Responses by natural enemies to semiochemicals associated with mountain pine beetle (*Dendroctonus ponderosae*)

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Thesis Abstract

Mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is an aggressive bark beetle native to North America west of the Rocky Mountains and in the Black Hills of South Dakota. Outbreaks result in mortality to mature pines over many thousands of hectares. In western North America, mountain pine beetle's primary hosts are ponderosa (*Pinus ponderosae* Lawson) and lodgepole (*Pinus contorta* Douglas) pines. In recent decades, ameliorating winter temperatures have allowed populations of mountain pine beetle to expand their range across the biogeoclimatic barrier of the Rocky Mountains into stands of jack pine (*Pinus banksiana* Lamb.) in Alberta, Canada. The invaded forest is contiguous with the boreal forest of eastern North America, creating concern that mountain pine beetle will expand its range toward the Great Lakes region where it threatens evolutionarily naïve red (*Pinus resinosa* Ait.), eastern white (*Pinus strobus* L.), jack, and scots (*Pinus sylvestris* L.) pines. Mountain pine beetles procure hosts by responding to species-specific aggregation pheromones. Natural enemies use these and other host volatile cues as kairomones. If mountain pine beetle were to expand its range into the Great Lakes region, it would encounter new forest and insect communities that are evolutionarily naïve to its presence. My thesis investigates semiochemical responses of native insects to chemical lures of mountain pine beetle within its native range and in the potential invasion zone. In addition, I study direct competitive interactions of mountain pine beetle with the native non-aggressive bark beetle, the eastern five-spined ips (*Ips grandicollis* Eichoff). My work suggests very few natural enemies native to the Great Lakes region will respond to the chemical cues associated with mountain pine beetle if it were to arrive so may not be significant mortality factors.

Table of Contents

List of Tables

List of Figures

Figure 2.1 Box and whisker plots of number of (A) *Dendroctonus ponderosae,* (B) *Ips pini,* (C) *Ips grandicollis,* and (D) *Pityogenes knechteli* in each 14-day sample period per site captured in summers 2017 (solid line) and 2018 (dashed line). Variation within funnel trap captures at each collection period is displayed in box and whisker plots for 2017 (white) and 2018 (shaded). The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

Figure 2.2 Box and whisker plots of number of (A) *Temnoscheila virescens,* (B) *Platysoma* spp., and (C) Cleridae in each 14-day sample period per site captured in summers 2017 (solid line) and 2018 (dashed line). Variation within funnel trap captures at each collection period is displayed in box and whisker plots for 2017 (white) and 2018 (shaded). The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

Figure 2.3 Boxplots displaying the total trap captures of (A) *Dendroctonus ponderosae* and (B) *Dendroctonus valens* in South Dakota (top), Minnesota (middle), and Nebraska (bottom) by lure treatment in the collection periods of 2017 and 2018. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5

times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

Figure 2.4 Boxplots displaying the total trap captures of total trap captures of (A) *Ips pini*, (B) *Ips grandicollis*, and (C) *Pityogenes knechteli* in South Dakota (top), Minnesota (middle), and Nebraska (bottom) by pheromone treatment. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

Figure 2.5 Boxplots displaying the total trap captures of total trap captures of (A) *Hylurgops* spp. and (B) *Hylastes* spp. in South Dakota (top), Minnesota (middle), and Nebraska (bottom) by pheromone treatment. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

Figure 2.6 Boxplots displaying the total trap captures of total trap captures of (A) *Enoclerus sphegeus*, (B) *Madoniella* spp., and (C) *Thanasimus dubius* in South Dakota (top), Minnesota (middle), and Nebraska (bottom) by pheromone treatment. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile

minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

Figure 2.7 Boxplots displaying the total trap captures of total trap captures of (A) *Platysoma* spp. and (B) *Temnoscheila virescens* in South Dakota (top), Minnesota (middle), and Nebraska (bottom) by pheromone treatment. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

Thesis Introduction

Mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (Coleoptera: Curculionidae) is a univoltine, aggressive bark beetle native to western North America and the Black Hills of South Dakota. During outbreaks, mountain pine beetle acts as a natural disturbance agent, causing extensive mortality among mature pine trees (*Pinus* spp.) (Safranyik and Carroll, 2006). These epidemics foster ecosystem services such as wood deterioration and nutrient cycling (Wood, 1982; Mikkelson et al., 2013). At the same time, large-scale mortality of pines can negatively impact the economy of the region in which it occurs. In British Columbia, Canada, mountain pine beetle is predicted to attack fifty-three percent of the province's merchantable trees by 2021, resulting in a cumulative $57.37 billion loss to the provincial GDP over the 45 year course of the most recent outbreak (Forest Service BC, 2013; Corbett et al., 2016). Despite such impacts, tree-killing behavior by mountain pine beetle is rare among species of bark beetles (Lindgren and Raffa, 2013). Species that kill mature trees are mostly concentrated in the genus *Dendroctonus* (Wood, 1982). Most bark beetles are innocuous, non-aggressive species that only colonize stressed or dying trees (Lindgren and Raffa, 2013).

In order to colonize trees, mountain pine beetle uses pheromone-mediated mass attacks to overwhelm and exhaust host defenses when at outbreak levels (Safranyik et al. 1975; Raffa and Berryman 1983). Conversely, when at endemic population levels, persistence of mountain pine beetle is facilitated by non-aggressive bark beetles (Smith et al., 2011). Smith and colleagues (2011) found that endemic populations of mountain pine beetle infest trees already infested by *Pseudips mexicanus* in greater abundances and emerge earlier than trees infested with solely mountain pine beetle. Non-aggressive

colonizers may facilitate mountain pine beetle persistence in the environment until populations of mountain pine beetle can increase to levels necessary to mass attack susceptible pines, often when stressors reduce stand resistance (Safranyik and Carroll, 2006). Species-specific aggregation pheromones help bark beetles locate conspecifics for mating and often inhibit colonization by interspecific competitors (Byers and Wood, 1980; Byers, 1989b). Aggregation pheromones can facilitate spatial separation of multiple species of bark beetle throughout the bole of the same tree (Byers, 1989b), although interspecific interactions still occur under the bark and range from facilitation to competition (Lindgren and Raffa, 2013). Under experimental conditions, for example, when aggressive bark beetles such as mountain pine beetle infest logs simultaneously with a non-aggressive species such as *Ips pini* Say, both species experience decreased reproductive rates compared to infesting logs alone (Rankin and Borden, 1991; Schlyter and Anderbrandt, 1993).

In recent decades, British Columbia has experienced the largest mountain pine beetle outbreak in recorded history (Aukema et al., 2006). Ameliorating winter temperatures have allowed mountain pine beetle to expand its range over the biogeoclimatic barrier of the Rocky Mountains into stands of pure jack pine (*Pinus banksiana* Lamb.) and hybrids of jack pine x lodgepole pine (*Pinus contorta* Douglas) in northwestern Alberta (Kurz et al., 2008; Safranyik et al., 2010; Cullingham et al., 2011; de la Giroday et al., 2012; Lusebrink et al., 2013). The invaded forest is contiguous with the boreal forest of eastern North America, including the Great Lakes region. Of emerging concern is the possibility that mountain pine beetle will continue expanding its range across the North American continent (Cooke and Carroll, 2017). Previous research

has shown that mountain pine beetle is capable of colonizing and reproducing within cut logs of naïve eastern species of pine including jack pine, red pine (*Pinus resinosa* Ait.), eastern white pine (*Pinus strobus* L.), and Scots pine (*Pinus sylvestris* L.) (Rosenberger et al., 2017b; Cale et al., 2017). Moreover, age-classes and distribution of these species in the Great Lakes region are considered highly susceptible to infestation from mountain pine beetle when compared to traditional western hosts (Windmuller-Campione, 2018).

