggregation by eruptive bark beetles is accomplished by the use of pheromones, which attract conspecifics to hosts to overwhelm defenses (Shepherd, 1966; Pitman et al., 1968; Pureswaran and Borden, 2003; D. Wood, 1982). These pheromones are often synthesized directly from resin components (Keeling and Bohlmann, 2006; Pierce et al., 1987), which may mean that successful aggregation by eruptive bark beetles is actually reliant upon the defensive chemicals that evolved to combat them. This is particularly important in the face of a changing climate and subsequent range expansion; bark beetles have invaded forests of novel host species with varying chemical compositions (Cullingham et al., 2010; Safranyik et al., 2010), and of novel host populations which may exhibit qualitatively different defensive resin characteristics than those of the same species in the beetles' native range (Clark et al., 2010, 2014). It may be that population thresholds required for eruption could be different due to variations in quantitative and qualitative resin characteristics in novel forests. A thorough

understanding of the consequences of shifting defensive chemistry due to climate-induced range-shifts on the eruptive potential of bark beetles is needed to improve the accuracy of future predictions.

1.4 The mountain pine beetle as a model system

Here, I use the mountain pine beetle (*Dendroctonus ponderosae* Hopkins, 1902) system in western Canada to address knowledge gaps associated with eruptive herbivore dynamics in a warming climate. I believe that this long-studied and well-understood system is a good representation of the general behaviors and biology of eruptive bark beetles, and so therefore results of experimentation in this system are applicable to others, especially other species of *Dendroctonus*. The mountain pine beetle is the most influential eruptive forest insect in North America, and the most recent outbreak that began in the late 1990s and is ongoing at the time of this writing, is unprecedented in size and severity. The principle host of mountain pine beetle in Canada is lodgepole pine (*Pinus contorta* var. *latifolia*) (Safranyik and Carroll, 2006); however the beetle is oligophagous within *Pinus*, and can reproduce in most native and introduced species (Furniss and Schenk, 1969). The beetle commonly attacks ponderosa pine [*Pinus ponderosa*] (Pierce et al., 1987) and has invaded populations of the high-elevation species whitebark pine [*Pinus albicaulis*] (Bentz and Schen-Langenheim, 2007; Raffa et al., 2013) in the United States and Canada. By 2011, over 700 million m³ of timber in 18.1 million ha of pine forests in British Columbia had been killed by the beetle, which amounts to ca. 50% of merchantable pine timber in the province (Nealis and Cooke, 2014). By the mid-2000's, beetles became so numerous in British Columbia that they breached the Rocky Mountains and entered Alberta to the east, and have undergone a species shift into the novel host jack pine (*Pinus banksiana*) in the mosaic

hybrid zone of the montane-boreal transition (see: Cullingham et al., 2011) and boreal forests to the east (Cullingham et al., 2011; de la Giroday et al., 2011, 2012; Safranyik et al., 2010).

Interestingly, this range shift was predicted by Logan and Powell (2001) based on their generalization of stage-specific models of the influence of temperature on the mountain pine beetle. The threat of movement through the boreal forests into eastern Canada and the United States is a major concern for governments of both countries, and enormous effort is underway to try and mitigate the spread (<http://www.nrcan.gc.ca/forests/fire-insects-disturbances/top-insects/13381> [accessed October 18th, 2016]).

Previously limited by cold climates to areas south of N56° and west of the Rocky Mountains, warming and fire suppression have led to the northward shift in range for the mountain pine beetle in British Columbia, to within 100 km of the border of the Yukon Territory, and east into Alberta (Carroll et al., 2004; Nealis and Cooke, 2014). Newly invaded forests of both lodgepole and jack pine have no historical experience with mountain pine beetle outbreaks, and are considered evolutionarily naïve (Cudmore et al., 2010). In novel lodgepole pine forests, evidence suggests that the beetle actually performs better; researchers have observed higher reproductive potential (Cudmore et al., 2010) and higher attack densities (Clark et al., 2010), potentially resulting in higher measured rates of spread and impact (Robinson, 2015) compared to native range forests. Comparison of constitutive and induced resin defenses from novel and native forests suggests that defensive capacity of the former is reduced, and may contribute to increased susceptibility (Clark et al., 2010, 2014). It is not entirely clear whether this is true across a wide range of lodgepole pine forests, nor whether this discrepancy is the result of absent or weak co-evolutionary relationships (e.g. Raffa et al., 2013) within the contiguous range of the principal host.

Boreal expansion and potential invasion of eastern forests of North America may be facilitated by the defensive characteristics of jack pine. Jack pine contains higher concentrations of the defensive resin volatile monoterpene α -pinene (Clark et al., 2014, Taft et al., 2015b), that is converted by female mountain pine beetles to their main aggregation pheromone, *trans*-verbenol (Pitman et al., 1968; Renwick et al., 1976; Pierce et al., 1987), that attracts beetles of both sexes in a dose-dependent fashion (D. Miller et al., 2005). Laboratory experiments have demonstrated that higher concentrations of α -pinenes (irrespective of stereochemistry) in infested tissues lead to greater production of pheromones including (-)-*trans*-verbenol (Erbilgin et al., 2014; Taft et al., 2015a), which may aid the spread of beetles into eastern forests. To date, it is not clear whether the potential for increased pheromone production will lead to higher rates of aggregation *in situ*.

These discrepancies between native and novel forests provide a natural laboratory to study the effects of climate change on eruptive species. Native and novel lodgepole pine forests of British Columbia and Alberta are highly similar in species composition and potential trophic interactions, which allow for the isolation of factors that may contribute to continued spread of beetles. There are differences in defensive characteristics however, and here I exploited this to assess the contributions of these characteristics as they pertain to eruptive dynamics of bark beetles. In addition, the mountain pine beetle has spread through a mosaic hybrid zone in the montane-boreal transition forests in Alberta, and I exploited this gradient to assess the consequences of a species shift on the complex communication and population dynamics of eruptive bark beetles.

1.5 Research Goals

The goal of my research has been to use the mountain pine beetle system to assess the consequences of climate-induced range expansion on the population dynamics and ecology of forest insects. Mountain pine beetles have been studied for a long time in North America (Hopkins, 1905), and much is known about their biology in southern British Columbia and the western United States (see Safranyik and Carroll, 2006). However, it is uncertain, but increasingly likely that their behavior and potential host interactions, and therefore population dynamics, are different in the newly expanded range. This dissertation addresses knowledge gaps concerning the consequences of their continued spread, and relates findings back to ecological theory to advance our understanding of population dynamics and the biological consequences of climate change.

In chapter 2, I conducted a manipulative experiment in the laboratory to assess the proximate causes that lead to the transition from the endemic to epidemic population phase in mountain pine beetle, and how it relates to defensive resin composition. Mountain pine beetles exhibit population state-dependent life history strategies, and Boone et al. (2011) suggested state-dependent host selection is based on gustatory assessment of monoterpene composition in potential hosts. The authors found that endemic beetles selected impaired hosts with low monoterpene content, and incipient and epidemic beetles selected hosts with high monoterpene concentrations. I tested this directly, and exploited this preference to assess what conditions influence the transition in host selection behavior. I developed a phloem-based artificial diet and bioassay to address the following question: What environmental conditions lead to population-phase transition in the mountain pine beetle? My hypothesis was that rearing conditions and natal experience, as a consequence of maternal host selection, dictate the distribution of host

selection behaviors in offspring via context-dependent maternal effects (*sensu* Plaistow and Benton, 2009). As the endemic phase is characterized by isolated attacks on impaired hosts, as opposed to cooperative attacks on healthy hosts in an epidemic, I predicted that beetles reared in isolation as a consequence of endemic maternal host selection would prefer simulated phloem with low concentrations of monoterpenes, whereas those reared in epidemic-phase simulation would prefer high concentrations of monoterpenes.

In chapter 3, I conducted a manipulative experiment in the field to assess the effect of constitutive α -pinene content on the ability of mountain pine beetles to initiate aggregation. Once a host has been initially selected, the beetle must initiate secondary attraction of conspecifics to that host (aggregation) to successfully reproduce. Laboratory research has revealed that beetles tunneling in jack pine bolts, which contain elevated levels of α -pinene, produce significantly higher quantities of pheromones (Erbilgin et al., 2014; Taft et al., 2015a). This is a major concern in Canada and the United States, as jack pine extends across North America and varies significantly in chemical composition (Taft et al., 2015b). I developed a field-based bioassay to address the following question: What is the effect of differences in the defensive characteristics of potential hosts on the aggregation capacity of mountain pine beetles? My hypothesis was that the potential for increased pheromone production in trees with higher α -pinene content would render them individually more attractive when infested. I predicted that bolts of various pine types with increasing concentrations of α -pinene will be increasingly attractive when infested at known densities and exposed to the same natural population of mountain pine beetles.

In chapter 4, I conducted a manipulative experiment on mature lodgepole pines across western Canada, to assess the effects of historic exposure to mountain pine beetle epidemics on

the induced defensive response of the hosts. Since the last glacial maximum, lodgepole pine migrated north mostly from southern refugia, with mountain pine beetles lagging behind (Bentz et al., 2010; MacDonald and Cwynar, 1985). However, while lodgepole pine range extends into the Yukon Territory to approximately N64° (Farrar, 1995), mountain pine beetles were limited historically by cold climate to approximately N56° latitude and west of the Rocky Mountains. Recently, global temperatures have risen and the beetle has been found much further north and east into Alberta (Carroll et al., 2004; Nealis and Cooke, 2014; Safranyik et al., 2010), in areas with no history of outbreaks (Taylor et al., 2006). These forests are highly similar and contiguous, but research has revealed that some defensive traits are different between native and novel lodgepole pine forests (Clark et al., 2014, 2010), and beetles perform better in naïve systems (Cudmore et al., 2010; Robinson, 2015). However, it is not known if these discrepancies are the result of natural selection by the beetle on the defensive characteristics of the host trees. I conducted a field-based bioassay using mature lodgepole pine trees to address the following question: What is the effect of prolonged exposure to tree-killing bark beetle epidemics on a population of hosts? My hypothesis is that, since tree-killing bark beetles must kill their host to reproduce, and the induced defense is the primary determinant of resistance, then prolonged exposure to beetle epidemics will lead to a specific adaptation to attack in the induced defensive response of hosts. I predicted that lodgepole pine trees with a greater degree of historic exposure to epidemic mountain pine beetle would react more strongly to simulated beetle challenge than would those from the newly invaded expansion zone.

The experiments described in chapters 2-4 address gaps in our knowledge about the biological consequences of global climate change. There is substantial evidence that diverse taxa are being affected by warming and precipitation trends, and that many, but not all taxa are

undergoing range shifts. What is a clear consequence of these changes is that we can expect species composition and trophic relationships to change, and this is sure to affect resilience and stability of ecosystems. Natural ecosystems are critical to our continued existence, and society must contend with the consequences of anthropogenic modification of the environment.

Research designed to address these consequences are vastly important if we are to mitigate the negative effects of future climate change such as the loss of ecosystem services and sustainable timber, and this dissertation describes my contribution to this body of knowledge.

2 Breeding matters: Natal experience influences population state-dependent host acceptance by an eruptive insect herbivore

2.1 Introduction

Herbivorous insects are highly influential agents of change in most coniferous forests types. Whereas most populations are highly regulated and are not prone to dramatic fluctuations in size (Royama, 1992), some intermittently experience widespread population eruptions and can inflict biome-scale disturbances (Cooke et al., 2007; Raffa et al., 2008). For eruptive species, the constraints on individuals from sub-outbreak (i.e., endemic) populations are often very different than those associated with outbreak (i.e. epidemic) populations (Eveleigh et al., 2007; Raffa et al., 2008). For example, strategies for dispersal, host selection, and mate location may differ by necessity when populations are low, and conspecifics rare, as compared to high-density populations where conspecifics are abundant. State-dependent life history strategies (Chitty, 1960; Houston and McNamara 1992; Luttbeg and Sih, 2010; McNamara and Houston, 1996; Sih et al., 2004a,b) have been demonstrated among diverse insect species with distinct population phases (Boone et al., 2011; Wallin and Raffa, 2002, 2004; White, 1976). Although it is clear that the evolution of population-state dependent behaviors is an ultimate adaptation to extremes in population density (Guo et al., 1991), for most eruptive forest insect species the proximate mechanism(s) by which individuals shift between endemic and epidemic strategies/behaviors remains unclear.

Maternal effects, defined as causal influences of the maternal genotype or phenotype on offspring phenotype (Wolf and Wade 2009), have been implicated in population dynamics of many taxa (reviewed by Inchausti and Ginzburg, 2009). For example, nutritional quality of food resources available to mothers may influence the health and fecundity of offspring (Ginzburg

and Tanneyhill, 1994), and maternal choices may change offspring growth rate or behaviors (Caro and Bateson, 1986; Emlen, 1997; Gross, 1996). However, their precise role remains controversial since fluctuations in species abundance may have many causes (e.g., Royama, 1992; Turchin, 2003). Recent evidence suggests that the expression of maternal effects may be context dependent, influencing different traits and/or creating unique patterns of trait expression under different conditions (reviewed by Plaistow and Benton 2009). For example, the influence of maternal size, age, or nutritional status on offspring fitness has been shown to depend on population density for a broad range of taxa (Czesak and Fox, 2003; Gliwicz and Guisande, 1992; Lardies et al., 2004; Plaistow and Benton, 2009; Räsänen et al., 2005). Context-dependent maternal effects may be critical to the eruptive dynamics of species with population state-dependent life history characteristics by reinforcing traits specific to endemic or epidemic populations. For instance, if cooperative attacks of well defended hosts, characteristic of many epidemic-state bark beetle species (Raffa et al., 2008), are associated with a density-dependent maternal effect, then the cooperative epidemic strategy will be reinforced across generations via enhanced access to additional well defended hosts associated with increased population density (i.e. positive feedback). Given evidence that the frequency and severity of outbreaks by some eruptive forest insects may be increasing due to climate change and other anthropogenic modifications to the environment (Raffa et al., 2015; Robert et al., 2012), a clear understanding of the complex constraints on endemic and epidemic phases of these species is required to predict and mitigate impending outbreaks.

Mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is an eruptive bark beetle (i.e. subcortical herbivore) that displays distinct endemic and epidemic population phases in pine forests of western North America (Safranyik and Carroll, 2006; Raffa et al., 2008; Boone et al.,

2011). The endemic state is the normative condition for the mountain pine beetle, and is characterized by very low-density populations (several females per hectare of forest) that preferentially colonize defensively impaired trees, that are often occupied by interspecific competitors (Safranyik and Carroll 2006; Boone et al., 2011; Smith et al., 2011). When conditions facilitate an increase in generation survival allowing numbers to increase beyond the threshold density for successful cooperative (i.e., mass) attack of large-diameter, healthy trees (Safranyik and Carroll, 2006), beetles undergo a density-dependent shift in host preference and will selectively colonize highly defended trees (Boone et al., 2011). Unique life-history strategies are associated with each population state. Endemic beetles must locate exceedingly rare and ephemeral, susceptible (i.e. defensively impaired) trees that comprise nutritionally suboptimal resources (thin phloem) (Amman and Pace, 1976), and then contend with potentially high levels of interspecific competition (Safranyik and Carroll, 2006). By contrast, virtually all mature pine trees are susceptible to epidemic beetles; however, the beetles must contend with the aggressive defensive chemicals produced by healthy trees, and accommodate high levels of intraspecific competition that results from mass attacks (Safranyik and Carroll, 2006). Cooperative mass attacks by epidemic beetles are facilitated by a complex synergism of host-produced (kairomones) and beetle-produced (pheromones) volatile chemicals (Borden et al., 1987), and by the introduction of mutualistic phytopathogenic ophiostomatoid fungi and various bacteria and other microbes during colonization (A. Adams et al., 2009; Boone et al., 2013; Safranyik et al., 1975; Six, 2003). Although the shift between endemic and epidemic behaviors by the mountain pine beetle is derived from changes in population density (Boone et al., 2011), the mechanism(s) by which beetles determine the appropriate behaviors and strategies have not been investigated.

The threshold population density that defines the transition between endemic and epidemic states for the mountain pine beetle is determined by the defensive capacity of healthy host trees with thick phloem (Safranyik and Carroll, 2006). For *Pinus* spp., defenses against bark beetles rely primarily on the production of secondary metabolites, most notably monoterpenes (e.g. Keeling and Bohlmann, 2006; Raffa and Berryman 1982a; 1983; Shrimpton and Whitney, 1968). Field studies suggest that foraging female mountain pine beetles assess monoterpene content gustatorially to determine host quality, quantify defensive capability, and accept or reject a potential host (Boone et al., 2011; Pureswaran and Borden, 2003, 2005; Raffa and Berryman, 1982b). As a result of state-dependent maternal host selection, offspring may find themselves feeding in host material of varying nutritional quality and vastly different conspecific densities. As a result, there is potential for maternal host choice to affect offspring biology as a maternal effect. Here, I investigated the role of maternal host choice (i.e. the state-dependent strategy) and offspring developmental experience (i.e. the context-dependent maternal effect) on host preference by mountain pine beetle. More specifically, using laboratory experiments I critically evaluated the hypothesis that host acceptance by foraging mountain pine beetles will be influenced by density-dependent maternal host choice. I predicted that the progeny of low-density females developing in poorly defended hosts (i.e. endemic behavior) will accept substrates indicative of low host defenses, whereas the progeny of high-density females from well defended hosts (i.e. epidemic behavior) will not.