As mountain pine beetle expands across eastern North America, it will encounter species of forest insects with which it shares no evolutionary history, resulting in novel ecological interactions. Natural enemies can have large impacts on bark beetle populations (Amman, 1984; Rankin and Borden, 1991; Aukema and Raffa, 2002) and the Great Lakes region has a high diversity and abundance of natural enemies that respond to semiochemicals associated with bark beetles as kairomones (Aukema et al., 2000; Erbilgin and Raffa, 2002). The complex of natural enemies in the Great Lakes region, however, has not co-evolved with mountain pine beetle and therefore may not recognize or be attracted to its associated semiochemicals (Pfammatter et al., 2015).

My thesis aims to study the potential novel interactions of mountain pine beetle with natural enemies of bark beetles in the Great Lakes region. In chapter 1, I look at potential cross attraction to lures associated with mountain pine beetle and eastern five-spined ips beetle, *Ips grandicollis*, in a 2x2 factorial experiment in the field. The latter insect is a ubiquitous, non-aggressive bark beetle present throughout the Great Lakes region (Drooz, 1985). Lures containing the respective aggregation pheromones of each species were attached to logs of jack pine sourced from Minnesota that were suspended at breast height in the Black Hills National Forest in South Dakota to allow bark beetles to

infest the logs. After field exposure, the logs were returned to a laboratory where they were stored and debarked at periods up to one year to measure colonization and reproduction of subcortical insects. In chapter 2, I investigate which predators and competitors were attracted to semiochemicals associated with mountain pine beetle and native species of bark beetles. Lures were deployed in field choice assays in Minnesota, the Nebraska National Forest at Halsey in Nebraska, and the Black Hills National Forest in South Dakota. Nebraska was chosen as an intermediate location that does not have mountain pine beetle but could be part of an invasion pathway from the Black Hills. I assess differences in the natural enemy complex of the native and non-native regions of mountain pine beetle, studying geographic variation in natural enemies' attraction to these semiochemicals and noting when each of these species occur phenologically.

The chapters of my thesis were prepared for publication in peer-reviewed journals. While, I am the lead author, the research reflects the combined efforts of multiple authors, so I use plural voice throughout. Chapter 1 is being prepared for submission to *Forest Ecology and Management* and Chapter 2 is being prepared for submission to *Oecologia*. The frameworks for each chapter may have small degrees of redundancy, which are included to preserve the integrity of each chapter as a stand-alone unit.

Chapter 1

Colonization and reproduction of potential competitors with mountain pine beetle in baited logs of jack pine

Synopsis

The mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is an aggressive bark beetle native to pine forests of western North America and the Black Hills of South Dakota. Drought and ameliorating winter temperatures in recent years have resulted in range expansion of mountain pine beetle over the geoclimatic barrier of the Rocky Mountains in Canada into stands of lodgepole pine (*Pinus contorta*) X jack pine (*Pinus banksiana*) hybrids. Potential further invasion toward pine forests of eastern North America will bring mountain pine beetle into contact with novel species of pines and associated insect fauna; interactions with which are largely unexplored. We baited logs of jack pine with lures for mountain pine beetle and eastern five-spined ips (*Ips grandicollis* [Eichoff]) alone and in combination in a 2x2 factorial design in the Black Hills of South Dakota. Both insects occur in this region, but not jack pine, a common species in eastern pine forests at risk of invasion by mountain pine beetle. We measured attraction and reproduction of insects that colonized the logs. Eastern five-spined ips were significantly more attracted to logs baited with their aggregation pheromone, ipsenol, than unbaited logs or those baited with pheromones of mountain pine beetle and myrcene, a host volatile. Colonization by eastern five-spined ips was inhibited by the presence of lures for mountain pine beetle. We also found larvae of longhorn borers, likely *Monochamus* spp., infesting logs. These borers, which act as competitors and facultative predators of bark beetles, were significantly attracted to logs baited with ipsenol over those baited with lures for mountain pine beetle. Our results suggest that if mountain pine beetle were to invade the Great Lakes Region, common bark and wood-boring species such as eastern five-spined ips and longhorn borers would not compete

with mountain pine beetles at tree-colonizing stages, and thus could pose little resistance to invasion.

Introduction

In forested ecosystems, bark beetles (Coleoptera: Curculionidae) provide critical ecosystem services and impact carbon dynamics by promoting wood deterioration, nutrient cycling, and biodiversity (Wood, 1982; Kurz et al., 2008; Mikkelson et al., 2013; Beudert, et al., 2014). Most species of bark beetles are innocuous and non-aggressive, infesting dying or stressed trees and consuming the subcortical cambial tissues (Wood, 1982; Raffa and Lindgren, 2013). Conversely, a minority of bark beetle species can become aggressive, capable of killing mature, live trees at landscape scales. As natural components of disturbance regimes, such species can alter successional trajectories of biomes (Taylor and Safranyik, 2003; Raffa, et al., 2008).

Several environmental requirements must be met for an aggressive species of bark beetle to reach outbreak levels. The first condition is favorable climate. Depending on the species, conditions may include drought that stress host trees or warm minimum winter temperatures that foster brood survival (Safranyik et al., 1978; Carroll et al., 2004; Klutsch et al., 2017). The second requirement is an abundant supply of susceptible host trees (Safranyik et al., 1978). Finally, outbreaking species must be released from potential regulatory effects of natural enemies such as predators and parasitoids and other subcortical woodborers that compete for resources. Each of these guilds may depress the reproductive capabilities of aggressive bark beetles at endemic levels (Rankin and Borden, 1991; Raffa and Lindgren, 2013; Aukema et al., 2016).

Competition may be especially pronounced among bark beetles as an endophytic feeding guild (Raffa and Lindgren, 2013), frequently mediating population dynamics, especially between aggressive and non-aggressive species (Rankin and Borden, 1991; Aukema et al., 2016). Often, several sympatric species of bark beetles simultaneously infest the same tree (Rankin and Borden, 1991; Byers, 1989a). The overlap in the subcortical layer elicits the need for resource partitioning (Byers, 1989a). Interspecific competition can be avoided in two ways: temporally (e.g. exploiting differences in phenology) and spatially (e.g. infesting different parts of the tree). Host procurement and resource partitioning is frequently mediated by semiochemicals. Aggregation pheromones attract conspecifics, while inhibitory allomones push heterospecifics toward other resources (Paine et al., 1981; Byers, 1989b; Ayres et al, 2001). *Ips pini* (Say), *I. paraconfusus* (Lanier), and *Dendroctonus brevicomis* (LeConte), for example, may simultaneously infest a ponderosa pine (*Pinus ponderosae*) but spatially separate themselves throughout the bole (Birch and Wood 1975; Paine et al., 1981; Byers, 1989a). Despite temporal and spatial partitioning strategies among species, however, a broad range of overlap can still exist (Paine et al., 1981; Byers, 1989a; Ayres et al, 2001;).

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), is an aggressive bark beetle native to North America west of the Rocky Mountains and the Black Hills of South Dakota and Wyoming, USA (Safranyik and Carroll, 2006). While mountain pine beetle is a generalist, feeding on most species of sympatric pines in these regions, its principal hosts have historically been ponderosa pine (*Pinus ponderosae* Dougl. ex Laws) and lodgepole pine (*Pinus contorta* Dougl. ex Loud) (Safranyik and Carrol, 2006; Raffa et al, 2008). Outbreaks of mountain pine beetle can

lead to landscape-level mortality of mature pines over many thousands of hectares (Safranyik and Carroll, 2006; Bentz et al., 2010; Hicke et al., 2012). While populations of mountain pine beetle were historically restricted in their northern range due to climatic and geological barriers, ameliorating winter temperatures have permitted northward range shifts (Cudmore et al., 2010, Cullingham et al., 2011). In recent years, British Columbia, Canada has experienced the largest outbreak of *D. ponderosae* in recorded history (Aukema et al., 2006), expanding the insect's range over the geoclimatic barrier of the Rocky Mountains into stands of jack pine (*Pinus banksiana* Lamb.) and its hybrids with lodgepole pine (*Pinus contorta* Douglas. ex Loud. Var. *latifolia* Engelm.) in northwestern Alberta (Kurz et al., 2008; Safranyik et al., 2010; Cullingham et al., 2011; De la Giroday et al., 2012; Lusebrink et al., 2013). Of emerging concern is potential range expansion of mountain pine beetle to the Great Lakes Region of North America with evolutionarily naïve host species that include jack pine, red pine (*Pinus resinosa* Ait.), eastern white pine (*Pinus strobus* L.), and introduced Scots pine (*Pinus sylvestris* L.) (Cooke and Carroll, 2017; Rosenberger et al., 2017b). Recent studies have shown that age classes and distributions of these species exist on the landscape in categories considered highly susceptible to this insect based on comparative studies with suitable and susceptible hosts in western North America (Windmuller-Campione, 2018). While little is known about susceptibility of live potential hosts in the threatened range, it is apparent that mountain pine beetle is able to colonize, tunnel, attract mates, and reproduce within cut logs of all four species of pine (Rosenberger et al., 2017b; Cale et al., 2017).

Despite ecosystem-level changes when mountain pine beetle is at outbreak levels, the species typically exists for decades in an endemic state in which it is unable to

procure hosts through pheromone-mediated mass attacks (Burke and Carroll, 2016). In such states, persistence of mountain pine beetle in the ecosystem may be facilitated by species of non-aggressive bark beetles that allow mountain pine beetle to co-colonize dead or weakened trees in low numbers (Safranyik and Carroll, 2006; Smith et al., 2011). When an environmental stress lowers stand resistance, increasing populations of mountain pine beetle begin partially or mass-attacking mature, live hosts in a population phase transition across the incipient-eruptive threshold (Safranyik and Carroll, 2006). At high levels, non-aggressive species may then inhibit the population growth of mountain pine beetle. Rankin and Borden (1991), for example, showed that forcing a non-aggressive bark beetle such as the pine engraver, *Ips pini* Say, to compete with mountain pine beetle in synchronously co-colonized logs resulted in fewer progeny for both species than if logs were infested independently.

If mountain pine beetle were to arrive in the Great Lakes Region of North America, it may encounter species of non-aggressive bark beetles and woodborers with which it shares no evolutionary history. This mixing would result in novel ecological interactions that may either facilitate or impede the invasion of mountain pine beetle. The Great Lakes Region has several native species of non-aggressive bark beetles such as the pine engraver, *I. pini*, the red turpentine beetle, *Dendroctonus valens* LeConte, and the eastern five-spined ips, *Ips grandicollis* Eichoff. The eastern five-spined ips is an especially common, multivoltine secondary bark beetle native to the Great Lakes Region that typically infests dying or stressed pines (Erbilgin et al., 2002; Lombardero et al., 2006).

It is unknown how mountain pine beetle and the eastern five-spined ips might interact if they were to become sympatric in the Great Lakes region. While pheromones of bark beetles of the genera *Dendroctonus* and *Ips* have been shown to inhibit each other's response in several cases (Rankin and Borden, 1991; Byers, 1989a; Symonds and Elgar, 2004), the responses of the eastern five-spined ips and mountain pine beetle to each other's pheromones have never been studied. The eastern five-spined ips utilizes a species-specific aggregation pheromone, consisting mainly of ipsenol (2-methyl-6-methylideneoct-7-en-4-ol), to attract conspecifics and find mates (Witanachchi and Morgan, 1981). Mountain pine beetle uses both *trans*-verbenol and (±)-*exo*-brevicomin as its aggregation pheromones (Conn et al., 1983). In this study, we aim to elucidate how colonization behavior of each species in logs of jack pine native to the Great Lakes region is affected by the presence of the other's aggregation pheromones. Further, we examine the patterns of reproduction of bark and woodboring beetles in logs baited with each type of pheromone. We hypothesize that there will be no cross attraction or inhibition between the eastern five-spined *Ips* and mountain pine beetle. Our goal is to understand whether cross attraction and thus competitive interactions might exist between the eastern five-spined ips as a bark beetle native to the Great Lakes Region and a potential aggressive invader, mountain pine beetle.

Materials & Methods

Host material

Jack pine was used as a representative novel species of pine native to the Great Lakes Region. Mountain pine beetle is known to colonize and reproduce in jack pine in western forests (Cullingham et al., 2011) and can similarly colonize and reproduce in cut

logs of jack pine from Minnesota (Rosenberger et al., 2017a; Rosenberger et al., 2017b). Twelve trees of 25-27 cm in diameter at 1.4m were harvested from a single-aged stand of jack pine approximately 40 years old at the University of Minnesota Cloquet Forestry Center (CFC) in Cloquet, Minnesota (46.704490˚ N, -92.525310˚ E) on 23 July 2018 and again on 22 July 2019. The site from which trees were harvested is classified as a fire dependent ecosystem in the Laurentian Mixed Forest province of the state, with a climate moderately modified by Lake Superior and lying within USDA Cold Hardiness Zone 4a (Cloquet Forestry Center Management Plan 2016-2025).

Harvested trees were visibly free of infestation of bark beetles or other insects. The twelve felled trees were cut into 48 sections ca. 56 cm in length each year. The ends of these logs were then immediately sealed using melted paraffin wax (Gulf Wax, Roswell, Georgia) applied with a paint brush to reduce the desiccation rate of the logs. Once the wax cooled, logs were enclosed in a 13-gallon drawstring plastic bag (GLAD, Oakland, California) and placed in the bed of a covered pickup truck to prevent unwanted infestation from cerambycid beetles or other borers.

Experimental design

Each year the logs were transported to the Black Hills National Forest near Rapid City, South Dakota (44.072086˚ N, -103.234799˚ E) where populations of mountain pine beetle and the eastern five-spined ips currently exist sympatrically. The logs were deployed at 12 different sites in stands of mature ponderosa pine within 48 hours of being felled. Sites were separated by at least 0.5 km. At each site, four logs were suspended at breast height using MIL-C-5040 Type III 550 lb nylon paracord (Paracord Planet, Fargo, North Dakota) in a square formation at 25m spacing between logs. Each position within

the square was randomly assigned a commercially available pheromone treatment (Alpha Scents, Inc., West Linn, Oregon) at a given site in a 2x2 factorial design. The treatments included mountain pine beetle lure (myrcene, 2-(2H-benzotriazol-2-yl)-4-methyl-phenol, butylated hydroxytoluene, trans-verbenol, and exo-brevicomin), an eastern five-spined ips lure containing ipsenol, both lure types, and a control with no lures in combination. Amounts and elution rates of lure compounds are shown in Table 1.1. Each lure packet was attached to the log using a nail through the outer bark.