2.2 Materials and methods

2.2.1 Mountain pine beetle source

In September of 2012, 2013, and 2014, I felled several naturally mass-attacked lodgepole pine trees from epidemic infestations near Lillooet (N 50.684°, W 121.936°), Baldy Mountain (N 49.110°, W 119.117°), and Grand Forks (N 49.029°, W 118.445°), southern British Columbia, Canada. These were cut into ca. 75-cm-long bolts and transported to the Forest Insect Disturbance Ecology Laboratory (FIDEL) at The University of British Columbia in Vancouver. The ends of the bolts were sealed with paraffin wax to prevent desiccation, and the bolts were placed in cages (2m × 2m × 1m wooden boxes, with 0.5 mm screen side panels) at room temperature until beetles completed their development and emerged (~4-6 weeks). Beetles were collected from the sides and top of the cages, segregated by sex based upon stridulation [males stridulate when disturbed (Michael and Rudinsky, 1972)], and either assigned to an endemic or epidemic population state simulation as described below.

2.2.2 Endemic simulation

Given that endemic mountain pine beetle populations are characterized by isolated attacks on vigor-impaired, small-diameter trees with thin phloem (Safranyik and Carroll, 2006; Smith et al., 2011), I recreated these conditions in the laboratory to produce beetles with the potential for endemic behavior. Several small [<20 cm diameter at 1.3 m] lodgepole pine trees were harvested in the autumn from an uninfested, mature stand near Whistler, British Columbia (N 50.115°, W 122.957°), cut into 50-cm-long bolts, sealed with paraffin wax and transported to the laboratory. In the lab, one 8-mm hole was punched through the bark near the bottom of each bolt. A newly emerged female beetle was placed into a 2 mL Eppendorf® vial (Fisher-

Scientific®) with the end snipped off and the open end placed into the hole, such that the female could initiate tunneling beneath the bark. Vials were left in place for 24 hours and then assessed for the presence of boring dust indicative of successful entry. Females that did not enter were replaced by another female for a new 24-hour period, until successful. Once females had successfully established in the bolts, one male was rechecked for stridulation, then placed into the gallery entrance, where they immediately entered and initiated mating. Only one mating pair was introduced per bolt, and in this way I simulated endemic maternal host choice.

Infested bolts were placed in individual endemic simulation chambers created from a 19 L (30 cm dia × 36 cm ht) paint bucket (The Home Depot®) with six, 5-cm-diameter mesh-covered holes on the sidewall (Fig. 2.1a). One bolt was placed in the bucket, and then the top was covered with screening and secured with string. Buckets were placed in individual rooms (≥ 10 m apart, separated by multiple walls) in the University of British Columbia Forest Sciences Centre to keep the chambers isolated from each other and minimize the potential for interaction between non-sibling conspecifics during offspring development. Ambient temperature in all rooms was maintained between 20-22°C.



Figure 2.1. Images of a) an endemic simulation chamber used to simulate low-density developmental conditions for mountain pine beetles, b) simulated phloem substrate arenas during a trial to assess the effect of population-phase simulation on host acceptance behaviors, and c) a beetle excavated from within the phloem substrate, after its acceptance of the substrate and initiation of tunneling.

Endemic simulation chambers were checked three times daily to minimize contact among emerging beetles, and any beetles found were segregated by sex. Female beetles were immediately placed into individual 20 mL glass vials, containing a small piece of folded filter paper, to assist them in staying upright. In empty glass vials, beetles will often overturn and be unable to right themselves, and may die from exhaustion (J. Burke – personal observation). Males were either kept for use in other trials, or discarded, as host selection by mountain pine beetles is conducted exclusively by females. Dispersing beetles will search for hosts for up to several days (Safranyik et al., 1992). Therefore, to simulate foraging conditions for low-density populations I placed individual females in their glass vials into 120 mL plastic specimen

containers, along with about 10 lodgepole pine needles and 1 mL of distilled water, which were then sealed and placed in a fume hood under natural light for 48 hours. Needles were provided as a cue to indicate that the beetles were in the vicinity of host trees. This method simulated the foraging period post-emergence, but without the potential for interaction with conspecifics.

2.2.3 Epidemic simulation

Epidemic simulation was achieved using beetles from the source population. The bolts collected from the naturally mass-attacked trees (> 25 cm diameter at 1.3 m) from active infestations represented *in situ* epidemic maternal host choice. To ensure the potential for epidemic-state maternal effects to manifest in progeny, bolts were grouped within emergence cages in a single room, and beetles were collected every 48 hours for use in trials, to maximize their potential for interaction with conspecifics. Beetles were always collected from the top or the sides of the cage and never from the log surface or the floor to ensure they were vigorous and minimize the chance of collecting a newly emerged beetle that had not had a chance to interact with conspecifics.

2.2.4 Simulated phloem

I used a semi-artificial phloem diet comprised of denatured lodgepole pine phloem, agar, water, and synthetic monoterpenes to simulate the sub-cortical environment occupied by the mountain pine beetle. My approach was modeled after previous, successful experiments using other bark beetle-conifer systems (Klepzig et al., 1996; Wallin and Raffa, 2004, 2002; Wallin et al., 2002). Additional lodgepole pine bolts from mature trees collected from the Whistler stand were stripped of their outer bark, which was discarded, then of the phloem tissue beneath, using

a draw knife. Harvested phloem tissue was submerged in liquid nitrogen, crushed into small (1x1 cm) pieces using a large mortar and pestle, and then quickly placed into a coffee grinder (Black and Decker® model CBG100S) and pulverized. Ground phloem was then run through a 0.5 mm sieve, and then put into an autoclave at 105°C for 20 mins to remove the existing volatile monoterpenes (Wallin and Raffa, 2000). A mixture of agar (Fisher Scientific® BP1423-500) and distilled water at 60 g/L was created, and 300 mL of agar solution was added to 200 g of ground phloem and mixed evenly (adapted from Klepzig et al., 1996). The resulting mixture was ~70% moisture, by weight. Moisture content was checked for each new phloem batch by weighing, then drying samples in a drying oven at 70°C for 24 hours and reweighing.

Small (60 mm diam. × 15 mm ht.) Petri plates (Fisherbrand® FB0875713A) were textured on the inside using coarse-grit sandpaper to allow beetles to maneuver within the arena and achieve purchase on the plastic surface when boring into the simulated phloem. A triangular piece of wood (60 × 42 × 42 mm) was inserted in to the arena, with the hypotenuse of the triangle bisecting the arena, and approximately 10 g of diet was pressed into the empty half. Prepared dishes were placed into a drying oven at 70°C for 1.5 hours, and after drying, the wood insert was removed (Fig. 2.1b).

2.2.5 Monoterpene blend

Mountain pine beetles can successfully reproduce in most North American *Pinus* species (Furniss and Schenk, 1969; S. Wood, 1982). Consequently, beetles must contend with a range of monoterpenes during colonization. For example, two of the most common monoterpenes in defensive resin of pines, (+)- and (-)- α -pinene, comprise as much as ~50-90% of jack pine (*Pinus banksiana*) resin (Burke and Carroll, 2016; Clark et al., 2014; Erbilgin et al., 2014; Taft et al.,

2015a, 2015b; Hall et al., 2013; Wallin and Raffa, 1999), but only ~7-12% of whitebark pine (*Pinus albicaulis*) resin (Raffa et al., 2013). I created a blend of monoterpenes intended to represent the major components present in potential hosts. I began with a monoterpene solution containing 50% (-)- β -phellandrene distilled from lodgepole pine turpentine (C. Breuil, unpubl.). At the time of this experiment, β -phellandrene was not available for purchase in purified form. This chemical was required as it is the major monoterpene component of lodgepole pine phloem resin (Burke and Carroll, 2016; Clark et al., 2010; Hall et al., 2013). The solution contained additional monoterpenes, all of which I intended to use, so rather than distilling it further to isolate β -phellandrene, I augmented it with (+)- and (-)- α -pinene, (-)- β -pinene, myrcene, 3-carene, and limonene from Sigma-Aldrich® (www.sigma-aldrich.com). The final solution comprised the following component ratios: 32.6% racemic α -pinene, 32.6% (-)- β -pinene, 3.8% 3-carene, 10.6% myrcene, 3.2% (-)-limonene, 15.3% β -phellandrene, and 1.9% terpinolene. (-)- β -pinene and β -phellandrene are major components of lodgepole pine resin (Burke and Carroll, 2016), and the remaining components have various biological functions in this system; 3-carene and limonene are toxic to beetles (Reid and Purcell, 2011), and myrcene and terpinolene are pheromone synergists (Borden et al., 2008). Alpha-pinene is particularly important, as it is both detrimental (A. Adams et al., 2009) and beneficial (Burke and Carroll, 2016; Erbilgin et al., 2014; Pitman, 1971; Taft et al., 2015a) to foraging beetles. Aliquots of the monoterpene mixture were then diluted in pentane (Wallin and Raffa, 2000) to 10% and 50% (for 10 and 50 mg/g treatments), with the remaining left at 100% monoterpene concentration (for 100 mg/g treatments). I then added ~115 μ L of the appropriate monoterpene mixture (10, 50, 100%) per gram of simulated phloem (~700 μ L) to the surface of the substrate in the petri dishes, allowing the liquid to penetrate and distribute evenly. Arenas were then left uncovered under a fume hood

for 1 hour to allow the pentane to evaporate (Wallin and Raffa, 2000). In this way, I added the same amount of liquid to each arena, while producing different concentrations of monoterpenes.

2.2.6 Substrate acceptance versus population state

Once the arenas were prepared, one female mountain pine beetle, either from the mass-attacked bolts maintained in the large emergence cages (epidemic simulation), or the smaller bolts in the isolated endemic simulation chambers, was placed into the empty side of an arena. The lid of the petri dish was labeled with a marker and placed onto the arena. These were then placed onto a foam board, and secured standing at a 45° angle to the board with insect pins (Fig. 2.1b). This configuration ensured that beetles could right themselves if they tipped over (J. Burke – personal observation). Between 6 and 8 replicates of each concentration treatment were used in each trial. A small fan was placed near the arena board to gently move air over the surface and ensure volatiles would not accumulate over the arenas. Each arena was angled in the same direction, facing a 120-watt equivalent, full spectrum LED flood lamp (Philips® model PAR38), to approximate daylight. Due to the process of rearing, epidemic simulation beetles were tested first, then endemic simulation beetles were tested ~4-6 weeks later, under identical conditions.

Once all beetles and arenas were in place, the arena board was situated underneath a Logitech® HD Pro C920 webcam video camera, which recorded beetle activity for 8 hours. To clearly see the beetles, I set the camera to capture in greyscale (0% color saturation) and low color contrast. The black beetles were clearly visible against the grey and white background of the arena and foam board under these recording conditions. I created a time-lapse series of photographs using Chronolapse® software version 1.0.7 (2010, Collin Green,

<https://code.google.com/p/chronolapse/>), which is an open-source software application (<http://opensource.org/licenses/mit-license.php>). A picture was recorded every 30 minutes for 8 hours, at 2304 x 1296 resolution. A beetle was considered to have accepted the simulated phloem when she had entered it completely and her entire body was no longer visible. After 8 hours, the arenas were frozen at -30°C for 48 hours, and the dead beetle was excavated and the width of her pronotum was measured (Fig. 2.1c). Preliminary, 24-hour trials revealed very little change in acceptance ratio after 8 hours, so subsequent trials were stopped at this time-point. I then removed the elytra and checked each beetle for sexually dimorphic characteristics, as reviewed by Amman and Cole(1983), to confirm the subject was female. Of 292 total beetles trialed over the course of the experiment, only 4 were males (that did not stridulate), and these were removed from the analysis.

In total, 38 trials were run with three concentration treatments. Seven trials of 8 beetles per concentration treatment were run with beetles from epidemic simulations (168 beetles). Number of trials run per concentration treatment and beetles per trial varied within endemic simulation, as rearing beetles at such low density limits the number of offspring available to trial at any one time. Six trials of 6-8 beetles were run for 10 and 50 mg/g treatments, and 5 trials of 8 beetles were run for 100 mg/g treatments (124 beetles).

2.2.7 Statistical analyses

All data were prepared using Microsoft® Excel© version 16.0 (Microsoft®, 2015, Redmond WA USA), and all statistical tests were performed using SAS® Statistical Software version 9.4 (SAS® Institute, 2014, Cary NC USA)., I calculated the mean acceptance ratio (entered/non-entered) for each trial of 6-8 beetles, at each hour of the eight hour trials (n=38 x 8).

All data were arcsine (square-root) transformed to account for the truncated distribution of proportion data (Dowdy et al., 2004). I then applied PROC UNIVARIATE to test the assumptions of normality and equality of variance.

I conducted a two-way analysis of variance with interactions using PROC GLM, with population-state simulation and concentration treatments as main effects, on the acceptance ratio after 8 hours. To more thoroughly examine the influence of juvenile rearing conditions on subsequent adult host acceptance, I also used PROC GLM to assess variation in substrate acceptance by simulated endemic and epidemic beetles within each concentration treatment after 8 hours.

To assess the effect of developmental experience on the rate of acceptance, I performed a repeated-measures analysis of variance on data in time-series from 1-8 hours of exposure, for each concentration treatment using PROC GLM with the REPEATED statement. The interaction of time (i.e. “rate”) and simulation determined if the rate of acceptance differed between simulations, within each concentration treatment.

I used PROC GLM to perform one-way analysis of variance to determine if simulation treatment affected offspring size. Since the thin phloem of defensively impaired trees may lead to smaller brood beetles (Amman and Cole, 1983; Amman and Pace, 1976), and small beetles may be less able to overcome defenses of larger, healthy hosts (Graf et al., 2012), I also assessed the effect of body size (pronotal width) on beetle acceptance using logistic regression (PROC LOGISTIC) on the original, binary (0/1) data at 8 hours, within simulation, and within concentration treatments, to determine if larger beetles were more likely to accept high monoterpene substrates.

2.3 Results

When considered all together, female mountain pine beetles accepted (i.e. entered) simulated phloem about 50% of the time (Fig. 2.2). Approximately 85% of beetles that accepted the simulated phloem did so after 5 hours. Those that did not initiate tunneling spent most of their time walking on the surface of the simulated phloem and in the empty side of the arena. Three beetles remained motionless, and upon inspection after 8 hours were found to have died during the trial. These were removed from analyses. Once initiated, tunneling activity lasted for about 2-3 hours (observed by accumulating material at the entrance site). Occasionally, beetles emerged from the simulated phloem, likely due to limited tunneling space, but these were still considered to have accepted the diet.

The concentration of monoterpenes within the simulated phloem significantly affected the likelihood that a female beetle would mine within it ($F_{2,37}=6.69$, $p=0.004$) (Fig. 2.2, Table 2.1). Overall, beetles were more likely to accept the phloem with the highest concentration (100 mg/g) over lower concentrations at all time-steps, and within the epidemic simulation acceptance ratio was directly related to monoterpene concentration at all time-steps (Fig. 2.2). Although there was no overall effect of beetle rearing condition on acceptance of the phloem substrates among monoterpene concentrations, nor a detectable interaction between simulated population state and concentration (Fig. 2.3, Table 2.1), acceptance of the phloem substrate with the lowest monoterpene concentration (10 mg/g) differed significantly between epidemic and endemic beetles ($F_{1,12}=6.24$, $p=0.03$). The effect of population state did not persist at the higher monoterpene concentration levels (Fig. 2.3, Table 2.1).

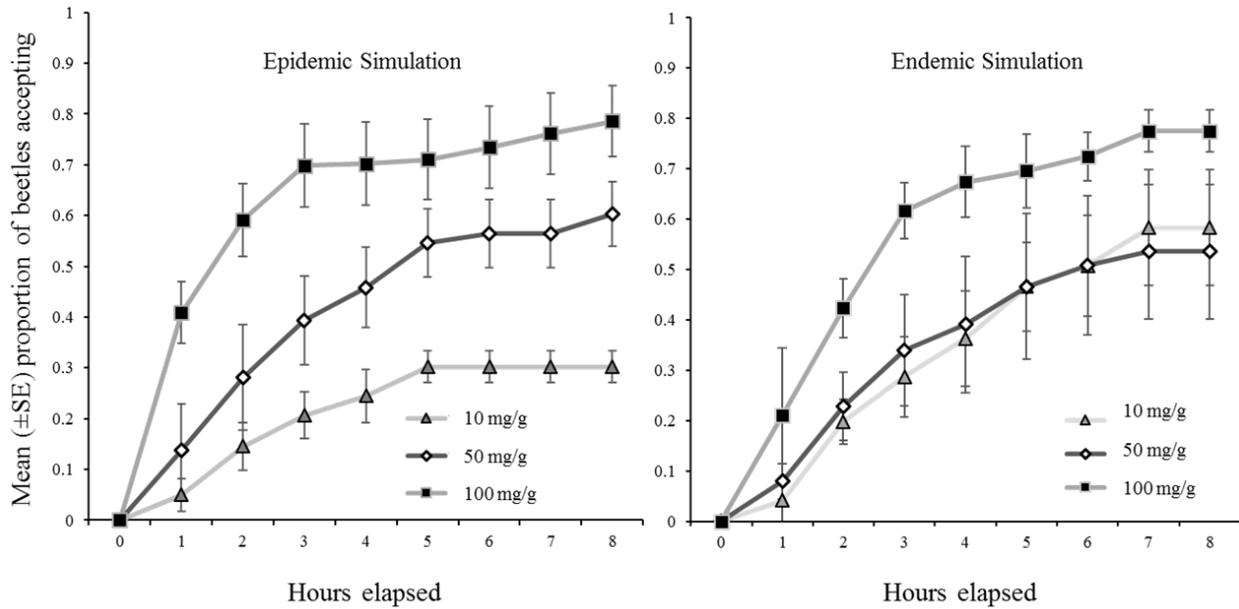


Figure 2.2. Mean (\pm SE) acceptance ratio (accept/reject) per trial (38 trials, 292 total beetles) of simulated phloem amended with three monoterpene concentrations by female mountain pine beetles reared in two population-density simulations over the course of 8-hour trials.