Logs were exposed to field conditions from July 25-August 8 of the years 2018 and 2019 to correspond to the flight period of mountain pine beetle (Safranyik and Carroll, 2006) and allow potential infestation by both mountain pine beetle and the eastern five-spined ips, which is typically abundant throughout the summer (Erbilgin et al. 2002). Logs were then retrieved from the field and placed inside rearing tubes at the proximate Wheaton College Science Station (44.061693˚ N, -103.407476˚ E) under ambient temperature conditions where any colonizing insects were allowed to continue development undisturbed (Rosenberger et al, 2018).

For each annual replicate, we removed the logs from the rearing tubes and debarked them in two batches: one half after one month (i.e., mid-September), and the other half in July of the following year (n=24 each time). Logs debarked in the first batch of each year were chosen by randomly selecting six of the twelve sites and then debarking all logs from that site to ensure equal sampling of treatments. We chose to debark logs at these two different time periods in order to elucidate differences in insect community composition and development after one month and after one year. Outer bark was removed with a wood chisel, exposing the internal larval galleries created by insects.

All adults, larvae, and pupae of insects were counted from each gallery and placed in vials of 95% ethanol for identification. All bark beetle larvae found were assumed to be from the species that had constructed the parental gallery. After debarking, a clear plastic sheet was laid over phloem layer of the peeled barked and markers of different colors were used to trace and color-code galleries of bark beetles, creating a map of subcortical utilization. A Scalex PlanWheel XL was used to measure the one-dimensional length of bark beetle parental galleries. In the second year of the study, most of the eastern five-spined ips brood in the initial set of peeled logs had matured to adults by the time logs were peeled in mid-September. Because we could not confidently distinguish initial colonizing beetles from new progeny that had reach adult life stages, we report total counts of bark beetles in each log for each year without assigning colonizer and brood statuses.

The remaining sets of logs (*n*=24 each year) were peeled in July of the following year, approximately one year after field exposure to allow any univoltine species such as mountain pine beetle to complete development (Rosenberger et al., 2018). Larval *Monochamus* were found to have consumed most of the phloem resource, destroying most, if not all, of the bark beetle galleries within. Due to the amorphous nature of the cerambycid galleries we chose to use imageJ (Schneider et al., 2012) to measure the area of phloem consumed by cerambycids rather than gallery length. As such, data analyzed from logs left in rearing tubes for one year only includes the total area of cambial subcortical resource consumed by *Monochamus* spp. larvae.

Statistical analysis

For the logs peeled after six weeks of field exposure and incubation time each year, analysis of variance (ANOVA) with a random effect of site was used to evaluate how response variables such as the numbers of bark beetle larvae, pupae, and adults, as well as the number and length of their galleries, varied among different lure treatments in the 2x2 factorial design (i.e., mountain pine beetle pheromone lures, eastern five-spined ips pheromone lures, and their interaction). A random effect term for year was originally included in the models, low variation between years induced issues of model singularity, so the term for year was removed. Data from one site (n= 4 logs) was excluded from our analysis due to labeling error obscuring treatment. A square root transformation was used on the beetle count data as well as gallery lengths to satisfy the assumptions of a normal distribution of residuals and homogenous variances. Assumptions were assessed by visual inspection of residual plots. ANOVA was also used to evaluate the effect of lure treatments on response variables such as the number of *Monochamus* spp. larvae and the total phloem surface area consumed, with a random effect term of site. A square root transformation was used for both the number of *Monochamus* spp. larvae and their respective area consumed to satisfy the assumptions of a normal distribution of residuals and homogenous variances. Assumptions were asses by visual inspection of residual plots. All statistical analyses were conducted using R version 3.5.3 (R Core team, 2020).

Results

Eastern five-spined ips readily colonized the logs of jack pine in the field but numbers varied with lure type. The ipsenol treatment significantly affected colonization by eastern five-spined ips ($F_{1,31}$= 97.57, P<0.0001). Logs baited with ipsenol had a mean

of 4.6 ± 1.3 SE ovipositional galleries of eastern five-spined ips per log compared to a mean of 0.045 ± 0.064 galleries without (Fig. 1.1A). Conversely, the presence of mountain pine beetle pheromone lures appeared to have a significant negative effect on the colonization behavior of eastern five-spined ips ($F_{1,31}$= 12.13, P= 0.0012). Logs baited with myrcene and the aggregation pheromones of mountain pine beetle had a mean of 1.1 ± 0.3 ovipositional galleries of eastern five-spined ips per log, compared to 3.6 ± 1.1 without. There was a significant interaction effect between the two treatments ($F_{1,31}$= 8.99, P=0.0047) as fewer ovipositional galleries of eastern five-spined ips were constructed in logs baited with both ipsenol and mountain pine beetle lures than would be expected if both main effects were additive (Fig. 1.1A). Thus, the presence of the mountain pine beetle lure inhibits the response of eastern five-spined ips to its own pheromone.

Unsurprisingly, the effect of pheromone treatments on the number of insects found under the bark was very similar to the effects noted on the number of ovipositional galleries found above (Fig. 1.1B). We observed significantly more eastern five-spined ips inside logs baited with ipsenol with a mean of 135.5 ± 39.9 compared to those logs without the pheromones ($F_{1,31}$= 42.73, P<0.0001). In contrast, very few eastern five-spined ips were found in logs baited with mountain pine beetle lures versus those without ($F_{1,31}$=10.04, P=0.0034) (Fig. 1.1B). Again, we observed a significant interaction effect between the two lure types: we found significantly fewer eastern five-spined ips in logs baited with both pheromones than would be expected with an additive effect of the two lures ($F_{1,31}$=5.62, P=0.0242). In these co-baited logs, we collected a mean of 32.0 ± 14.5

eastern five-spined ips per log. These results confirm an aversive response from eastern five-spined ips to the pheromone lure of mountain pine beetle.

In logs left in rearing tubes for one month, significantly more *Monochamus* spp. larvae were found in logs baited with ipsenol than those baited with mountain pine beetle aggregation pheromones (Fig. 1.2). A mean of 19.1 ± 4.9 SE *Monochamus* spp. were found in logs baited with ipsenol compared to 14.2 ± 4.2 larvae found in logs without ipsenol ($F_{1, 31}$=11.62, P=0.0019). There was no overall effect of the mountain pine beetle lure on the numbers of *Monochamus* larvae noted with a mean of 12.7 ± 3.8 *Monochamus* spp. larvae collected ($F_{1, 31}$=0.77, P=0.37). We did note a significant interaction effect between the two lure types with 15.7 ± 4.7 *Monochamus* spp. larvae counted ($F_{1, 31}$=8.41, P=0.0069) likely due to more longhorn borers than would be expected in the logs with mountain pine beetle lure but no ipsenol.

We did not find any mountain pine beetles in the logs peeled in the fall of each year or the sets that were allowed to develop for one year. Any potential colonizers may have been destroyed by cerambycidae, however, as up to 90% of the surface area of the logs' phloem had been chewed apart by developing larvae. We noted small but statistically significant differences of phloem area consumed by *Monochamus* spp. larvae between lure treatments in logs left in rearing tubes for one year (Fig. 1.3). In logs baited with ipsenol, *Monochamus* spp. larvae consumed a mean of 2,015 ± 308 cm^2 ($F_{1, 31}$=4.46, P=0.04242) which was significantly more than logs baited with no lures that had a mean area of 1,084 cm^2 ± 290 cm^2 of phloem consumed per log. Logs baited with mountain pine beetle lures and those baited with both lures had 1733 cm^2 ± 262 cm^2 ($F_{1, 31}$=1.18, P=0.29) and 1,762 cm^2 ± 289 cm^2 ($F_{1, 31}$=2.76, P=0.11) of phloem consumed by

Monochamus spp. respectively. Neither treatment was significantly different from those logs without baits.

Discussion

Our results that the eastern five-spined pine engraver constructed fewer galleries and produced fewer offspring in logs that were baited with mountain pine beetle lures, even in the presence of their own aggregation pheromones, suggests that they will not compete directly mountain pine beetle if the latter were to arrive in the Great Lakes region. These species have no historic sympatric association in the jack pine forests of Minnesota from where the logs originated. The aversive behavior of eastern five-spined pine engraver to pheromones of mountain pine beetle reflects long conserved traits unique to both genera, consistent with interaction between *Ips* spp. and *Dendroctonus* spp. elsewhere. For example, Byers and Wood (1980) demonstrated that *Ips paraconfusus* and *Dendroctonus brevicomis* are both captured in traps in smaller quantities when in the presence of logs infested by both species rather than just conspecifics. Additionally, response of mountain pine beetle to its aggregation pheromones has been shown in both laboratory and field bioassays to be inhibited by the addition of ipsdienol, a component of the aggregation pheromone produced by *I. pini* (Hunt and Borden, 1988). Conversely, response of *I. pini* to ipsdienol is inhibited by the presence of mountain pine beetle aggregation pheromones involving myrcene, *trans*-verbenol, and *exo*-brevicomin or some combination of the three (Hunt and Borden, 1988).

The aversion of bark beetles to aggregation pheromones of other species facilitates pheromone-mediated niche partitioning, regulating interspecific competition

within the tree (Paine et al., 1981; Byers, 1989a). Several species of *Ips* in the southern United States compete with the southern pine beetle, *Dendroctonus frontalis* (Zimmerman), for example (Stephen, 2011). Typically arriving after *D. frontalis*, *I. avulsis* (Eichoff), *I. grandicollis*, and *I. calligraphus* (Germar) may all compete for resources within southern pine species, usually resulting in less phloem resource being consumed by each species when simultaneously infesting a tree than if they were individually infesting that tree (Stephen, 2011). While *D. frontalis* has been shown to be unresponsive to the pheromones produced by the southern *Ips* spp. complex, each of the *Ips* species in this system respond to pheromones of others *Ips* with only *I. grandicollis* responding to pheromones produced by *D. frontalis* (Svihra et al., 1980; Stephen, 2011). Interestingly, the response of *I. grandicollis* to pheromones of *D. frontalis* described by Svihra and others (1980) was one of attraction, which contrasts with our results. This pattern could indicate that sympatric coevolution of *I. grandicollis* with *D. frontalis* has resulted in the attraction of *I. grandicollis* to its pheromones while the lack thereof with mountain pine beetle has maintained its inhibitory response.

Most studies of pheromone responses in bark beetles have been conducted in areas of high populations, which may obscure responses to semiochemicals that vary with population density (Wallin and Raffa, 2004). Responses of both *I. pini* and *Ips latidens* (LeConte) to their respective aggregation pheromones, for example, are interrupted by verbenone, the anti-aggregation pheromone of mountain pine beetle, with increasing levels of interruption with increasing verbenone release rates (Borden et al., 1992; Miller et al., 1995). These examples of niche separation occur with bark beetles at high population levels. It is possible that facilitative interactions may emerge when at

endemic population levels. Previous infestation of pines by *Pseudips mexicanus* Hopkins, for example, has been shown to create more suitable resources for mountain pine beetle when mountain pine beetle is in endemic population sizes. Mountain pine beetle was found to be attacking in higher densities while excavating similar sized galleries in trees previously infested by *P. mexicanus* rather than those not previously infested (Smith et al, 2011).

Species of *Monochamus* exist naturally in the Black Hills National Forest as well as the Great Lakes Region. Larvae of *Monochamus* beetles infest and feed on the phloem of pines. *Monochamus* spp. are thus potential subcortical competitors with mountain pine beetle and the eastern five-spined ips as well as facultative predators (Dodds, 2001; Schenk and Benjamin, 1969; Schoeller et al., 2012). These findings are consistent with previous field and laboratory studies indicating that beetles in the *Monochamus* genus are kairomonally responsive to aggregation pheromones of *Ips* species but not those of *Dendroctonus* (Dodds, 2001; Allison et al., 2003). These findings further indicate that species of *Monochamus* may also not act as significant agents of competition or predation on mountain pine beetle if it were to arrive in the Great Lakes Region but may instead compete more with native species of *Ips*.

While we noted aversion of eastern five-spined ips to mountain pine beetle lures, a lack of mountain pine beetles colonizing the logs precludes ability to make conclusions concerning the response of mountain pine beetle to pheromones of eastern five-spined ips. We also know little about potential responses of natural enemies of bark beetles to pheromones of mountain pine beetle in the Great Lakes Region (Pfammatter et al., 2015). It is possible that lack of coevolution with mountain pine beetle will correspond to a lack

of response to the native complex of bark beetle predators and parasitoids, functionally releasing invasive populations of mountain pine beetle from the pressures of natural enemies. Additionally, our results suggest that insects such as the eastern five-spined ips may avoid trees being mass attacked by mountain pine beetle. Future work should focus on the kairomonal and/or allomonal responses of the diverse bark beetle predators as well as other potential competitors native to the Great Lakes Region to the aggregation pheromones produced by mountain pine beetle (Chapter 2).

This research adds to existing bodies of literature of competition and interspecific pheromonal response of aggressive and non-aggressive bark beetles as well as the response of cerambycid beetles to the aggregation pheromones of bark beetles. It is also the first, to our knowledge, to investigate the novel, and potentially competitive, ecological interactions of the non-aggressive eastern five-spined ips and aggressive mountain pine beetle. Understanding these interactions is especially important given the emerging threat to eastern North America by mountain pine beetle (Cudmore et al., 2010; Cullingham et al., 2011; Rosenberger et al., 2017b; Rosenberger et al., 2018).

Table 1.1 Components within each lure treatment used and the relative release rate of each compound.