The effect of time (rate) was always significant within concentration treatments ($p < 0.001$) [Table 2.2]. Furthermore, at the lowest and highest monoterpene concentrations, the rate of acceptance was dependent upon simulated population state. The rate of acceptance by endemic beetles of 10 mg/g treatments was significantly higher ($F_{7,77}=2.15$, $p=0.498$), while their rate of acceptance of 100 mg/g treatments was significantly lower ($F_{7,63}=3.77$, $p=0.002$) [Table 2.2] than that of epidemic beetles. Interestingly, beetles reared under epidemic conditions were more likely to accept phloem substrates amended with the highest monoterpene concentration within 3 hours when compared to endemic beetles (Fig. 2.3).

Pronotal widths ranged from 1.8 – 2.6 mm with a mean of 2.27 (± 0.01 SE). Contrary to expectation, body size of beetles from each simulation were not different ($p=0.65$) and there was no effect of beetle body size on the likelihood of acceptance of phloem at any monoterpene concentration ($p=0.64-0.96$).

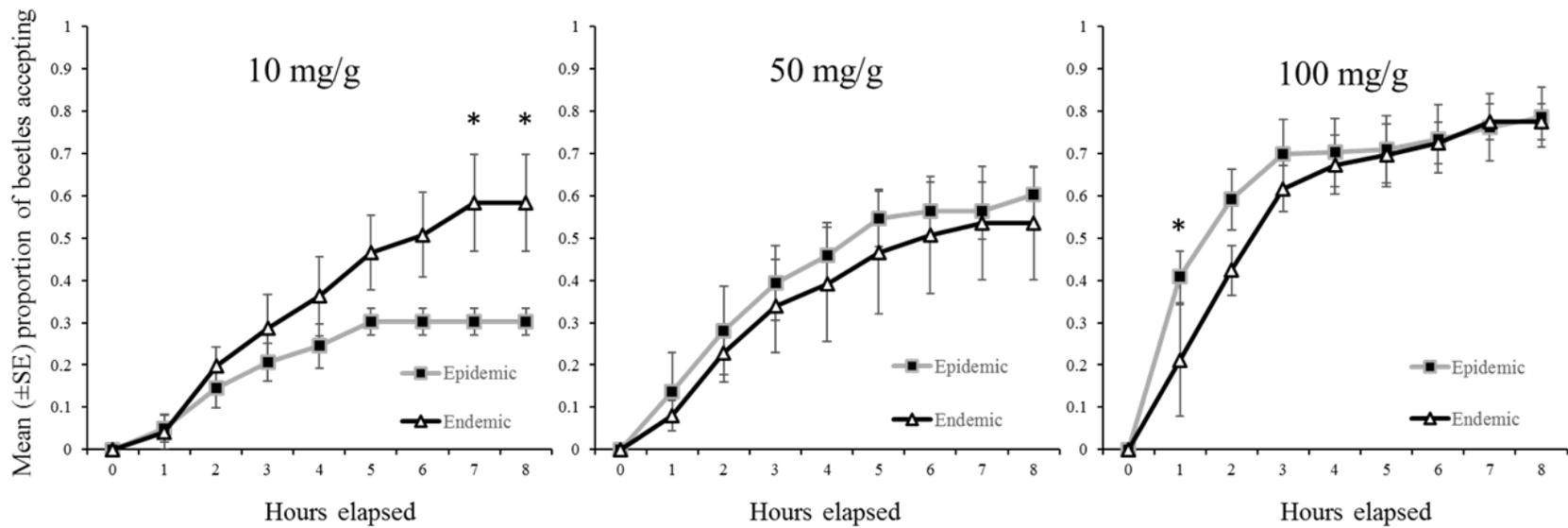


Figure 2.3. Mean (\pm SE) acceptance ratio (accept/reject) per trial (38 trials, 292 total beetles) of simulated phloem amended with three monoterpene concentration treatments by female mountain pine beetles, reared in two population-density simulations over the course of 8-hour trials. Asterisks (*) indicate significant differences ($p < 0.05$) determined by analysis of variance.

Table 2.1. Summary statistics for analysis of variance tests of the effect of monoterpene concentration and simulated population state on total acceptance ratio of simulated phloem by female mountain pine beetles, after 8 hours, and for tests segregated by concentration treatment. Significant ($p < 0.05$) effects are shown in ***bold italics***.

<i>Source</i>	<i>df</i>	<i>Type III SS</i>	<i>F</i>	<i>p</i>
Monoterpene concentration	2	0.983	6.05	<i>0.006</i>
Simulated population state	1	0.023	0.29	0.592
Concentration*simulation	2	0.299	1.84	0.175
Total	37			
<hr/> <i>10 mg/g</i> <hr/>				
Simulated population state	1	0.293	6.24	<i>0.030</i>
Total	12			
<hr/> <i>50 mg/g</i> <hr/>				
Simulated population state	1	0.016	0.13	0.723
Total	12			
<hr/> <i>100 mg/g</i> <hr/>				
Simulated population state	1	0.012	0.17	0.686
Total	10			

Table 2.2. Summary statistics for repeated measures analysis of variance tests on the effect of simulated population state on the rate of acceptance of simulated phloem amended with different monoterpene concentrations by female mountain pine beetles after 8 hours (overall). Significant ($p < 0.05$) effects are shown in ***bold italics***.

<i>Source</i>	<i>df</i>	<i>Type III SS</i>	<i>F</i>	<i>p</i>
<hr/> <i>10 mg/g</i> <hr/>				
Rate	7	4.24	29.19	<i><0.001</i>
Simulation	1	0.63	2.45	0.146
Rate*simulation	7	0.33	2.15	<i>0.049</i>
Error(rate)	77	1.55		
<hr/> <i>50 mg/g</i> <hr/>				
Rate	7	4.37	19.51	<i><0.001</i>
Simulation	1	0.11	0.14	0.711
Rate*simulation	7	0.01	0.04	0.999
Error(rate)	77	2.47		
<hr/> <i>100 mg/g</i> <hr/>				
Rate	7	3.87	35.55	<i><0.001</i>
Simulation	1	0.58	1.19	0.304
Rate*simulation	7	0.41	3.77	<i>0.002</i>
Error(rate)	63	0.98		

2.4 Discussion

State-dependent life history strategies are influenced by context-dependent maternal effects in the mountain pine beetle. The propensity for maternal experience to influence the pattern of offspring host acceptance within population states can be used to elucidate critical aspects of herbivore-tree interactions that underlay eruptive population dynamics. Initial host selection by female mountain pine beetles begins with foraging individuals randomly landing on host trees (Hynum and Berryman, 1980), followed by gustatory assessment of resin components upon initial breach of the outer bark (Raffa and Berryman, 1982a). A beetle will then accept or reject that host as a function of population state, and putatively, the concentration of monoterpenes in phloem resin (Boone et al., 2011). In bioassays, decisions regarding host acceptance were made based on monoterpene concentrations in phloem tissue even in the absence of other cues. This supports the assertions of Raffa et al. (1982a) that beetles use gustatory stimulation to assess host tree condition, and Boone et al. (2011) that density-dependent host selection by the mountain pine beetle is based on assessment of monoterpene concentration in resin, and that this association is not an artifact of other search criteria employed by foraging beetles. The result that population-state simulation changed acceptance patterns further reinforces the assertion by Boone et al. (2011) that their preferences for low or high concentrations are a function of population-state.

Contrary to expectations, beetle body size had no influence on host acceptance. Previous work has shown that mountain pine beetle body size is positively related to phloem thickness of natal host trees (Amman, 1972; Amman and Cole, 1983; Amman and Pace, 1976), and has potentially important ramifications to beetle fitness through its influence on energy reserves (Elkin and Reid, 2005), and ability to detoxify host-tree defenses (Graf et al., 2012). Therefore,

the absence of a positive relationship between body size and acceptance of increasingly defensive substrates was surprising. However, parental beetles used in this investigation were on average larger and not representative of the full range of sizes observed in previous assessments that considered mountain pine beetle body size (Graf et al., 2012; McGhehey, 1971), and therefore any effect of body size may have been masked. The potential for reduced offspring body size resulting from development in nutritionally suboptimal hosts would likely reinforce their preference for low concentrations of monoterpenes, given the relationship between size and detoxification potential (Graf et al., 2012). A similar mechanism was proposed by Ginzburg and Tanneyhill (1994) as a regulator of population cycles in forest Lepidoptera.

Variation in the likelihood of acceptance of host-tree defenses as a function of maternal experience provides valuable insights into the state-dependent life histories of the mountain pine beetle and other eruptive bark beetles, and the transition between states leading to population eruptions and collapse. Maternal endemic behavior (isolated attacks of suboptimal hosts), results in offspring that are more likely to accept poorly defended substrates, whereas maternal epidemic behavior (mass attacks of healthy trees) leads to offspring that are more likely to reject poorly defended substrates. This suggests that in nature, offspring from mothers which select impaired hosts will have a greater propensity to also select an impaired host, increasing the chances of persistence over local extirpation in low-densities due to the strong Allee effects associated with a threshold density required to attack healthy trees (Berryman, 1978; Chubaty et al., 2009; Powell and Bentz, 2014). Endemic offspring were slow to accept, but did not reject defensive substrates after 8 hours. However, this may be due in part to the feeding-stimulant properties of monoterpenes (Raffa and Berryman, 1982a) combined with the no-choice bioassay. Regardless, this variation has implications for the transition from the endemic to the epidemic phase. During

the endemic phase, if generation mortality is reduced due to a density-independent perturbation such as mild winter temperatures or drought leading to reduced tree defensive capacity (Kolb et al., 2016; Thomson and Shrimpton, 1984), then offspring will interact with more conspecifics during development, potentially triggering epidemic behavior and attacks on defensive hosts. Furthermore, phenotypic plasticity in the response of endemic offspring may lead to a synergistic reinforcement of this transition by those offspring with the capacity to accept well-defended hosts, thus facilitating the rapid transition from endemic to epidemic population-phase characteristic of mountain pine beetle dynamics (Boone et al., 2011; Safranyik and Carroll, 2006).

In contrast, offspring from epidemic mothers were likely to reject low concentrations of monoterpenes and those that did accept were slow to do so, even when given no choice. This means that an epidemic population *in situ* has a very strong propensity to persist, and is resilient to collapse due to a mortality event like an extremely cold winter (see Safranyik and Carroll, 2006). The strong reinforcement of epidemic host selection by maternal experience is the proximate mechanism behind positive feedbacks associated with this phase at the stand and landscape scale (Raffa et al., 2008). Offspring of epidemic parents are most likely to pursue a well-defended, nutritionally optimal host under most conditions. If optimal hosts are abundant, reinforcement of the epidemic choice will result in many of these hosts being colonized, increasing the potential to colonize even more optimal hosts in the following year, rapidly leading to a stand-level outbreak. If optimal stands are abundant on the landscape, beetles dispersing into adjacent stands will take with them a strong propensity for epidemic selection, even if they initially arrive without many conspecifics. If enough beetles join them, aggregation will be successful, the stand-level epidemic process will initiate, and the outbreak will propagate.

Thus, reinforcement of epidemic-phase host selection behavior by context-dependent maternal experience can lead to a landscape-scale disturbance event.

Strong reinforcement of epidemic behavior by maternal experience also means mountain pine beetle populations will rapidly deplete the local supply of optimal hosts and collapse; a common observation during epidemics (Amman and Cole, 1983; Robinson, 2015; Safranyik and Carroll, 2006; Safranyik et al., 1975; Safranyik et al., 2010). Eventual host depletion means it is inevitable that a generation of mothers will find themselves without large trees to attack or insufficient conspecifics to aggregate, and be at high risk for local extinction. Given that maternal experience influences offspring host acceptance, the propensity for endemic behavior will increase and populations will transition back to the endemic state. Indeed, this transition appears to happen very quickly. Endemic mothers employed in experiments were reared as epidemic offspring until introduced into defensively impaired logs in isolated conditions, leading to endemic behavior in their offspring. Thus, the influence of population state-dependent maternal effects can manifest in a single generation to both minimize the likelihood of local extirpation, and maximize the potential for outbreak.

Evidence for the influence of context-dependent maternal effects on mountain pine beetle eruptive dynamics can be extended to the consideration of the impacts of climate change on landscape-scale forest disturbances by other eruptive forest insect systems. Herbivorous forest insects in Holarctic regions often do not occupy the more northern range of their hosts, meaning that the potential for native invasion is high (Carroll et al., 2004; Jönsson et al., 2007; Netherer and Schopf, 2010; Safranyik et al., 2010). Indeed, increasing temperatures have led to range expansion and shifts into novel habitats for several eruptive forest insects (Carroll et al., 2004; Hagen et al., 2007; Jepsen et al., 2009; Tr an et al., 2007). Furthermore, novel habitats may

exhibit insufficiently coevolved defensive traits that increase host susceptibility (Burke and Carroll, 2016; Erbilgin et al., 2014; Raffa et al., 2013; Robinson, 2015; Taft et al., 2015a, 2015b). Weak host defenses may lead to reduced generation mortality of eruptive insects, increasing the propensity for epidemic behavior, enhance positive feedbacks associated with this phase, and exacerbate the frequency and magnitude of outbreaks. A more thorough understanding of the proximate causes leading to eruptions is required to improve predictions and mitigate the biological consequences of continued climate change.

3 The influence of variation in host tree monoterpene composition on secondary attraction by an invasive bark beetle: Implications for range expansion and potential host shift by the mountain pine beetle

3.1 Introduction

Due to their short life cycles, high reproductive potential and mobility, and physiological sensitivity to temperature changes, herbivorous insects are highly susceptible to variations in weather and climate (Deutsch et al., 2008; Logan et al., 2003; Musolin, 2007; Parmesan, 2006). To date, the most common response by insect species to a warming environment involves distributional changes, including both range expansions (Hickling et al., 2006, 2005; Parmesan et al., 1999), and range contractions (Visser and Holleman, 2001; Wilson et al., 2005). Range shifts have often resulted in novel herbivore-host plant associations (Braschler and Hill, 2007; Cullingham et al., 2011), and where these have occurred, herbivore impacts may be greater than expected due to insufficiently co-evolved plant defenses (Cudmore et al., 2010; Raffa et al., 2013).

The recent, unprecedented outbreak by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, in western North America has seen the species expand its range north in latitude and up in elevation into novel habitats (Carroll et al., 2004; Raffa et al., 2008). Historically, the mountain pine beetle was restricted to areas west of the Rocky Mountains and at latitudes $<56^{\circ}$ N (Safranyik and Carroll, 2006). Its distribution was not limited by host availability, but by the adverse effects of climate (Bentz et al., 2010; Carroll et al., 2004; Safranyik, 1978; Sambaraju et al., 2011). The beetle has expanded its range due in part to increases in temperature resulting from climate change (Carroll et al., 2004), and to forest management practices that have favored highly susceptible forests conditions (Taylor and

Carroll, 2004). In 2002, dispersing mountain pine beetles breached the Rocky Mountain geoclimatic barrier and spread into north-central Alberta (de la Giroday et al., 2011; Safranyik and Carroll, 2006; Safranyik et al., 2010). Since then, infestations have been detected in the Northwest Territory (north of 60° latitude), and within 50 km of the Alberta/Saskatchewan border (110° W longitude) (A L Carroll - personal observation). In the historic range in western Canada, the principal host of the mountain pine beetle is lodgepole pine (*Pinus contorta* Douglas var. *latifolia*), but it can successfully reproduce in most native and introduced *Pinus* species in North America (Furniss and Schenk, 1969). As a consequence of its abrupt recent range expansion, the beetle has established within habitats comprising novel [i.e. putatively evolutionarily naïve (Cudmore et al., 2010)] populations of lodgepole pine, a novel host species in jack pine (*Pinus banksiana* Lamb.), and the intermediate hybrid (*P. contorta* × *P. banksiana*) zone (Cullingham et al., 2011).

Jack pine is a principal species of the transcontinental boreal forest, and the rapid expansion by mountain pine beetle into this novel host type has raised concern that the beetle could spread across North America (Logan and Powell, 2001; Nealis and Peter, 2008; Safranyik et al., 2010). It has been observed that mountain pine beetle reproductive performance is enhanced in evolutionarily naïve lodgepole pine forests as compared to evolutionarily experienced forests, in the form of increased attack densities (Clark et al., 2010) and the number of surviving offspring produced per female (Cudmore et al., 2010). Population genetic studies have revealed that the beetle has been undergoing a post-Pleistocene demographic expansion northward (Bentz et al., 2010; Mock et al., 2007), following behind an earlier expansion by lodgepole pine (Cwynar and MacDonald, 1987; MacDonald and Cwynar, 1985). Thus, the beetle has likely been absent from the boreal region since at least the last glaciation period.

Mountain pine beetles feed in the phloem tissue of their host trees, and therefore must overcome the complex physical and chemical defenses produced by the trees. Sub-outbreak (i.e. endemic) populations characteristically comprise very low densities that are restricted to colonizing trees with impaired defenses (Safranyik and Carroll, 2006). These trees are usually weakened by biotic or abiotic agents, and are normally occupied by other species of bark beetles that are specialists on defensively impaired trees (Safranyik and Carroll, 2006; Smith et al., 2011). If conditions (e.g., mild winters that increase over-winter survival; hot/dry summers that increase colonization success) facilitate reduced generation mortality, populations may increase through an incipient-epidemic phase to the outbreak, or epidemic phase, in which they can colonize and kill many hectares of large-diameter, thick-phloem trees by way of pheromone-mediated aggregation and mass attack (Berryman, 1982). Attraction to infested hosts is termed “secondary attraction”, as opposed to “primary attraction” by foraging pioneer beetles in response to host characteristics (Pureswaran and Borden, 2003). Boone et al. (2011) demonstrated that host selection behavior and preference changes with population density, where endemic mountain pine beetles prefer trees with weak defenses, and epidemic beetles prefer trees with strong defenses (measured by the content of defensive chemicals in the resin). Trees with strong defenses are generally healthier, have thicker phloem, and therefore provide greater quantity and quality of resources for offspring development. These trees also tend to be very abundant in mature pine-dominated landscapes, and so this behavior is an adaptation that provides access to plentiful, high-quality resources in the absence of interspecific competition (Raffa et al., 2008).