Pheromone Lure	Component	Release Rate
Ipsenol	ipsenol	0.5 mg/day
Mountain pine beetle	myrcene	200 mg/day
	transverbenol	2.2 mg/day
	exo-brevicomin	1.1 mg/day

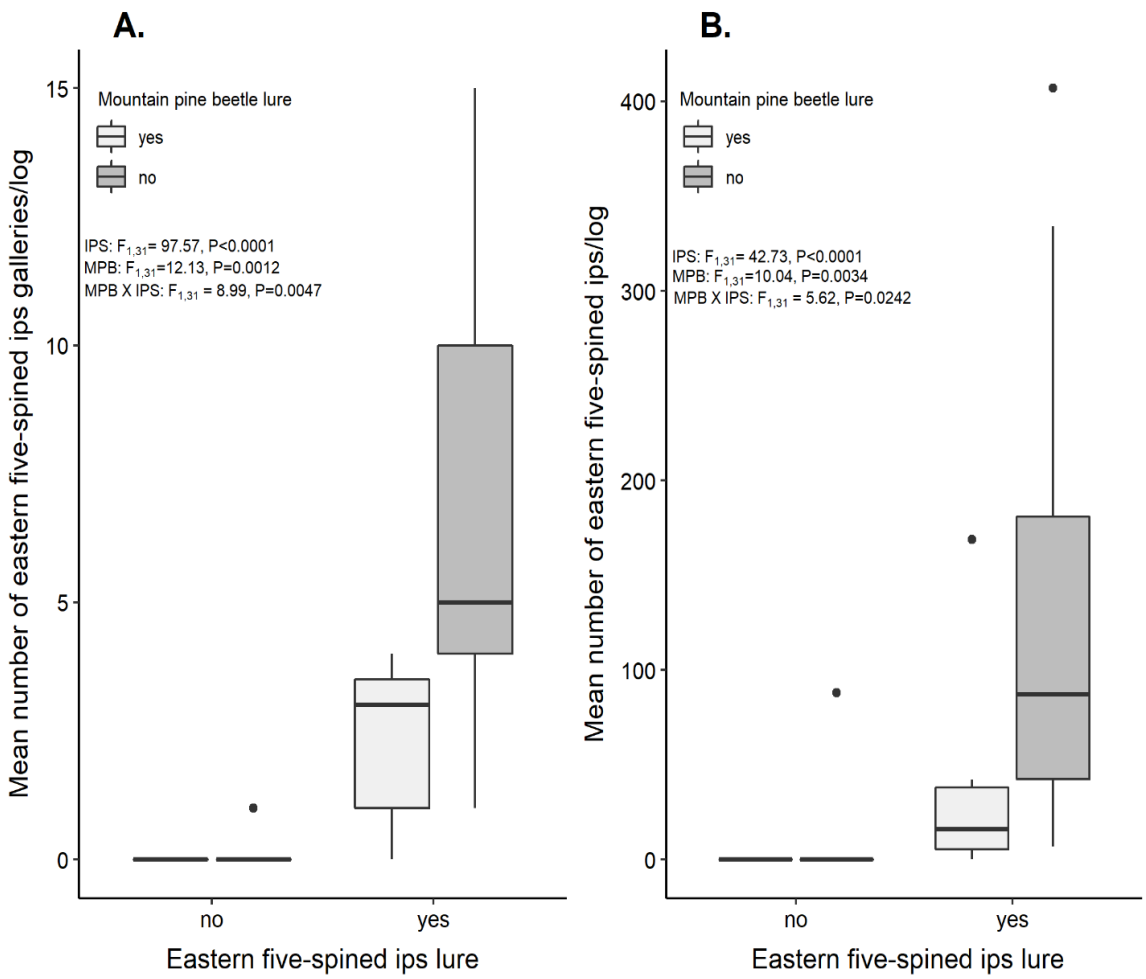

Figure 1.1 Box and whisker plots of A) the number of galleries formed by *I. grandicollis* and B) the combined number of *I. grandicollis* adults, pupae, and larvae collected from logs baited with the different aggregation pheromones and peeled after thirty days in emergence tubes (*n* = 44 logs). The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

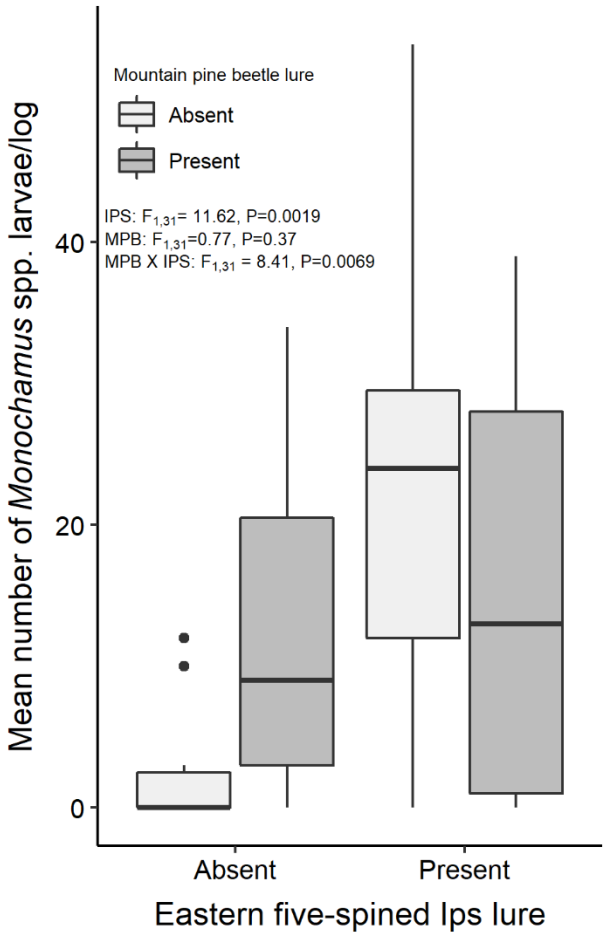

Figure 1.2 Box and whisker plots of number of cerambycid larvae collected from logs peeled after thirty days in emergence tubes of each treatment (*n* = 44 logs). The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

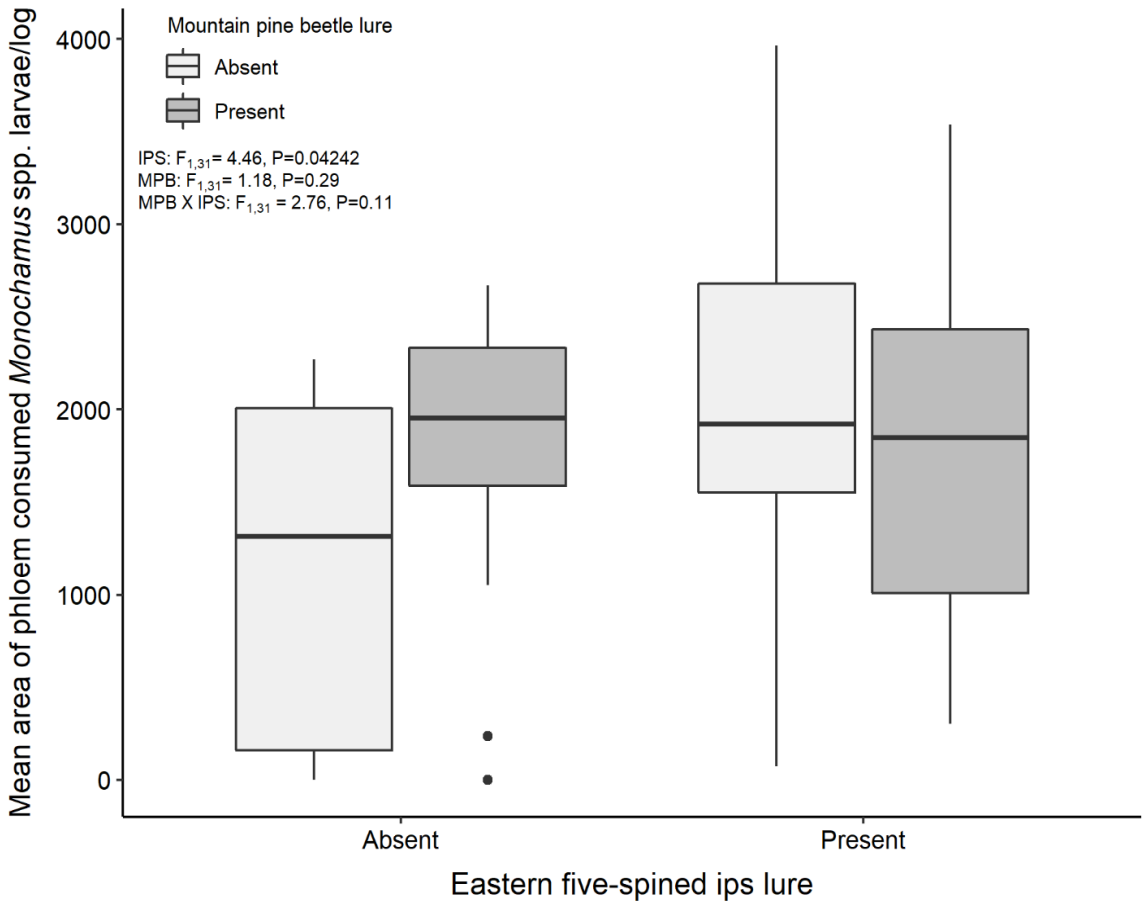

Figure 1.3 Box and whisker plots of the area of phloem resource consumed by cerambycid larvae in logs of each treatment peeled after one year (*n* = 48 logs) in emergence tubes. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

Chapter 2

Natural enemy and competitor sympatric and allopatric response to chemical cues associated with an eruptive herbivore expanding its range

Synopsis

Mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is an aggressive bark beetle native to western North America and the Black Hills of South Dakota that exerts landscape-level mortality to mature pines during outbreaks. Outbreaks of mountain pine beetle occur when landscape configurations of suitable host and warm winter temperatures coincide, facilitating population release from endemic population constraints of tree vigor, natural enemies and competitors. In the past twenty years, a hyperepidemic of mountain pine beetle in western Canada has resulted in seed populations that invaded stands of lodgepole pine (*Pinus contorta* Douglas) X jack pine (*Pinus banksiana* Lamb.) hybrids in northwest Alberta, Canada, causing concern of further eastward expansion into naïve pine forests with no coevolutionary history with this insect. In its native range, numerous insects compete with and predate on mountain pine beetle. However, little is known about how natural enemies beyond its native range might respond to pheromones of mountain pine beetle, with whom they have no historic interaction. This study aims to determine the ability of bark beetle predators and competitors to recognize and respond to species-specific bark beetle aggregation pheromones in three distinct regions falling within and outside the current range of mountain pine beetle. Our study demonstrates regional variation in the response to bark beetle pheromones from natural enemies but strong fidelity to cues associated with local prey, suggesting that associated insects in the Great Lakes Region may not optimally recognize mountain pine beetle aggregation pheromones if the insect were to arrive.

Introduction

Detections of nonindigenous insects, especially those that bore into wood, have increased in recent decades with increased international trade (Aukema et al., 2010). Although few of these non-native species establish, those that do can exert dramatic effects on ecosystems (Liebhold, 1995, Aukema et al., 2010). For example, invasive forest insects in the United States such as emerald ash borer (*Agrilus planipennis* Fairmaire) and hemlock woolly adelgid (*Adelges tsugae* Annand) have resulted in direct mortality to vigorous host trees. Other introduced species such as the European elm bark beetle (*Scolytus multistriatus* Marsh.) vector lethal pathogens, contributing to Dutch elm disease. Collectively, many non-native, introduced species have eliminated dominant species of trees, indirectly altered successional processes, and facilitated encroachment of invasive plants (Small et al., 2005; Gandhi and Herms, 2010; Straw et al., 2013; Herms and McCullough, 2014; Morin et al., 2017).

In the native range of alien species, co-evolved natural enemies can impose regulatory pressures on populations of species that are invasive elsewhere, restricting populations from increasing to destructive numbers in their invaded regions (Liebhold, 1995). In many cases, destructive invasive insect pest populations can be brought under control via classical biological control methods in which the pest's natural enemies are transported from their native range to the pest's invaded range (McGugan and Coppel, 1962; Embree, 1971; Ryan, 1990). Historically, successful cases of classical biological control among defoliating insects have included larch casebearer (*Coleophora laricella* Hubner) (Ryan, 1990), winter moth (*Operophtera bruamata* Linnaeus) (Embree, 1971),

and European spruce sawfly (*Gilpinia hercyniae* Hartig) (McGugan and Coppel, 1962; Liebhold 1995).

Among bark and woodboring insect feeding guilds, biological control and plant defensive responses are important to regulating herbivore populations. For example, the European wood wasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) is innocuous and non-aggressive in its native range of Eurasia and northern Africa. However, in invaded regions such as New Zealand (Nuttall, 1989), Australia (Haugen, 1990), South Africa (Tribe and Cillie, 2004), and South America (Iede et al., 1989), it has become one of the most destructive pests in exotic pine plantations in the southern hemisphere especially where host resistance has decreased due to drought or other environmental stresses (Lantschner et al., 2019). *Sirex noctilio* has also established in the eastern United States and Canada with the first detection in New York state in 2004 (Hoebeke et al., 2005). Although *S. noctilio* has successfully reproduced in many species of pine found in eastern North America such as eastern white pine (*Pinus strobus* L.), red pine (*Pinus resinosa* Aiton), jack pine (*Pinus banksiana* Lamb.), and the insect's native host, scots pine (*Pinus sylvestris* L.), (Dodds and de Groot, 2012), as well as many North American pines introduced to South America (Hoebeke et al., 2005), it has not had major consequences in North America. Native wood boring species may compete with *S. noctilio*. Moreover, many of same species of parasitoids in its native range are also naturally present in pine forests of North America (Dodds and de Groot, 2012).

Mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is an aggressive bark beetle native to forests of lodgepole pine (*Pinus contorta* Dougl.) and ponderosa pine (*Pinus ponderosae* Dougl.) in western North America (Safranyik and Carroll, 2006;

Raffa et al, 2008). Populations of mountain pine beetle persist for decades at endemic levels, where their behavior and brood productivity are poorly understood (Safranyik and Carroll, 2006; Bleiker et al., 2014; Aukema et al., 2016). Persistence in the environment at the endemic population phase is likely facilitated by non-aggressive species of bark beetles such *Pseudips mexicanus* Hopkins (Smith et al., 2011). When conducive conditions coalesce, such as consecutive years of warm winters that foster brood survival (Safranyik, 1978; Cole, 1981; Hicke et al., 2006; Aukema et al., 2008), droughts that lower stand resistance (Bentz et al., 2010; Klutsch et al., 2017), and landscape composition of hosts in susceptible age classes (Fettig et al., 2014), populations of mountain pine beetle can erupt into epidemics (Safranyik and Carroll, 2006; Bentz et al., 2010; Hicke et al., 2012).