Pinus spp., such as lodgepole pine, have evolved highly effective defenses against attack by bark beetles (Franceschi et al., 2005; Keeling and Bohlmann, 2006). When severed,

constitutive resin ducts in the bark exude resin that physically and chemically repels attacking beetles (Berryman, 1972; Shrimpton and Whitney, 1968). If beetles overwhelm the constitutive defenses, penetrate the bark and encounter the live tissues beneath, an induced response is initiated that involves the breakdown of parenchyma cells, formation of traumatic resin ducts, and the production of secondary resin containing higher concentrations of toxic terpenes and phenolic chemicals (Franceschi et al., 2005; Safranyik and Carroll, 2006). Mountain pine beetles have evolved to take advantage of host defensive chemicals, particularly in the use of the monoterpene resin constituents (+)- and (-)- α -pinene as pheromone precursors. Alpha-pinenes are converted to *trans*-verbenols via cytochrome p-450 mediated hydroxylation by females upon initiation of attack (Blomquist et al., 2010; Gries et al., 1990; Pierce et al., 1987). There are other aggregation pheromones utilized by mountain pine beetles, such as *exo*-brevicomin, but *trans*-verbenol, particularly (-)-*trans*-verbenol, is the primary semiochemical for initiation of mass attack (Borden et al., 1987; Progar et al., 2014). Epidemic mountain pine beetles respond in a dose-dependent fashion to *trans*-verbenol, but not to *exo*-brevicomin, which is synthesized *de novo* by males and is therefore mostly independent of host tree chemistry (Blomquist et al., 2010; D. Miller et al., 2005). Mountain pine beetle's dependence on *trans*-verbenol for secondary attraction suggests that variations in host tree α -pinene content across populations and species may affect the beetle's aggregation rates and attack success, as (-)- α -pinene is the direct precursor of (-)-*trans*-verbenol. Mountain pine beetles in newly invaded pine forests have begun to encounter elevated concentrations of α -pinenes; comparison of lodgepole pine phloem chemistry reveals more α -pinenes in naïve populations (Clark et al., 2010), and jack pines are comprised of even greater concentrations of α -pinenes in stem tissues (Clark et al., 2014; Hall et al., 2013; Taft et al., 2015b). Recent studies have also shown α -pinene content of the phloem is directly related

to the production of (-)-*trans*-verbenol by feeding female mountain pine beetles, irrespective of the stereochemistry of the precursor (Erbilgin et al., 2014; Taft et al., 2015a). In this study, I evaluated the hypothesis that α -pinene content will influence the secondary attraction of mountain pine beetles in an active infestation. My prediction was that higher α -pinene content in infested bolts will render them more attractive to foraging beetles.

3.2 Methods and Materials

3.2.1 Experimental Site Selection

To assess aggregation by mountain pine beetles in response to differing levels of α -pinene within their hosts, I conducted a field study within the historic range of the beetle (Safranyik and Carroll, 2006) near Oliver, British Columbia, Canada (N49.110°, W119.177°; elevation ~1300 m) in July of 2012. Sites were established in mature lodgepole pine stands, with a few interspersed aspen (*Populus tremuloides* Michx.) and western larch (*Larix occidentalis* Nutt.). Stands were 80-100 years old and averaged ~20 m²/ha basal area with ~600 stems per hectare, which are conditions considered highly suitable for mountain pine beetle outbreak (Shore and Safranyik, 1992). Stands were located in the transition between the Interior Douglas-fir and Montane Spruce biogeoclimatic zones (<https://www.for.gov.bc.ca/hre/becweb/> [accessed October 18, 2016]). Epidemic mountain pine beetle infestations were observed (i.e. clusters of large-diameter trees with foliage “fading” from yellow to red) sporadically throughout the area.

3.2.2 Trap Bolt Selection and Preparation

Naïve lodgepole (LPN), jack pine (JP), and lodgepole × jack pine hybrid (HYB) bolts were harvested near Slave Lake, Alberta, Canada [LPN: N54°51' W115°18', elev. 900 m; JP: N55°04' W114°06', elev. 577 m; HYB: N55°33' W114°53', elev. 652 m], from sites that had been thoroughly genotyped by Cullingham et al. (2011), and immediately transported to the experimental site. Experienced lodgepole pine (LPS) bolts were cut from a stand adjacent to the experimental site. I chose these source locations, and host populations and species to maximize the potential range of α -pinene content. Individual trees were not necessarily expected to be representative of populations or species. Bolts were sectioned from two trees from each site, using the lower part of the bole where diameter was 25-35 cm. Each bolt was cut to 70 cm long and both ends were immediately sealed using paraffin wax at the harvesting location to prevent volatiles from escaping and to prevent desiccation. All bolts (n=36) had an eye hook installed in one end, and were hung vertically between two trees on a line supported by a tripod in the middle. Bolts were hung at least 2 meters from each other and the support trees, and their bottom was about 1 meter from the ground. Bolts were deployed in trials within two days of harvesting. One bolt of each type (4) was randomly assigned to a replicate (9), and 3 replicates were randomly assigned to three different stands.

3.2.3 Artificial infestations and sampling

In May 2012, two naturally infested trees were harvested from the experimental site and transported to the UBC Forest Insect Disturbance Ecology Lab (FIDEL) in Vancouver, BC. In the laboratory, beetles were allowed to complete their development and emerge. Following emergence, beetles were segregated by sex and transported back to the experimental site. Once

all bolts were installed, two 8 mm holes were punched at randomly selected opposing cardinal directions approximately 5 cm from the base, to a depth ~1 mm into the sapwood. One female mountain pine beetle was placed into a gelatin capsule, and one half of the gelatin capsule was inserted into each hole. The beetles were left in this position for 24 hours. After that period, the capsules were examined for the presence of boring dust, indicating host acceptance and initiation of gallery construction. If the beetles failed to initiate gallery formation, they were replaced with a second beetle in the same manner, and the replacement beetle was left in this position for 24 hours. After this period, all gelatin capsules were removed regardless of introduction success. The bolts were then left in the stands for 7 days, after which they were removed and transported back to the FIDEL lab.

In the laboratory, phloem thickness was determined by taking four equidistant, 1-cm-diameter samples from each bolt, and calculating the mean. Outer bark from these samples was removed, and remaining phloem tissue was stored at -30°C for chemical analysis. Then, all the outer bark and phloem was removed from each bolt and the number and lengths of all initial and subsequent attack galleries were measured.

3.2.4 Chemical Analyses

Samples collected for chemical analysis were dissected and extracted in *tert*-butyl methyl ether [MTBE] for 24 hours, with 75 ppm iso-butyl benzene [IBB] as an internal standard.

Phloem terpene concentrations and ratios were calculated using an Agilent 6890 Gas Chromatograph 5973 Mass Spectrometer with a SGS Solgelwax column [30 m x 250 µm, 250 µm thickness]. Oven initial temperature was 40°C, stepped up to 85°C at 3°C per minute, then to 180°C at 10°C per minute, then to a final temperature of 260°C at 15°C per minute and held for

10 minutes. Pulsed splitless injection at 20.0 psi was used with a helium carrier, with a pulse time of 0.05 minutes.

3.2.5 Statistical Analyses

All statistical tests were conducted using SAS® Statistical Software version 9.4 (SAS Institute, Cary NC, USA, 2014). I applied PROC UNIVARIATE to perform Shapiro-Wilk tests of normality on absolute and relative terpene concentration data. Absolute phloem terpene concentration data were square-root transformed to achieve normality, and data for relative concentrations were arcsine (square-root) transformed to account for the distribution of proportion data (Dowdy et al., 2004). I applied PROC GLM to perform analysis of variance where bolt type is fixed and block and replicate are random effects. I used the MEANS statement with the TUKEY option to perform post-hoc comparison of means on monoterpene and attack data.

To assess the effect of α -pinene content on secondary attraction, I performed a series of tests using only those bolts with at least one successful introduction. First, I plotted data from this subset against the arcsine(square-root) transformed relative concentrations for α -pinene, and fit a curve using R® version 3.3.1 (R Core Team, Vienna, Austria, 2016). As these are count data, I then performed poisson regression using the glm function in R® (Dowdy et al., 2004).

3.3 Results

None of the bolts in any of the replicates in block 1 were colonized, likely due to the natural initiation during the experiment of a mass attack on a large tree (> 40 cm diameter) within the stand which would have acted as a strong sink for foraging beetles. This stand was

removed from the analyses, and all subsequent results were generated from the two remaining stands. Over half (54%) of the bolts had at least one successful introduction, indicated by the presence of boring dust in the capsule, and there was no effect of bolt type on the number of successful introductions ($F_{3,23}=0.83$, $p=0.497$). Phloem thickness was not different across types ($F_{3,23}= 2.43$, $p=0.120$).

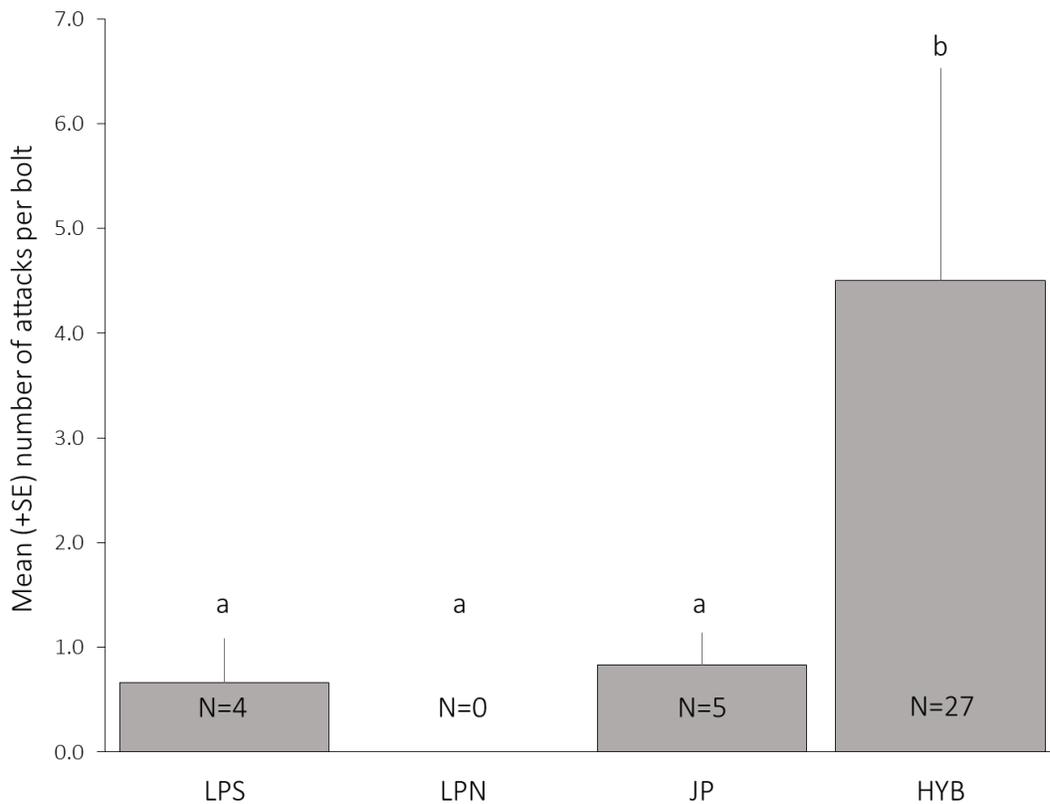


Figure 3.1: Mean (+SE) number of attacks per bolt, by bolt origin location. LPS are lodgepole pine bolts from southern British Columbia, LPN are lodgepole pine bolts from Alberta, JP are jack pine bolts from Alberta, and HYB are lodgepole/jack hybrid bolts from Alberta. N is the total number of attacks. Different letters indicate significant differences based on Tukey’s post-hoc comparison of means.

There was a significant effect of bolt type on the number of attacks ($F_{3,23}= 4.48$, $p=0.027$), with 75% of attacks occurring on the hybrid type [Fig. 3.1]. Total monoterpene content was approximately 2x higher in the two lodgepole pine bolt types ($F_{3,23}=6.75$, $p=0.007$), and this pattern was reflected in the other monoterpenes with the exception of α -pinenes

($F_{3,23}=1.94$, $p=0.162$) and 3-carene ($F_{3,23}=2.96$, $p=0.062$). However, no measure of absolute monoterpene concentrations could explain the pattern of attacks. The relative concentrations of individual monoterpenes differed significantly among bolt types [Fig. 3.2], and in keeping with the pattern of attacks, phloem from the hybrid bolts had the highest α -pinene content as a percent of total monoterpenes (HYB: ~80%, LPS: ~24%, LPN: ~26%, JP: ~50%). There was little variation in the relative concentrations within bolt types [Fig. 3.2], probably due to the fact that harvested trees were adjacent to each other and likely to be highly related (Ott et al., 2011). The number of attacks on infested bolts was significantly affected by the relative concentration of α -pinene ($Z_{13}=4.95$, $p<0.001$), and attacks increased exponentially with increasing relative α -pinene concentrations ($r^2=0.535$) [Fig. 3.3].

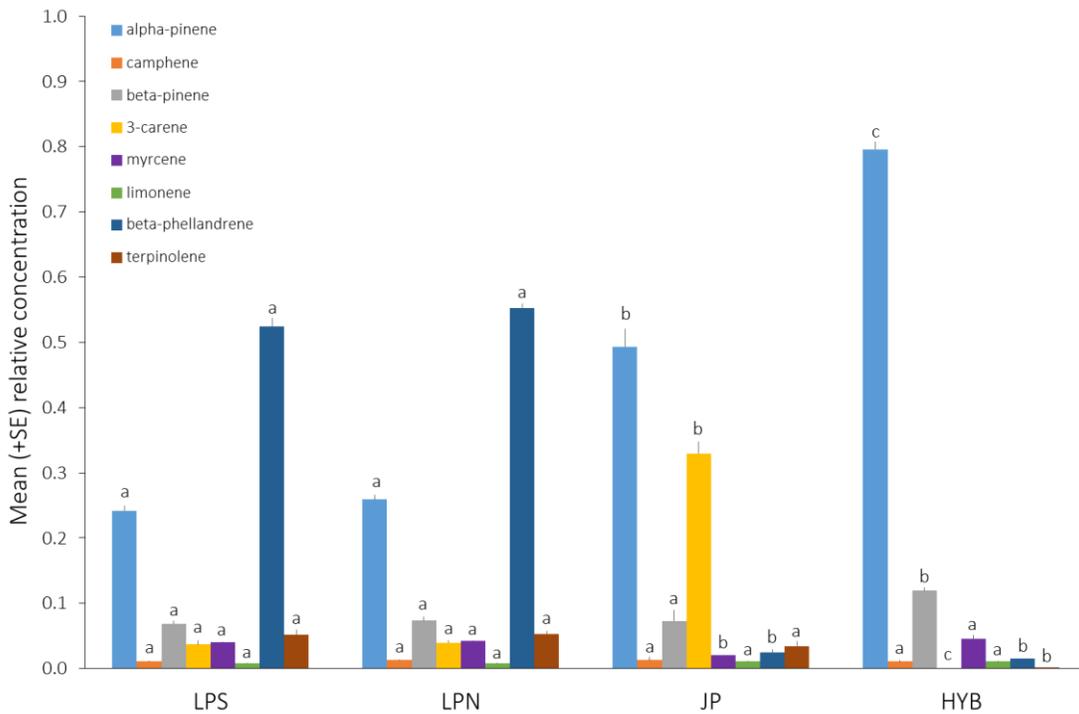


Figure 3.2: Mean (+SE) relative concentration of the 8 major (>2.5% of total) monoterpene components in phloem tissue. Different letters indicate significant differences among bolt origin locations, based on Tukey's post-hoc comparison of means. LPS are lodgepole pine bolts from southern British Columbia, LPN are lodgepole pine bolts from Alberta, JP are jack pine bolts from Alberta, and HYB are lodgepole/jack hybrid bolts from Alberta.

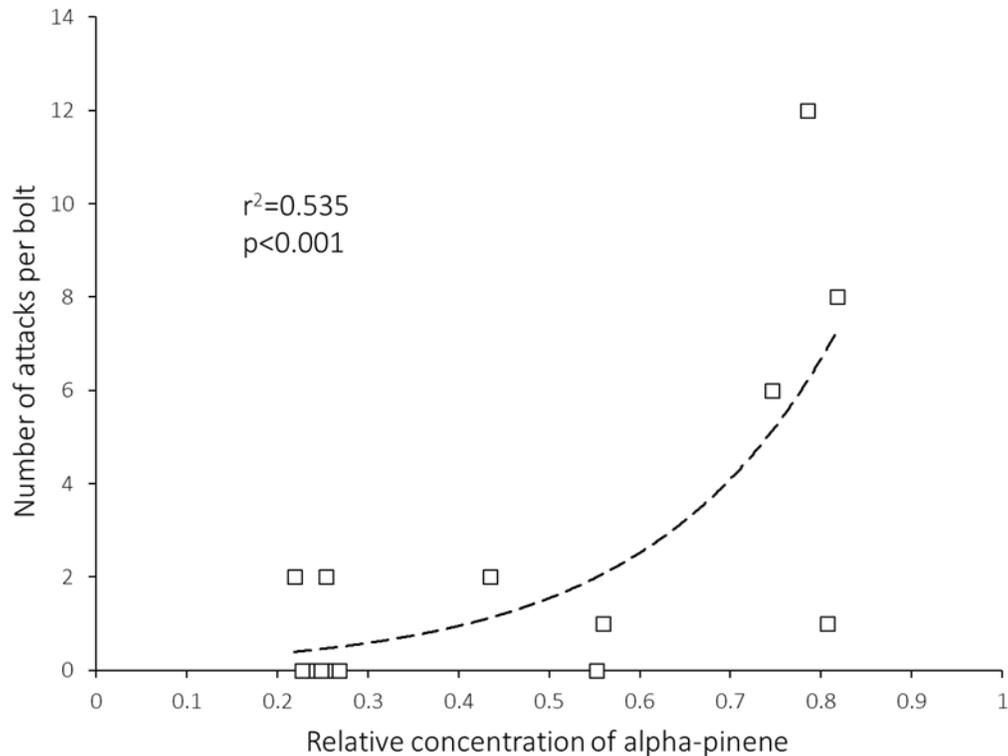


Figure 3.3: Number of attacks as a function of relative α -pinene content, for those bolts with at least one successful introduction of a female mountain pine beetle prior to exposure to natural populations. Dashed line is the fitted exponential curve, and the p-value is associated with poisson regression.

3.4 Discussion

Elevated concentrations of terpenoid pheromone precursors in evolutionarily naïve hosts significantly affected the dynamics of secondary attraction by the mountain pine beetle. The increased rate of aggregation to host material with high relative α -pinene content demonstrated in my study suggests that patterns of host selection and colonization may be altered by shifting host chemistry as the beetles expand their range eastward and northward. These results, in addition to the observations that naïve hosts are less able to resist attack (Clark et al., 2014, 2010; Erbilgin et al., 2014; Raffa et al., 2013), and that beetle reproductive output is enhanced in novel hosts

(Clark et al., 2010; Cudmore et al., 2010) compared to experienced hosts, support assertions (Cudmore et al., 2010; Raffa et al., 2013) that the recent invasion of naïve pine forests is analogous in many ways to an exotic introduction, where the herbivore and host trees have insufficiently coevolved. Although considerable caution must be exercised when extrapolating results derived from the tree and stand scale to the level of landscapes, my results suggest that the tendency for naïve pine populations and species in recently invaded areas to contain higher levels of α -pinene than host trees within the beetle's native range (Clark et al., 2014, 2010; Erbilgin et al., 2014; Taft et al., 2015a, 2015b) could be a critical determinant in the surprisingly rapid spread of the mountain pine beetle through the western boreal forest (Safranyik et al., 2010) since its arrival east of the Rocky Mountains.

The result that relative α -pinene content was the best predictor of attraction was somewhat surprising, given that my original prediction was focused on absolute concentration. However, results of recent studies lend support to my interpretation. Erbilgin et al. (2014) found that beetles feeding in jack pine bolts produced about twice the amount of *trans*-verbenol than did those feeding in lodgepole pine. The jack pine bolts in their study comprised of approximately 2.5 times the relative concentration of α -pinene in relation to lodgepole pine, and a similar magnitude of difference was seen in my study, thereby suggesting that my results are driven by increased production of *trans*-verbenol. Taft et al. (2015a) also found similar results; *trans*-verbenol production was enhanced for beetles feeding in logs from the group with the highest relative concentrations of α -pinenes. In addition, I observed that among the hybrid type bolts in my study, those that had at least one successful introduction were always attacked, and those with no introductions were not attacked. This lends further support to the interpretation that my results are due to *trans*-verbenol production and not to any characteristic of the bolts

themselves, such as bark morphology or volatiles associated with primary attraction.

Additionally, the number of attacks in relation to the relative α -pinene content of bolts was best described using an exponential curve suggesting that *trans*-verbenol production increases at an increasing rate as more beetles arrive and begin feeding.

The implications of higher aggregation rates toward host trees with higher relative α -pinene content are considerable in terms of the potential for the mountain pine beetle to persist within the novel pine forests of the western boreal region, and continue its movement into forests further north and east. Jack pine is reported to contain 30-90% α -pinenes (Clark et al., 2014; Erbilgin et al., 2014; Hall et al., 2013; Taft et al., 2015a, 2015b; Wallin and Raffa, 1999), which is significantly more than any of the other major host species within the beetle's historic range in western Canada; 7-25% in ponderosa pine [*Pinus ponderosa* Douglas] (Farentinos et al., 1981; Sturgeon, 1979), 5-25% in lodgepole pine (Clark et al., 2014, 2010; Erbilgin et al., 2014), and 7-12% in whitebark pine [*Pinus albicaulis* Englem.] (Raffa et al., 2013). Thus, colonized jack pine trees may represent a supernormal stimulus (Tinbergen and Perdeck, 1950) to foraging mountain pine beetles, and fewer beetles may be required to initiate mass attack. If host location potential is enhanced, as suggested by Clark et al. (2010), it may also be possible for mountain pine beetles to overcome some of the constraints to establishment and movement through the boreal, such as low pine volume and stand connectivity (Safranyik et al., 2010). If a beetle can find an infested jack pine tree from further away, for example, then she may be able to overcome the difficulties of low relative host abundance.

Anthropogenic drivers, such as climate change and forest management activities that affect the distribution and abundance of susceptible trees, can directly and indirectly affect the complex dynamics of biotic disturbance agents over extensive landscapes (Raffa et al., 2008).

Indeed, climate change-induced range expansion, a shift to novel host populations and species, and resultant exposure to altered trophic interactions by the mountain pine beetle is an example of such modified dynamics. Long-term associations among species through evolutionary time act to maintain ecosystem stability and resilience (Dawkins and Krebs, 1979). Where herbivore outbreaks are common, they are often essential aspects of plant life histories and are therefore considered normative (Herms and Mattson, 1992; Logan and Powell, 2001). Accumulating evidence suggests that rapid distributional changes by an eruptive forest insect herbivore in response to a warming environment can lead to extraordinary outbreaks and extensive disturbance to inexperienced ecosystems (Raffa et al., 2008; Safranyik et al., 2010) with the potential for considerable economic and ecological damage. Additional research is required to fully understand the potential for native forest insect herbivores to become invasive due to climate change-induced range shifts.

4 Coevolution, climate-change, and the emergence of a native invasive herbivore

4.1 Introduction

Introductions of invasive insect species have been increasing exponentially across the world, as a consequence of anthropogenic climate change and globalization (Hulme, 2009; Liebhold et al., 1995; Mattson et al., 1994; Ramsfield et al., 2016). Within climatically and geographically suitable habitats, the ability of an invasive insect herbivore to establish is influenced by interspecific interactions, host-plant defense potential, and biological traits of the invading species (Cullingham et al., 2011; Davis, 2009; Liebhold et al., 2013; Raje et al., 2016; Rochlin et al., 2013). The invasive potential of insect herbivores is often enhanced in new habitats where they encounter both enemy- and defense-free space, resulting from a lack of co-evolutionary association with those elements of the invaded habitats (Gandhi and Herms, 2010; Jeffries and Lawton, 1984). As a consequence of missing or ineffective population regulators, invasive arthropods can have devastating consequences on invaded habitats and lead to cascading effects at multiple trophic levels (Burke et al., 2011; Gandhi and Herms, 2010; Hanula et al., 2008; Herms and McCullough, 2014; MacFarlane and Meyer, 2005; Poland and McCullough, 2005).

Global climate change has affected species from all taxonomic groups, often in the form of altered ranges (Parmesan and Yohe, 2003; Parmesan, 2006). Terrestrial ectothermic organisms are particularly susceptible to variations in weather and climate due to their sensitivity to temperature and its direct and indirect effects on critical life history parameters such as development rate, life cycle duration, winter survival, and phenological synchrony (Deutsch et al., 2008; Jönsson et al., 2009; Musolin, 2007). Throughout the Holarctic region, the effects of climate warming on herbivorous insects have often resulted in range expansions into previously

thermally unsuitable habitats (Hickling et al., 2006, 2005), especially in forest ecosystems where the herbivores are not constrained by their host's range (Carroll et al., 2004; Jönsson et al., 2009; Logan et al., 2003; Netherer and Schopf, 2010). Rapid range expansion in response to climate change may result in novel herbivore-host interactions, potentially exacerbating herbivore impacts and accelerating range shifts due to weakly coevolved or evolutionarily naïve host defenses (Braschler and Hill, 2007; Cudmore et al., 2010; Cullingham et al., 2011; Raffa et al., 2013).

The mountain pine beetle [*Dendroctonus ponderosae* Hopkins] is an exceptional example of climate-induced range expansion by an insect herbivore (Carroll et al., 2004; Sambaraju et al., 2011), having drastically expanded in recent years to higher latitudes and elevations in North America, well beyond previous geographical limits (de la Giroday et al., 2011; Safranyik et al., 2010). Mountain pine beetle populations were previously constrained by the effects of extreme low temperatures on over-winter survival (Carroll et al., 2004; Safranyik, 1978), and the possible effects of climate on temperature-based developmental synchrony (Bentz et al., 1991; Logan and Powell, 2001). As a result of increasing temperatures, the amount of thermally benign habitat in western Canada that is available to the mountain pine beetle has dramatically increased (Carroll et al., 2004).

Although the range of the mountain pine beetle was previously restricted to areas of western North America south of 56°N (Safranyik and Carroll, 2006), the distribution of its principal host, lodgepole pine [*Pinus contorta* var *latifolia* Douglas], stretches north into the Yukon Territory [~64°N latitude] and east of the Rocky Mountains into Alberta, Canada [~115°W longitude] (Farrar, 1995). Recently, epidemic mountain pine beetle populations have been detected as far north as 59°N in British Columbia, only 100 km from the border of the

Yukon Territory (Nealis and Cooke, 2014) [Fig. 4.1]. Moreover, in 2002 the beetle was found to have breached the geoclimatic barrier of the Rocky Mountains to the east, and infestations are now established in hybrid lodgepole pine × jack pine (*P. contorta* × *P. banksiana* Lamb.) stands in the montane-boreal transition zone (Safranyik et al., 2010), and in pure jack pine stands in northwestern Alberta (Cullingham et al., 2011). The beetles also now occupy, and are proving detrimental to, high elevation whitebark pine forests [*Pinus albicaulis* Englemann] in the United States and Canada (Bentz and Schen-Langenheim, 2007; Raffa et al., 2013).

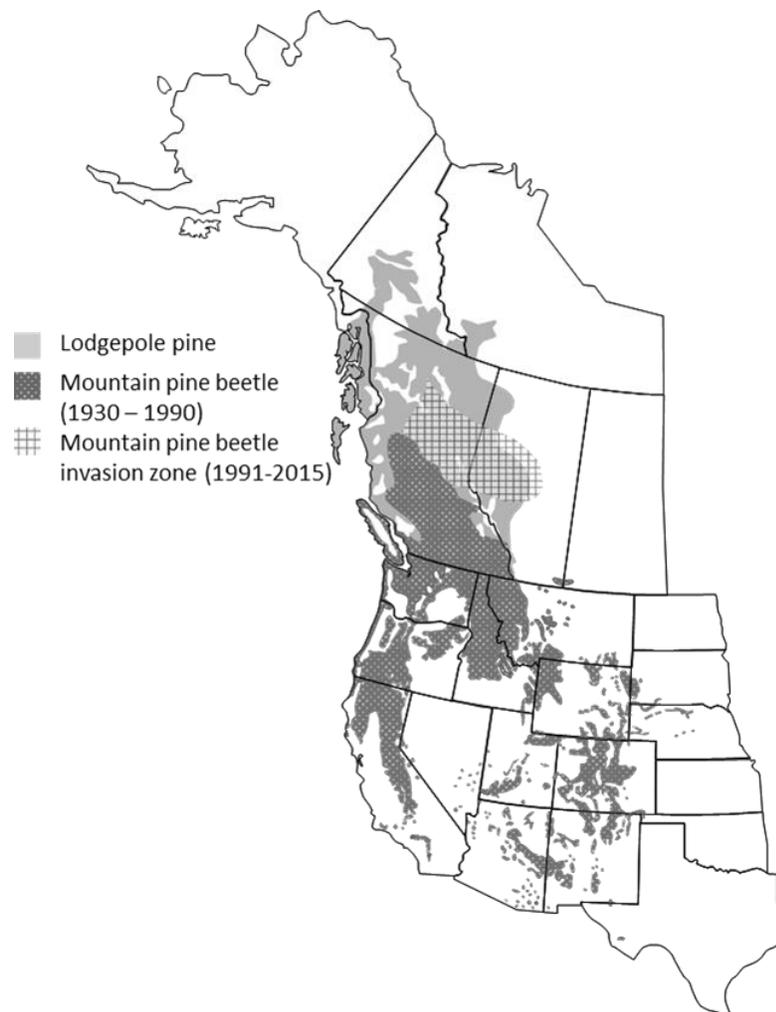


Figure 4.1: Range of lodgepole pine (*Pinus contorta* var. *latifolia*), historic (1930 – 1990) range of mountain pine beetle (*Dendroctonus ponderosae*), and estimated area of recent expansion zone of mountain pine beetle in western North America. Lodgepole pine range adapted from Farrar (1995), historic mountain pine beetle range adapted from Safranyik and Carroll (2006), and expanded range adapted from Nealis and Cooke (2014).

Mountain pine beetles are subcortical herbivores that feed within the phloem of their host trees. They are generalists within the genus *Pinus* in western North America, and have been shown to successfully reproduce in a wide variety of native and exotic pine species (Furniss and Schenk, 1969; S. Wood, 1982). Emergence and dispersal from the natal host tree occurs in July or August, and foraging females select a new host based on a range of factors pertaining to beetle population size and host tree chemistry and physiology (Boone et al., 2011; Burke and Carroll, 2016; Pureswaran and Borden, 2003). The normative population state is the low-density endemic phase, where beetles occupy defensively compromised hosts and are often associated with other, less aggressive subcortical insects (Boone et al., 2011; Safranyik and Carroll, 2006; Smith et al., 2011). During periodic epidemic phases, they employ pheromone-mediated mass attack to overwhelm the defenses of vigorous, mature host trees and can impact forests on a landscape scale (Boone et al., 2011; Kurz et al., 2008; Raffa et al., 2008; Safranyik and Carroll, 2006; D. L. Wood, 1982).

Successful colonization by the mountain pine beetle depends on the death of its host tree, thereby creating potentially intense selection pressure for the evolution of effective mechanisms in trees to resist attacks (Safranyik and Carroll, 2006). Host trees defend against mountain pine beetle attacks in two ways. Constitutive, preformed resin ducts in subcortical tissues contain defensive material under hydraulic pressure, and when severed during attack these exude resin that physically and chemically repels beetles (Berryman, 1972; Shrimpton and Whitney, 1968). If beetles successfully penetrate the outer bark and enter the phloem beneath, a highly complex response is initiated that may involve the breakdown of parenchyma cells, formation of traumatic resin ducts, and the increased production of resin terpenoids (Franceschi et al., 2005; Keeling and Bohlmann, 2006; Raffa and Berryman, 1983a; Raffa et al., 2005; Safranyik and Carroll,

2006). In conifers, there is a great diversity of resin terpenoids as a likely result of selection pressure from insects, as they adapt to increasingly complex chemical defenses in a long-fought evolutionary arms race (Dawkins and Krebs, 1979; Keeling and Bohlmann, 2006). As a result of this complex chemical diversity, induced resinosis is highly toxic to most subcortical insects and their associated microorganisms (Raffa et al., 2005). Eventually, the induced defense reaction will lead to the formation of auto-necrotic lesions in the host that isolate the infested/infected area from surrounding tissues, thereby halting progression by the insect and/or pathogen (Paine et al., 1987; Raffa and Berryman, 1983a; Wallin and Raffa, 1999).

The recent range expansion by the mountain pine beetle, both within the range of lodgepole pine and into novel host types, comprises an invasion of novel habitats. The population genetic structure of the mountain pine beetle shows a gradual expansion northward (Bentz et al., 2010; Mock et al., 2007), which lagged behind the earlier, post-glacial expansion of lodgepole pine (Cwynar and MacDonald, 1987; MacDonald and Cwynar, 1985; Wheeler and Guries, 1982). This suggests that forests north and/or east of the historic mountain pine beetle range have been under no recent evolutionary selection pressure from beetle attacks.

Constitutive and induced defensive resin in novel host-tree populations is both quantitatively and qualitatively different when compared to lodgepole pine trees in the native range (Clark et al., 2014, 2010). In addition, lodgepole pines from novel habitats contain elevated α -pinene content in constitutive resin (Clark et al., 2010); (+)- and (-)- α -pinene are pheromone precursors and lead to higher quantities of pheromone produced by beetles in the laboratory (Erbilgin et al., 2014; Taft et al., 2015a), and greater rates of aggregation by foraging beetles *in situ* (Burke and Carroll, 2016). Lower defensive capacity and greater apparency of trees has been suggested as the source of observed higher attack densities (Clark et al., 2010) and productivity (Cudmore et al., 2010)

by beetles in lodgepole pine trees from previously unoccupied regions, and higher rates of spread and impact by outbreak populations in these novel habitats (Robinson, 2015). However, it remains unclear whether these phenomena are a result of limited evolutionary exposure to the mountain pine beetle among populations of lodgepole pines beyond the beetles' historic range.

Variation in the induction of monoterpene defenses has been shown to be the primary determinant of susceptibility to mountain pine beetle-induced mortality for individual lodgepole pine trees (Boone et al., 2011; Raffa and Berryman, 1982b). Given the expectation of intense selective pressure by mountain pine beetles on its host trees, I conducted an extensive field study to critically evaluate the hypothesis that prolonged exposure to epidemic mountain pine beetles will select for a specific induced response to beetle attack. If this hypothesis is correct, I predicted that the quantity of induced monoterpenes expressed in response to simulated attack, relative to a generic defensive response elicitor, will increase in lodgepole pine populations with increasing historical exposure to epidemic mountain pine beetle impacts.

4.2 Methods and Materials

4.2.1 Study sites

Estimates of historic climatic suitability (HCS) (Carroll et al., 2004) were used as a proxy for the degree of evolutionary association between the mountain pine beetle and populations of lodgepole pine. Historic climatic suitability is derived from calculations of the joint likelihood of four conditions shown to be critical to the establishment and persistence of mountain pine beetle populations (Carroll et al., 2004; Safranyik et al., 1975): a univoltine life cycle, temperatures conducive to over-winter survival, optimal emergence/dispersal conditions, and the adequacy and variability of spring precipitation as an estimate of relative tree defensive capacity (see: Carroll et al., 2004). Maps of historic climatic suitability estimates for western Canada

were produced using BioSIM©, a spatially explicit, climate-driven simulation tool (Régnière, 1996; Régnière et al., 2014), and historic weather records from the Environment Canada Meteorological Service for the period 1921 – 1950. This time window predates any recorded shifts in mountain pine beetle range (Carroll et al., 2004) and was therefore considered a conservative representation of historic conditions. Mapped outputs comprised grid coverage of historic climatic suitability values within 64-ha cells (see: Carroll et al. 2004) for British Columbia and Alberta.

To assess variation in the induced defensive response by populations of lodgepole pine trees with different degrees of historical exposure to the mountain pine beetle, stands of trees were selected from regions representing a range of historic climatic suitability. In July of 2013 and 2014, two lodgepole pine stands from 4 regions of western Canada with varying HCS were selected (8 total stands; Fig. 4.2, Table 4.1). In Tumbler Ridge and Hinton ($HCS \leq 0.01$), no mountain pine beetle infestations had been recorded prior to 1970, at Baldy Mountain ($HCS 0.10 - 0.15$) infestations have occurred periodically, and at Merritt ($HCS > 0.25$) infestations have occurred regularly during the previous century (Taylor et al., 2006). In each stand, 11 lodgepole pine trees were selected for analysis ($n=88$ total trees). Each tree was > 20 cm (Table 4.1) diameter at breast height (1.3 m), with a clear, one-stem bole and no apparent evidence of disturbance by biotic or abiotic stressors. Trees directly on an edge were not selected. None of the stands had any signs of current-year mountain pine beetle activity at the time of the experiment. The mensurational characteristics of each stand were determined from three, 100 m^2 plots per site (Table 4.1).

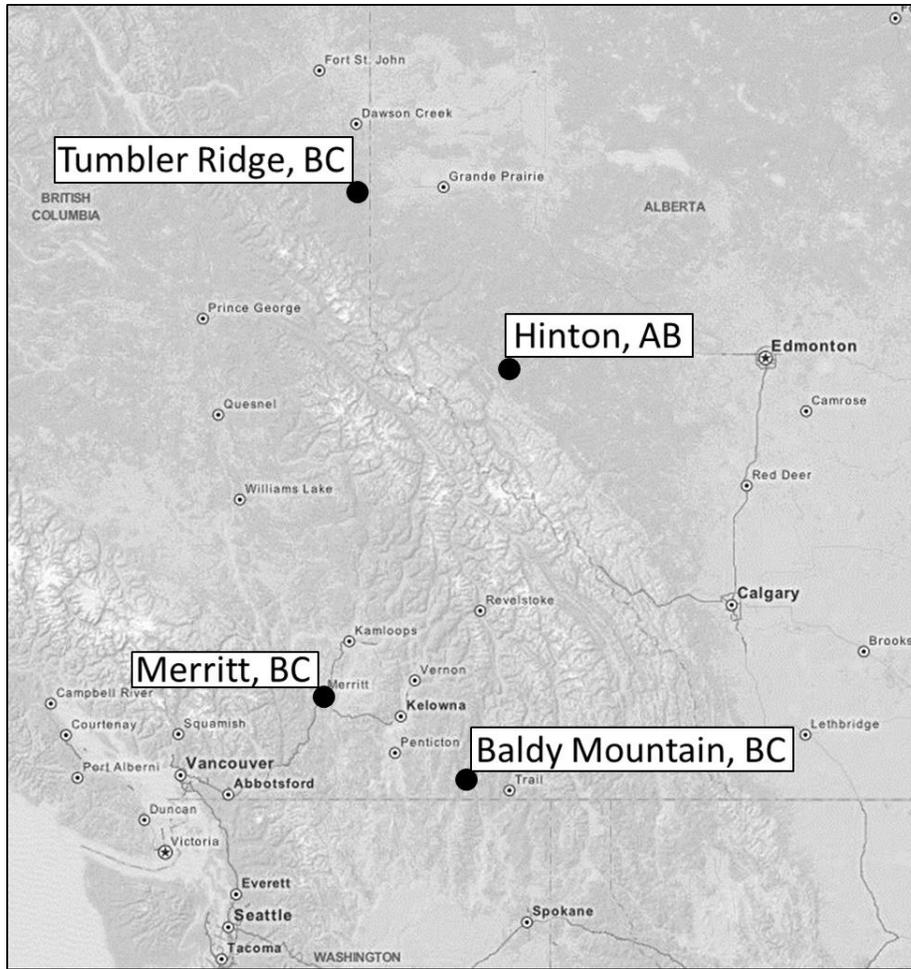


Figure 4.2: Location of lodgepole pine (*Pinus contorta*) stands in western Canada with varying degrees of historic exposure to the mountain pine beetle (*Dendroctonus ponderosae*) determined from calculations of historic climatic suitability (*sensu* Carroll et al. 2004). Map created using Open Street Map© Software (openstreetmap.org, OpenStreetMap contributors, under ODbI, <http://www.openstreetmap.org/copyright>, 2015).

4.2.2 Experimental treatments

Assessments of variation in tree defenses in response to subcortical challenges often encounter enormous inter-tree variability, making it difficult to isolate and identify treatment effects (Clark et al., 2014; B. Miller et al., 2005; Raffa and Smalley, 1995, 1988). To control for inter-tree variation and allow direct comparison of treatment effects among sites, I exploited the propensity for *Pinus* species to respond to minor wounding through localized reactions, rather than systemic physiological alterations (Raffa and Smalley, 1988; Wallin and Raffa, 1999).

Paired treatment and control manipulations were applied to each tree allowing the difference of the two to be assessed as the treatment effect, thereby equalizing the potential influences of inter-tree variation across both manipulations.

4.2.2.1 Mountain pine beetle simulation

Variation in the induced defensive response against the mountain pine beetle among the different lodgepole pine populations was assessed using simulated attacks comprising a mechanical wound combined with a single inoculation of the beetle's fungal symbiont *Grosmannia clavigera* (Robinson-Jeffrey & R.W. Davidson). The *G. clavigera* strain was isolated from lodgepole pine trees harvested in Alberta, Canada in 2011 near Grande Prairie (55.16 N, 118.80 W), and a voucher specimen is maintained at the University of British Columbia Forest Pathology Laboratory (D. Alayon and R. Hamelin, unpubl). Application of this fungus as an analog for mountain pine beetle attack is an established method, which elicits a reaction similar to live beetles (Boone et al., 2011; Raffa and Berryman, 1982b; Wallin and Raffa, 1999). Agar plugs containing the fungus were removed from cold storage and propagated on petri plates two weeks prior to their application in the field. To prepare these plates, an even mixture of malt extract agar (MEA) and unammended agar (Fisher-Scientific) was mixed with distilled water at 33 g/L. After autoclaving, the agar media was poured into 10 cm petri plates (~2 mL/plate) and left to cool. Agar plugs containing the fungus were then placed in the center of the plates, and these were sealed and incubated at 20°C for 7 days, until mycelium had reached the edge of the plate. Plates were stored at 5°C on ice for transport to the forest. In the field, plates were removed from cold storage and maintained at ambient temperature for 24 hours before use, to allow growth to resume.

4.2.2.2 Methyl jasmonate

The potential for specificity of induced defenses to mountain pine beetle attack was determined by comparing the response associated with simulated beetle attacks to the response associated with application of a general plant defensive response elicitor. Methyl jasmonate (MeJA) is a phytohormone that is involved in the induced defensive response of all plants (Creelman and Mullet, 1997), and has been used extensively to examine the defensive response of conifers (Franceschi et al., 2002, 2005; Graves et al., 2008; Hudgins et al., 2003; Krokene et al., 2008; Martin et al., 2002; Zeneli et al., 2006; Zulak and Bohlmann, 2010). In conifers, methyl jasmonate up-regulates genes and proteins associated with defense signaling and secondary metabolism, and its application induces a broad defense response (e.g. Hamberger et al., 2011; Miller et al., 2005; Zulak et al., 2009). A MeJA solution was prepared by mixing MeJA (95% purity, Sigma-Aldrich) with Tween© 20 (Sigma-Aldrich) in water both at 0.1% v/v concentration (Hudgins et al., 2003) and was stored at 5°C until use.

Table 4.1. Mensurational characteristics of eight lodgepole pine (*Pinus contorta* var. *latifolia*) stands (i.e. populations) assessed for the specificity of their defensive response against the mountain pine beetle (*Dendroctonus ponderosae*). Historic climate suitability score for mountain pine beetles is determined using the methods of Carroll et al. (2004) and climate normals from the period 1921-1951. Mean diameter is that of experimental lodgepole pine trees. Density and species composition is that of all trees >7.5 cm diameter at 1.3 m, determined from three, 100 m² plots per site.

Region		Location	Elevation (m)	Historic climate suitability score*	Mean Diameter (cm) at 1.3 m [\pm SE]	Density (trees/ha)	Percent live <i>P. contorta</i>	Percent dead <i>P. contorta</i>	Percent non-host**
Merritt, BC	1	N49.783, W120.734	927	0.32	23.5 [1.02]	1466	79.6	2.5	17.7
	2	N49.985, W120.590	995	0.27	26.7 [0.98]	2700	50.6	48.3	1.1
Baldy Mtn, BC	1	N49.111, W119.177	1291	0.15	24.3 [0.87]	933	71.1	11.1	17.8
	2	N49.108, W119.275	1422	0.12	23.8 [0.69]	533	76.3	16.0	7.7
Tumbler Ridge, BC	1	N54.991, W120.844	1027	0.01	26.8 [1.07]	1866	79.2	16.4	4.2
	2	N54.744, W120.551	1194	0.01	26.9 [1.05]	2166	44.1	18.0	37.9
Hinton, AB	1	N53.559, W117.931	1252	0.00	27.8 [0.95]	1400	94.2	5.7	0.0
	2	N53.583, W118.038	1380	0.00	28.8 [1.07]	1300	82.7	12.2	5.1

* Scores as they relate to suitability (Carroll et al., 2004): 0 = very low; 0.01–0.05 = low; 0.06–0.15 = moderate; 0.16–0.35 = high

** Non-host species include Douglas-fir (*Pseudotsuga menziesii*), subalpine fir (*Abies lasiocarpa*), spruce (*Picea spp.*), larch (*Larix spp.*), and trembling aspen (*Populus tremuloides*).

4.2.2.3 Treatment application

At each tree, a random cardinal direction was selected and the mountain pine beetle attack simulation treatment was applied at breast height (1.3m). MeJA treatment was applied at the same time to the same tree at breast height on the opposite side of the bole. All treatments were applied with high-carbon steel tools sterilized and cleaned with 100% ethanol between each use. To apply treatments, the outer bark around each application point was scraped using a knife to remove debris and provide a flat area to punch through. A 1 cm round disk of outer bark and phloem was removed using a leather punch (C.S. Osborne, Harrison NJ, USA). If a lesion or discoloration was discovered in the xylem or phloem, a new punch was taken ~ 15 cm to the right, until clear wood was found. If unaffected tissue was not found after three punch-attempts, that tree was abandoned. To apply the fungal treatment, a flat-tipped knife was used to remove a 1.0 cm² piece of agar from the petri dish, which was then packed into the wound site. To apply the MeJA treatment, a small piece of sterile dentist cotton was packed into the hole, and ~0.5 mL of MeJA solution was applied to the cotton using a syringe. This amount fully saturated the cotton, but did not allow the solution to drip excessively down the bole. Treatments were applied within 10 seconds after the hole was punched, to ensure minimal contamination or excessive resin flow. The treatment areas were then wrapped tightly in plastic kitchen wrap, and then again with cloth duct tape to keep the treatment materials in place, and minimize the potential for contamination of the wound site.

After 4 days, the plastic wrap and tape were removed. Samples from the treated trees were collected and handled using the methods of Raffa et al. (2013). Using a chisel, a 2 cm wide by 5 cm tall section of phloem was removed, with the center of the section being the wound site.

Samples were placed on dry ice in the field, transported to the University of British Columbia in Vancouver, BC, Canada and stored at -35°C until processing.

4.2.3 Monoterpene analysis

Samples were removed from cold storage and prepared for chemical analysis. All tools and surfaces were cleaned and sterilized using 99.5% acetone (Fisher-Scientific) between every use. Autosampler vials (Fisher-Scientific) were labeled and weighed prior to dissection. Samples were then removed from the freezer and placed on a cooled block of aluminum, to minimize the loss of volatiles. Using a razor blade, any outer bark was excised and discarded, and a portion of phloem directly above the wound site was removed and the remaining phloem was stored for later use. The dissected sample was sliced into ~1 mm-thick strips and mixed, then evenly distributed between 3 vials, creating technical replicates. Each vial was filled with 1.3 mL of tert-butyl methyl ether [MTBE] (Fisher-Scientific), with 75 ppm isobutyl benzene [IBB] (Sigma-Aldrich) as an internal standard. Vials were placed on a shaker table at room temperature for 24 hours. 1 mL of each sample was transferred to a new vial containing 200 µL of 100 mM ammonium carbonate aqueous solution and mixed, to remove polar contaminants. Vials were centrifuged for 4 minutes at 2000 rpm and 20°C, then stored at -35°C until GC/MS analysis. Monoterpene concentrations were calculated with an Agilent 6890 Gas Chromatograph 5973 Mass Spectrometer, using an Agilent DB-WAX column (J&W 122-7062, 60 m, 0.25 mm, 0.25 µm). Oven initial temperature was 40°C, stepped up to 85°C at 15°C per minute, then to a final temperature of 250°C at 50°C per minute and held for 10 minutes. Split injection at 21.25 psi was used with a helium carrier. After samples were analyzed, vials were placed in a fume hood for 24 hours to allow solvents to evaporate and then placed in a drying oven at 60°C for 24

hours, and reweighed to determine dry mass of the sample for weight-correction of GC/MS output. The dry mass of each dissected sample varied with phloem thickness, with an average of 205 mg, and a range of 80 mg to 300 mg. Concentrations for each sample were determined by taking the mean of technical replicates prior to statistical analysis.

4.2.4 Statistical analyses

All data were prepared using Microsoft® Excel© version 16.0 (Microsoft®, 2015, Redmond WA USA), and all statistical tests were performed using SAS® Statistical Software version 9.4 (SAS® Institute, 2014, Cary NC USA). I calculated the concentration of monoterpenes (mg/g) expressed in response to mountain pine beetle simulation treatments, and the relative abundance of individual monoterpene constituents that comprised greater than 1% of the total. To determine if prolonged exposure to epidemic mountain pine beetle mortality will select for a specific induced response, I calculated the difference in concentration of monoterpenes in response to the two treatments. Differential concentrations were calculated by subtracting the concentration of induced monoterpenes (mg/g) in phloem tissues in response to generic treatment (MeJA) from that of the mountain pine beetle simulation for each tree. A positive differential means the tree produced more monoterpenes per gram of phloem (in mg/g) in response to mountain pine beetle simulation. I also calculated concentration differences in the same manner for each of the major monoterpene constituents individually.

Prior to analyses, data for monoterpene relative abundance were arcsine (square-root) transformed to account for the truncated distribution of proportional data (Dowdy et al., 2004). I checked all data for assumptions associated with the application of linear regression using PROC UNIVARIATE. Data contained high leverage points, and so I then applied PROC

ROBUSTREG with the DIAGNOSTICS option to detect outliers. I used the MM method, which combines Huber M estimation and high breakdown value estimation (Yohai, 1987), and the default cutoff value of three standard deviations from the mean (Dowdy et al., 2004). Detected outliers were removed from the mountain pine beetle simulation data (n=3 of 88 trees) and differential concentration data (n=6 of 88 trees) separately.

I applied PROC REG to perform linear regression to assess the relationship between monoterpene expression in response to mountain pine beetle simulation and historic climate suitability scores of each stand, for total and individual monoterpene data. To address my hypothesis directly, I used linear regression to assess the influence of historic climatic suitability on the mean differential concentration of total monoterpene expression and of individual monoterpenes. To determine if herbivory by the mountain pine beetle has selected for a qualitative change in the form of the induced response, I compared the slopes from the regression analyses assessing the relationships between historic climatic suitability and differentials of individual monoterpenes with that of the slope derived from the regression on total monoterpene expression differential. Divergent slopes for individual monoterpenes would indicate a qualitative shift in expression in response to selection by mountain pine beetle as compared to generic treatment. To assess differences in slopes, I standardized each monoterpene score, since they varied in relative abundance. To do this, I first expressed each concentration differential (with outliers removed), for individual monoterpenes and the total, as a percent of the concentration of monoterpenes in response to the generic treatment. This converted each concentration differential to “percent increase” relative to the generic treatment (sometimes a decrease). I applied analysis of covariance using PROC GLM to compare the slopes of the

regressions for the percent induction of each selected monoterpene in relation to historic climatic suitability.

4.3 Results

4.3.1 Field challenges

During treatment applications, discolored or lesioned phloem was uncovered under asymptomatic outer-bark in only 2 or 3 trees per stand, and only 3 were abandoned in total. The plastic wrap and duct tape covering was 100% effective in preventing treatment site exposure or contamination, and none were disturbed. Upon removal after 4 days, treatments were always still in place, however some trees did produce high quantities of resin which may have partially dissolved the agar plugs. Cotton plugs were often saturated with resin but otherwise intact. Xylem tissue and sampled phloem contained light-brown lesions that were always small (extending ca. 3-5 mm above/below treatment site), with no detectable morphological differences between treatments.

4.3.2 Defensive response to mountain pine beetle

Expression of total monoterpenes in response to mountain pine beetle simulation was positively related to historic climate suitability (Fig. 4.3). Lodgepole pine populations with the greatest degree of historic exposure to the mountain pine beetle (Merritt, HSC 0.27 – 0.32) expressed an average of 122.18 mg of monoterpenes per g dry weight - approximately twice the concentration presented in the more recently invaded host populations. Similar positive linear relationships were observed for individual monoterpenes ($p < 0.05$, $r^2 = 0.54-0.85$); however, there was no clear pattern of variation in expression of either 3-carene or terpinolene ($p > 0.05$) in relation to historic exposure of lodgepole pine populations to mountain pine beetle.

Concentrations of 3-carene were comparatively lower at Baldy Mountain (4.3 mg/g) and Hinton (6.5 mg/g), than those at Merritt (15.5 mg/g) and Tumbler Ridge (10.3 mg/g). Terpinolene results show a similar pattern, where Baldy Mountain (0.80 mg/g) and Hinton (0.95 mg/g) had comparatively lower concentrations than Merritt (2.07 mg/g) and Tumbler Ridge (1.37 mg/g).

Relative concentrations of monoterpenes were variable, with the greatest deviation among sites arising in β -pinene, 3-carene, and limonene (Fig. 4.4). However, variation in the relative abundance of monoterpenes in response to mountain pine beetle simulation could not be explained by historic climate suitability ($p=0.11-0.98$).

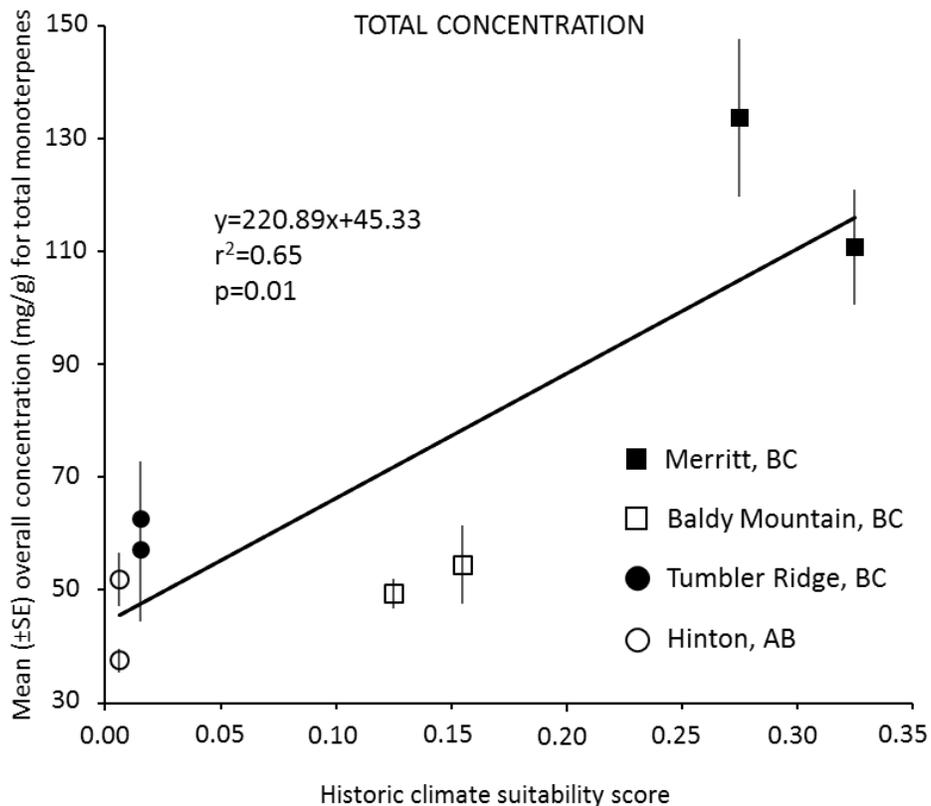


Figure 4.3: Effect of degree of historic exposure to the mountain pine beetle (*Dendroctonus ponderosae*) as indicated by historic climate suitability (1921-1950; see Carroll et al., 2004) on the mean (\pm SE) concentration of total monoterpenes in phloem tissue (mg/g) induced by simulated attacks for trees from 8 populations of lodgepole pine in western Canada. Line of best fit, and results of linear regression analysis are also displayed.

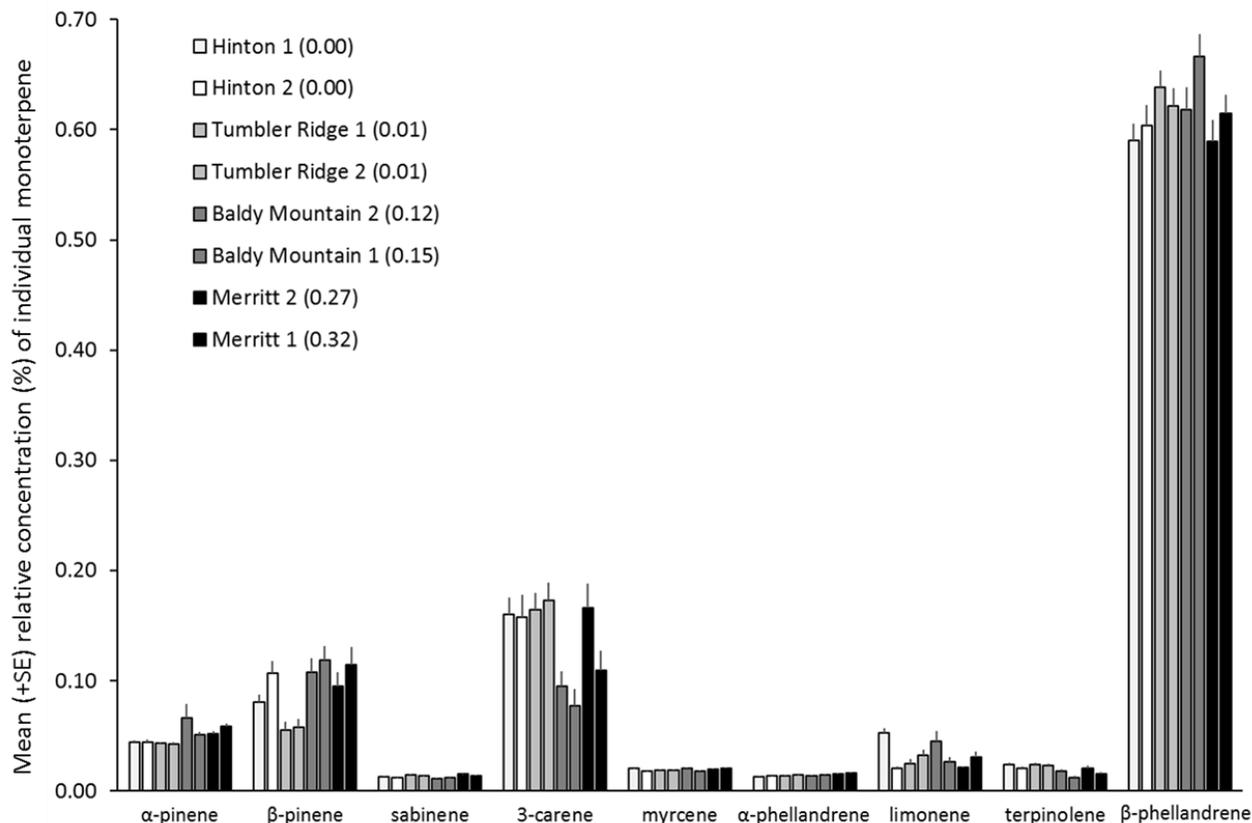


Figure 4.4. Mean (+SE) relative concentration (%) of individual monoterpene constituents induced in phloem tissue following simulated mountain pine beetle (*Dendroctonus ponderosae*) attacks, for 8 lodgepole pine (*Pinus contorta* var. *latifolia*) populations with varying degrees of historic exposure to herbivory as indicated by historic climatic suitability for the period 1921 - 1950 (scores in parentheses) as determined by Carroll et al. (2004). Populations are grouped in ascending order of historic climatic suitability (in brackets).

4.3.2 Differential defensive response between treatments

Assessment of the differential expression of monoterpenes associated with simulated mountain pine beetle attacks versus the response to MeJA within individual trees in relation to the degree of exposure of lodgepole pine populations to herbivory yielded results consistent with my prediction, and in support of my hypothesis. There was a positive linear relationship between historic climate suitability and the specificity of the induced defensive response (Fig. 4.5). Lodgepole pine with the greatest degree of historic exposure (Merritt, HCS 0.27-0.32) produced an average differential concentration of 46.26 mg/g, while those with the least degree of historic exposure (Hinton, HCS 0.00) expressed an average differential concentration of -8.23

mg/g. Trees at Tumbler Ridge (HCS 0.01) expressed a median average differential concentration of 0.96 mg/g. Both lodgepole pine populations at Baldy Mountain (HCS 0.12-0.15) were somewhat anomalous, and expressed lesser differential concentrations than anticipated (-2.91 mg/g) given their putative degree of historic association with the mountain pine beetle (Fig. 4.5). However, based on the steep terrain associated with Baldly Mountain, the historic climatic suitability estimate derived at a resolution of 64 ha may have inadvertently included lodgepole populations with lower historic suitability scores.

The above relationship was consistent among all monoterpene constituents tested (Table 4.2). Interestingly, I did not find any evidence for a qualitative shift in expression of monoterpenes between treatments. There was no difference between the slope of the total differential response in response to historic climate suitability and those of any individual monoterpene tested ($p > 0.05$).

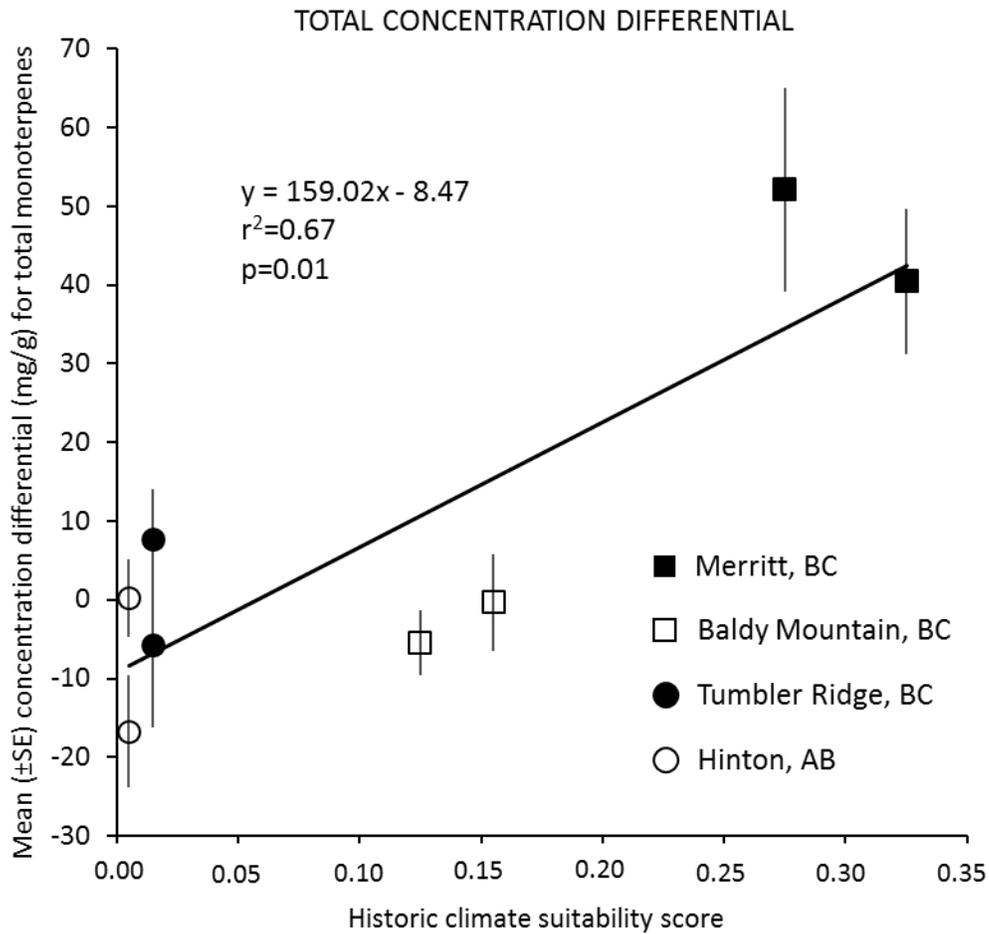


Figure 4.5: Effect of degree of historic exposure to the mountain pine beetle (*Dendroctonus ponderosae*) as indicated by historic climate suitability (1921-1950; see Carroll et al., 2004) on the specificity of induced defenses against the mountain pine beetle (*Dendroctonus ponderosae*) (determined as the mean (\pm SE) difference in concentration of total monoterpenes (mg/g) induced in phloem tissues within trees between simulated mountain pine beetle attacks and application of a generic defense elicitor) for trees from 8 populations of lodgepole pine in western Canada. Line of best fit, and results of linear regression analysis are also displayed.

Monoterpene component	df	Intercept			Historic Climate Suitability			
		Estimate	t-value	p	Estimate	t-value	p	r ²
TOTAL	7	-8.47	-1.26	0.25	159.02	3.86	0.01	0.67
α-pinene	7	-0.63	-1.54	0.18	10.66	4.23	0.01	0.70
β-pinene	7	-0.67	-0.77	0.47	18.00	3.36	0.02	0.60
sabinene	7	-0.13	-1.04	0.34	2.61	3.43	0.01	0.61
3-carene	7	-0.83	-0.90	0.40	17.73	3.11	0.02	0.55
myrcene	7	-0.17	-1.19	0.27	3.47	4.06	0.01	0.69
α-phellandrene	7	-0.15	-1.22	0.27	2.84	3.92	0.01	0.67
limonene	7	-0.32	-2.00	0.10	3.90	3.92	0.01	0.67
β-phellandrene	7	-5.18	-1.38	0.22	95.28	4.14	0.01	0.70
terpinolene	7	-0.02	-0.25	0.81	2.09	2.97	0.03	0.53

Table 4.2: Summary statistics for linear regression analyses evaluating the influence of the degree of historic association of 8 lodgepole pine (*Pinus contorta* var. *latifolia*) populations with the mountain pine beetle (*Dendroctonus ponderosae*) on the concentration differential of individual monoterpenes (determined as the mean difference in concentration of each monoterpene (mg/g) induced in phloem tissues within trees between simulated mountain pine beetle attacks and application of a generic defense elicitor). Historic climatic suitability for the period 1921-1950 for the mountain pine beetle (see Carroll et al., 2004) was used as a proxy for the degree of historic association between the beetle and lodgepole pine populations. Significant values (p<0.05) are in **bold italics**.

4.4 Discussion

Mountain pine beetle herbivory has apparently resulted in selection for a specific induced defensive response in populations of lodgepole pine with long-term association with its herbivory. Rapid and concentrated accumulation of monoterpenes is the most important factor determining an individual tree's survival, and the source for variability of resistance to mountain pine beetle among lodgepole pine trees (Boone et al., 2011; Raffa and Berryman, 1982b), and this trait varies in lodgepole pine populations in relation to the degree of historic exposure with the mountain pine beetle. This lack of an aggressive induced response by evolutionarily naïve lodgepole pines is likely the primary contributing factor to observed enhanced reproductive performance by the beetle in newly invaded habitats (Cudmore et al., 2010). Moreover, since the resistance of a stand of trees (i.e. defensive capacity) determines the population size at which mountain pine beetles can breach the endemic/epidemic threshold and begin attacking large diameter, highly defensive trees (Safranyik and Carroll, 2006), the lack of a specific evolved defensive response by naïve lodgepole pine populations suggests that the spread and impacts of mountain pine beetle may be exacerbated in newly invaded lodgepole pine forests. Indeed, Robinson (2015) recently demonstrated that landscape-level impacts of mountain pine beetle outbreaks were greater in areas with less historic association to mountain pine beetle. Similarly, weakly coevolved defenses in whitebark pines (*P. albicaulis*) have been implicated as the primary driver of widespread mortality in high elevation forests (Raffa et al, 2013). It is reasonable to expect future expansion of mountain pine beetle into novel habitats will be met with depressed stand-level resistance, although changing aspects of stand structure and competition may play a role in the potential for invasion of these systems.

Although the magnitude of the induced defensive response increased in populations of trees with increasing association with the mountain pine beetle, contrary to expectations there were no differences in the diversity and abundance of the individual monoterpene constituents in relation to historic climatic suitability. The mountain pine beetle has a complex relationship with the chemistry of its host trees, where interaction with certain monoterpene constituents will reduce generation survival whereas others will actually improve fitness (reviewed by Raffa et al., 2005; Safranyik and Carroll, 2006). For example, limonene and 3-carene are especially toxic to eggs (Raffa and Berryman, 1983b; Reid and Purcell, 2011), while myrcene and terpinolene are synergistic with pheromones and promote aggregation and mass attack (Borden et al., 2008, 1987). The monoterpenes (+)- and (-)- α -pinene are particularly interesting, as they are both toxic to beetles and their associated microorganisms (A. Adams et al., 2009; Reid and Purcell, 2011), and pheromone precursors that have been associated with higher rates of pheromone production (Erbilgin et al., 2014; Taft et al., 2015a) and higher rates of secondary attraction *in situ* (Burke and Carroll, 2016). Clark et al. (2010, 2014) found higher levels of α -pinenes in putatively evolutionarily naïve versus experienced lodgepole pine populations, and suggested that this was a consequence of selection by the mountain pine beetle for reduced host apparency by way of reduced α -pinene expression. However, my results show that the specificity of the response by evolutionarily experienced populations involves the up-regulation of the induced defensive response as a whole, which is in keeping with previous predictions that total monoterpene expression is the primary determinant of resistance (Boone et al., 2011; Raffa and Berryman, 1982; Reid and Purcell, 2011).

Understanding the underlying biochemical mechanism on which selection acts in this system is difficult, but my results may provide some insight. The induced response of conifers,

and the production of secondary resin metabolites, involves a multitude of multifunction enzymes and transcription factors (Hall et al., 2013; Keeling and Bohlmann, 2006; Zulak et al., 2009). The positive relationship between the degree of historic association with mountain pine beetle and accumulation of monoterpenes in response to attacks may be the result of changes in expression of terpene synthase genes or associated transcription factors, leading to a greater capacity to produce monoterpenes. The fact that evolutionarily experienced trees reacted more strongly to the simulated beetle infestation than to MeJA while naïve trees did not, may suggest that simulated beetle attack and MeJA activate the terpenoid defense response via different signaling pathways. Secondary resin metabolites are synthesized within traumatic resin ducts in the xylem of conifers, which form in response to injury (Franceschi et al., 2005; Krokene et al., 2008). If formation of these ducts is enhanced in response to the beetle in coevolved systems, such that there are more per unit xylem, this will lead to a greater abundance of monoterpenes without a shift in qualitative content (Schmidt et al., 2011). Plants are adapted to detect the presence of pathogen-associated molecular patterns, also known as PAMPs (Jones and Dangl, 2006). Plants recognize PAMPs (e.g. insect and/or fungal chitin) via specific receptors in tissues (Boller and Felix, 2009; Miya et al., 2007), leading to a cascade of defensive reactions as part of the plant immune system that is sensitive to the virulence of the invader (Jones and Dangl, 2006). This interaction of microbes with the immune system of plants has been implicated as a primary source of evolutionary pressure and change in plant immune-response, as each agent develops adaptations to detect, or suppress detection (Chisholm et al., 2006). One explanation of my results may be that coevolved lodgepole pine trees are more sensitive to the presence of insect and fungal chitin or other PAMPs associated with the beetle/fungus complex. Evolutionarily experienced lodgepole pines may be able to better “recognize” the beetle complex, leading to a

greater overall immune response and possibly a higher density of traumatic resin ducts. Further tests involving non-pathogenic fungi and anatomical comparisons between treatments to quantify traumatic resin duct density could elucidate this further.

While experienced populations of lodgepole pine are adapted to the mountain pine beetle, and naïve populations are not, it is not entirely clear whether long-term exposure to the beetle has selected for aggressive induced defenses, or lack of exposure has selected against them. The induced defensive response by conifers is energetically costly (Christiansen et al., 1987; Keeling and Bohlmann, 2006) and the growing season in northern latitudes is short. Therefore, trees may prioritize production of primary metabolites for growth over secondary metabolites for defense in the absence of significant herbivory (Herms and Mattson, 1992). Thus, it is conceivable that a lack of exposure to herbivory among lodgepole populations beyond the historic climatically constrained range of the mountain pine beetle may have led to selection against such a costly trait. Since the last glaciation (ca. 8000 years before present), lodgepole pine spread northward mostly from southern refugia to colonize western North America (Cwynar and MacDonald, 1987; MacDonald and Cwynar, 1985; Wheeler and Guries, 1982). Furthermore, the genus *Dendroctonus* has had a long evolutionary history with *Pinus* hosts (Kelley and Farrell, 1998), and would likely have been associated with ancestral lodgepole pine populations within the glacial refugia. Therefore, it is possible that the aggressive defensive traits were present in the expanding lodgepole pine populations post glaciation, but were lost once the trees migrated into regions from which the mountain pine beetle was climatically excluded.

Regardless of selection for an aggressive induced defensive response against mountain pine beetle within experienced lodgepole pine populations, or selection against it in naïve populations, recent climate warming has facilitated the expansion of mountain pine beetle into

defense-free space (*sensu* Gandhi and Herms, 2010). Results of this study strongly suggest that the mountain pine beetle is an invasive species within a contiguous population of its principle host, as a result of weak recent evolutionary relationships in novel lodgepole pine populations. The mountain pine beetle system allows for a detailed consideration of the role of plant defenses in invasion biology, as naïve lodgepole pine habitats are identical to experienced forests in most aspects (Robinson, 2015; Safranyik et al., 2010). By contrast, in exotic invasive systems, the invader's success is attributable to a lack of coevolution with the full suite of biotic elements of the invaded habitat (Gandhi and Herms, 2010; Jefferies and Lawton, 1984). For example, trophic insufficiencies including inadequate host defenses have recently been implicated in the rapid invasion and severe impacts of the emerald ash borer, *Agrilus plannipennis* (Cipollini et al., 2011; Duan et al., 2014; Herms and McCullough, 2014; Whitehill et al., 2011), redbay ambrosia beetle, *Xyleborus glabratus* (Kendra et al., 2013) and hemlock woolly adelgid, *Adelges tsugae* (Radville et al., 2011; Vieira et al., 2012) in North American host-tree populations. However, the direct influence of trophic interactions is largely unknown and potentially unknowable, as nearly all species in every niche will be different on different continents, including the host. In the case of the mountain pine beetle, similarities in ecosystem processes and trophic interactions in the native and newly invaded regions (Safranyik et al., 2010) lead us to conclude that the susceptibility to invasion of novel lodgepole pine forests is likely due to insufficient host defensive capacity, emphasizing the role of bottom-up forces in habitat invasion by herbivores. Improvements in the capacity to determine what traits contribute to the susceptibility of novel systems will allow for better mitigation of consequences of exotic introductions, and native invasions, in the future (Davis, 2009; Dukes et al., 2009; Liu and Trumble, 2007; Raje et al., 2016).

Holarctic forests are very likely to experience more native invasions with further climate warming and ever-increasing anthropogenic movement of people and products. In northern Europe, where forests extend far beyond the thermal range of most insects, evidence suggests imminent range expansion by both lepidopteran defoliators (Netherer and Schopf, 2010), and bark beetles (Jönsson et al., 2009) within the contiguous populations of their current hosts. The southern pine beetle (*Dendroctonus frontalis* Zimmermann), a highly destructive species related to the mountain pine beetle and native to the southeastern region of the United States, has significantly expanded its range northward as a consequence of warming winter temperatures (Trân et al., 2007). Analysis of the widespread and varied effects of climate change on insects and pathogens by Weed et al. (2013) suggests that in many cases, impacts have been greater than predicted by previous assessments [see Ayers and Lombardero (2000)]. Impacts are even more pronounced in the tree-killing species of bark beetles (which compete directly with humans for resources), for a number of reasons attributed to their biology and life histories (Raffa et al., 2015). An understanding of the traits that allow native insects to invade novel habitats of conspecific hosts will be increasingly important, as climates continue to warm.

5 General conclusions

The close association between eruptive bark beetle population dynamics and the defensive systems of their hosts (e.g. Raffa et al., 2013; Wallin and Raffa, 2002, 2004) means that perturbation of coevolved relationships due to warming-induced migration has the potential to exacerbate the influence of these insects on forests (Raffa et al., 2015). Migration of mountain pine beetles into novel habitats will enhance their ability to erupt and spread, as defensive characteristics of novel hosts are insufficiently adapted to this species and may result in a greater propensity for landscape-scale outbreaks.

5.1 Key findings and conclusions

5.1.1 Chapter 2

Population-state dependent host selection by female mountain pine beetles is reinforced by context-dependent maternal effects, and selections are made by gustatory assessment of defensive resin even in the absence of other cues. The offspring of mountain pine beetles that selected impaired hosts exhibited host selection behavior that reflected the experience of their parents. Endemic (i.e. low population density) maternal host selection led to a propensity in offspring to accept host material low in defensive volatile concentration, whereas epidemic maternal host selection led to rejection of low-concentration material in offspring and a strong preference for highly concentrated material. This intergenerational effect is partially responsible for the maintenance of, or transition from, one population state to another, as it reinforces population-state dependent behaviors in offspring, and allows for the persistence of behaviors through significant perturbation or long-distance dispersal.

These findings contribute to our understanding of how eruptive bark beetles can maintain huge populations over long periods, and why they may affect novel forests more severely.

Recent evidence, including results from chapter 4, have shown that the defensive capability of novel pines may be depressed as they apply to mountain pine beetle epidemics (Clark et al., 2010, 2014; Cudmore et al., 2010; Raffa et al., 2013; Robinson, 2015). Weak host defenses may reduce beetle generation mortality and increase conspecific interactions in offspring, thereby increasing the propensity for epidemic behavior. Subsequent generations will be increasingly likely to continue epidemic behaviors due to the reinforcement of phase-specific biology as a consequence of maternal host choice. More broadly, these results also demonstrate the importance of maternal effects in the biology and population dynamics of eruptive bark beetles. The influence of maternal effects on the population dynamics of insects is not widely agreed upon (Berryman, 1995; Myers and Cory, 2013; Räsänen and Kruuk, 2007), however these results clearly demonstrate that maternal experience and its effect on offspring behavior is a crucial element in the transition and maintenance of population-phases in eruptive bark beetles, a group which is highly influential in both natural forest ecosystems and human economies.

5.1.2 Chapter 3

Aggregation by eruptive bark beetles is a critical component of their epidemic life histories (D. Wood, 1982). The potential for enhanced aggregation in novel forests is an important aspect when considering how these insects will behave and spread in the face of future climate warming. The defensive characteristics of novel pines may enhance the ability of the mountain pine beetle to attract conspecifics and potentially overcome some of the constraints to continued expansion through the boreal forests. For example, greater relative abundance of the pheromone precursor α -pinene in novel pines may mean that a mountain pine beetle could find a novel host tree from further away, when compared with expectations from the beetle's primary

host, lodgepole pine, the species with which the insect has coevolved. Novel hosts such as jack pines therefore potentially represent a super-normal stimulus (*sensu* Tinbergen and Perdeck, 1950) to foraging beetles, and could lead to their ability to overcome the constraints to further boreal expansion presented by low pine volume and connectivity in that ecosystem (see Safranyik et al., 2010).

These results demonstrate one potential mechanism by which some forest insect species successfully invade novel systems. A simple assessment of the volume of defensive chemicals produced by novel hosts in response to an invading species may not be sufficient to predict the defensive capacity of a given population/species of host tree (e.g. Raffa and Berryman, 1982b). For example, resistance to the exotic invasive emerald ash borer (*Agrilus planipennis*) in different *Fraxinus* spp. appears to be a result of qualitative differences in chemical expression, as opposed to volume of defensive chemicals produced in response to attack (Cipolini et al., 2011). In the case of the mountain pine beetle, the relative concentration of monoterpenes may be more influential than absolute expression on the susceptibility of a novel species, since mass-attack effectively eliminates the direct lethality of resin components. Invasive insects are becoming more and more common in the face of climate change and globalization (Dukes et al., 2009; Hulme, 2009; Ramsfield et al., 2016), and detailed analysis of potential novel defensive systems in anticipation of migration will be increasingly important in the future.

5.1.3 Chapter 4

Eruptive bark beetles are becoming more influential and potentially very damaging with continued warming and anthropogenic modifications to the environment (Kolb et al., 2016; Raffa et al., 2008, 2015). Prolonged exposure to mountain pine beetle infestations has created

selection pressure on lodgepole pine populations to be more defensive, and a lack of coevolution in the expanded range has potentially assisted the spread of mountain pine beetle and likely led to more severe impacts (Cudmore et al., 2010; Robinson, 2015). The mountain pine beetle is acting as a native invasive species, even within the contiguous population of their principle host, as novel hosts lack specific defensive adaptations to the insect, similar to exotic invasive systems (Gandhi and Herms, 2010). The close association with the defensive system of their hosts has exacerbated the consequences of warming-induced range expansion; they not only outbreak in more forests, but these forests are also more susceptible to impacts. Increased mortality of trees in novel ranges exacerbates positive feedbacks associated with carbon deposition to the atmosphere (Kurz et al., 2008), contributing to warming and further increasing the likelihood of expansion into even more naïve forests.

This system is likely to be only the first example of a native invasive bark beetle entering novel habitats with lasting detrimental effect, in response to anthropogenic changes in climate and forest structure. Many Holarctic forest insects occupy only the southern range of their hosts, and therefore the potential for native invasion due to warming is high (Hagen et al., 2007; Jepsen et al., 2009; Netherer and Schopf, 2010). For example, in October 2014, the North American species southern pine beetle (*Dendroctonus frontalis*) was found for the first time in New York (U.S.A) in the Long Island region, a new northern limit for this economically and ecologically influential bark beetle (<http://www.dec.ny.gov/animals/99331.html> [accessed October 18, 2016]). There is substantial evidence to support that the spruce engraver beetle (*Ips typographus*) is being affected by climate change and threatens northward expansion in Scandinavia in Europe (Jönsson et al., 2009). Anthropogenic climate change can rapidly

introduce novel species into naïve habitats, thereby disrupting the delicate natural balance of co-evolutionary relationships that maintain ecosystem stability.

5.2 Limitations of research

Although all experiments comprised critical tests of my hypotheses and predictions, there were some limitations with the experimental design I used in chapter 2, mainly in the use of no-choice behavioral assays. Assays were not strictly no-choice, as the insects were allowed to reject the simulated phloem and were not forced to enter it; however, they could not leave the arena, and due to the feeding-stimulant properties of monoterpenes in general (Raffa and Berryman, 1983a), it is very likely that acceptance of the 100 mg/g treatment by endemic simulated beetles was artificially high. Were they allowed to leave, they probably would, and support for this is partially demonstrated by the slower acceptance rate of that treatment.

In chapter 3, I identified two limitations that should be corrected in future experiments employing that design. First, ideally more replicates should be used as a safeguard against environmental variation. Although the experiment I implemented comprised three blocks of three replicates of each treatment, I did not anticipate the difficulties associated with manually infesting all bolts before the time the experiment needed to begin. Doubling the replicates would have mitigated this, and provided more successfully infested bolts to challenge the treatments. Second, more attention to, and analysis of, stereochemistry of monoterpenes is needed. Previous ecological assessments of host chemistry as it relates to mountain pine beetle interactions have not addressed this (e.g. Clark et al., 2010; Boone et al., 2011), where chemistry-focused approaches have (e.g. Hall et al., 2013). In the future, I will always consider stereochemistry of monoterpenes.

Chapter 4 suffered mainly from one minor omission, which was to take a non-treated phloem sample from a third part of the bole, to assess constitutive monoterpene content prior to any induced response. Although the experiment was designed to test the induced response, assessment of the state of the constitutive defenses of each tree would have added valuable information regarding the starting state of the system. However, increasing the number of samples is very costly in terms of chemical analyses. Where possible, in future assessments using this method, I will not omit this measure.

5.3 Future directions

Building on the results of chapter 2, that there are two things that require further experimentation. First, a multi-generational endemic simulation would result in a stronger effect of population state. It is probable that as generations continue to occupy suboptimal material, the strength of the state-dependent maternal effect will increase. I expect that over time, rejection of high concentrations by endemic beetles would be observed, especially if the problem regarding the design I identified in section 5.2 can be resolved by a strict choice assay. Second, if endemic beetles were treated with *trans*-verbenols, they may revert back to rejection of weak phloem (and rapid acceptance of defended phloem), as this chemical is very likely the primary cue that foraging beetles use to determine whether conspecifics are present in sufficient numbers to mass-attack. Beetles from endemic simulation that are exposed to pheromones for a period of time before the assay begins would very likely exhibit different behaviors, demonstrating the importance of each pheromone in the detection of conspecifics.

The results of chapter 3 may raise more questions than they answered. First, there is still some mystery surrounding the production of *trans*-verbenol from α -pinene. The results of

Erbilgin et al. (2014) regarding the lack of influence of precursor chirality on pheromone stereochemistry, combined with the finding that relative concentrations of the precursor seems to determine volume of pheromone produced, suggests that *trans*-verbenol is not directly oxidized from α -pinene originating from the tree under attack. Ongoing research at UBC on the production of *trans*-verbenol may be able to elucidate this in the near future. Second, the consequences of elevated aggregation pheromone production in novel hosts is unclear. It is not known whether a jack pine tree growing in the boreal forest will be more susceptible to mortality, and whether elevated pheromone production would lower the epidemic threshold of an infested stand. This may be answered by measuring rates and densities of attack in these forests by the mountain pine beetle. At the time of this writing, I am involved in experiments to assess this question.

The results of chapter 4 demonstrate the usefulness of the dual-challenge approach to assess the dynamics of conifer induced defense. This method can be improved, and should be applied to more populations and species, both within the realm of the mountain pine beetle system and in other systems as well. Treatment of the trees with a non-pathogenic fungal species (such as *Agaricus bisporus*, commonly known as portobello) would be useful to determine the degree to which trees are adapted to the specific fungal pathogen intended to be tested. It is probable that amongst lodgepole pine populations within the native range, trees would respond with more vigor to this treatment than to MeJA, but less so than to *Grosmannia clavigera*. This would demonstrate more clearly that the trees are adapted specifically to the mountain pine beetle complex. This experiment should include populations of lodgepole pine with even longer potential association with the beetles, such as those in northern Arizona in the United States.

Additionally, this experiment should include populations of jack pine, ponderosa pine, whitebark pine, and the potential host red pine (*P. resinosa*).

5.4 Conclusion

This dissertation describes my attempts to address critical knowledge gaps associated with the ecology of eruptive species, and the continued biological consequences of global climate change. Species across the world are being affected by anthropogenic modifications to the atmosphere and biosphere, and predictions concerning the magnitude and distribution of changes, especially of those species that compete directly with humans for resources or infect us with disease, are paramount if mitigation efforts are to be successful. Natural ecosystems are highly complex, making mathematical description of their function difficult. Despite this, mathematical models are increasingly useful as technology expands, especially in this changing climate, and ecological experiments designed to better understand interactions are valuable for model development. Successful predictions rely on detailed ecological information, and continued climate change means this information will need to be constantly updated in order to remain as accurate as possible.

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