During outbreaks, the response of mountain pine beetles to their aggregation pheromone, *trans*-verbenol, is synergized by host volatiles to locate and mass attack mature, vigorous pines, overwhelming the host's defenses (Borden, 1982; Safranyik and Carroll, 2006). In this way, mountain pine beetle epidemics can result in mortality of mature vigorous pines over broad geographic scales (Safranyik and Carroll, 2006; Aukema et al., 2006). In recent decades, warmer winter temperatures, as well as the largest mountain pine beetle outbreak in British Columbia's history, have allowed the beetle's range to expand over the geoclimatic barrier of the Rocky Mountains in northern Alberta, Canada into evolutionarily naïve stands of jack pine (*Pinus banksiana* Lamb.) and jack pine x lodgepole pine hybrids (Aukema et al., 2006; Kurz et al., 2008; Cudmore et al., 2010, Safranyik et al., 2010; Cullingham et al., 2011; De la Giroday et al., 2012; Lusebrink et al., 2013). The recent range expansion has increased concern that mountain

pine beetle may eventually invade the boreal forest of the Great Lakes region where evolutionarily naïve species of eastern pines including jack pine, red pine, eastern white pine, and scots pine grow in abundance (Cooke and Carroll, 2017; Rosenberger et al., 2017b; Windmuller-Campione, 2018). While the susceptibility of live eastern pine hosts to pheromone mediated mass attack from mountain pine beetle is unknown, the insect has been shown to be able to successfully colonize and reproduce in cut logs of all four species (Rosenberger et al., 2017b; Cale et al., 2017).

Natural enemies of mountain pine beetle and other bark beetles use their prey's aggregation pheromones as kairomones to locate potential food sources (Aukema et al., 2000; Erbilgin and Raffa, 2001; Erbilgin et al., 2002; Boone et al., 2008). If mountain pine beetle were to invade the Great Lakes region, it would encounter a complex of natural enemies with which it has no evolutionary history (Pfammatter et al., 2015). While natural enemies in mountain pine beetle's native range respond to the beetle's aggregation pheromones (Amman, 1984; Boone et al., 2008), little is known about how the suite of natural enemies in the Great Lakes region would respond to the presence of mountain pine beetle's aggregation pheromones in the landscape (Pfammatter et al., 2015). Pfammatter and colleagues (2015) for example, conducted initial evaluations of predators and competitors attracted to the pheromone of mountain pine beetle in plantations of red pine in Wisconsin (U.S.A.) outside the range of mountain pine beetle. Here, we expand on that foundation by conducting surveys using a field choice assay that facilitates direct comparisons of behavioral responses of predators and competitors to chemical cues associated with native bark beetles vs. mountain pine beetle in regions where natural enemies have vs. have not coevolved with mountain pine beetle. We also

elucidate when responding insects occur phenologically, as temporal separation can allow escape of the primary herbivore in time. This information is imperative in understanding the response of native forest insects to the presence of mountain pine beetle on the landscape as well the novel ecological interactions that may form a component of biotic resistance against mountain pine beetle if the insect were to successfully invade the Great Lakes region.

Materials and Methods

Site Locations

Trapping experiments were conducted in three regions of central North America: Minnesota, South Dakota, and Nebraska (described below). Traps were sampled biweekly from ordinal day 107 (April 17) to ordinal day 285 (October 12) in 2017 and ordinal day 101 (April 11) to ordinal day 262 (September 19) in 2018.

The Black Hills National Forest near Rapid City, South Dakota (44.072086˚ N, -103.234799˚ E) is considered the easternmost historic range of *D. ponderosae.* Sites were composed of stands of pure ponderosa pine, which is the dominant forest type (Walters et al., 2011). Trees were approximately 40 years old. A large outbreak of *D. ponderosae* had subsided after a cold snap in November of 2014 (Rosenberger et al., 2017a) but there were still pockets of *D. ponderosae* activity throughout the region.

Minnesota was selected as a site within the Great Lakes Region due to its proximity to the invasion front of *D. ponderosae* and coverage of eastern pine species potentially susceptible to *D. ponderosae*. Traps were placed primarily in mature red pine plantations of approximately 50 years of age. A transect of sites from Stacy, MN to Sturgeon Lake, MN was installed to detect if populations of *D. ponderosae* had already

established in the region. Populations would presumably first establish in the northern latitudes of the state if arriving through jack pine forests of the Canadian boreal zone (Cooke and Carroll, 2017).

The Nebraska National Forest at Halsey near Halsey, Nebraska, USA (394850.16 E, 4639584.04) is a hand-planted forest within the Sandhills that is comprised of *P. ponderosae* sourced from the Black Hills National Forest and *P. banksiana* sourced from Minnesota. Sites were placed in in 60-100 year age classes. Due to its isolation, this forest represents a "pine island" in the Great Plains. Mountain pine beetle is found in western Nebraska but has never been found in the Nebraska National Forest at Halsey to date (Castello and Schaupp, 2011).

Experimental Design

Five Lindgren funnel traps were deployed 25 meters apart 1.3 meters off the ground in a pentagon arrangement at six sites within each region (Lindgren, 1983). Each site was separated by at least two kilometers. Funnel traps were each randomly baited with a different lure (Synergy Semiochemicals, British Columbia, Canada). The five treatments were as follows: a blend targeting *Ips grandicollis* (Eichoff) known hereafter as "ipsenol" (cis-verbenol, ipsenol), a blend targeting *Ips pini* (Say) hereafter known as "ipsdienol" (R (-) ipsdienol), mountain pine beetle aggregation pheromone lure combined with myrcene, thus referred to as MPB+Myr (exo-brevicomin, transverbenol, myrcene), mountain pine beetle aggregation pheromone lure with terpinolene, thus referred to as MPB+Terp (exo-brevicomin, transverbenol, terpinolene), and a blank control. Release rates for pheromone components are shown in Table 2.1. The collection cup on each funnel trap contained a 2 cm x 2 cm insecticidal strip containing 18.6% dichlorvos (Hot

Shot, St. Louis, Missouri, USA) to prevent insect escape and reduce consumption of bycatch by predators. Samples from each site were collected biweekly. Trap placement within each pentagon was randomized at every trap check using a random number generator (www.random.org/lists) and lures were replaced at every other trap check to maintain consistent elution rates.

Trap contents were stored in a freezer at -4º C until identification. Insects from collected samples were counted and identified to species with some exceptions that were identified to genus such as the genera *Phyllobaenus* (Dejean), *Madoniella* (Pic), *Platysoma* (Leach), *Hylurgops* (LeConte), and *Hylastes* (Erichson).

Statistical Analysis

Statistical analysis of insect species found in trap captures focused on species with counts higher than 60 total captures in the years 2017 and 2018. Insects were identified to species level, although certain groups were aggregated to genus or family levels. Analysis of variance (ANOVA) with a random effect term of site was used to evaluate variation in insect abundance between regions, response to treatments by region, and their interaction. Because the interaction term was significant for almost all species ($P<0.0001$), indicating that the pheromone response varied between regions, we present all data by region. A square root transformation was used on insect count data to satisfy the assumptions of a normal distribution of residuals and homogenous variances. Model assumptions were assessed by visual inspection of residual plots. All statistical analyses were conducted using R version 3.5.3 (R core team, 2020). Where significant treatment or region effects existed, the glht() function from the multcomp package was used for

post-hoc comparisons of means using Tukey's HSD test. Degrees of freedom for test statistics were approximated using Satterthwaites method (Hothorn et al., 2008).

Results

Seasonal Abundance

Several species of bark beetles were captured in this study, exhibiting disparate seasonalities. All *D. ponderosae* were captured between ordinal days 195 and 240 (corresponding to July 14 and August 28) with peak captures on day 212 (July 31) in 2017 and day 240 (Aug 28) in 2018 (Fig. 2.1A). In contrast, species such as *I. pini* and *I. grandicollis* were captured throughout the study (Fig. 2.1B, C). When trapping began in 2017, high numbers of *I. pini* were captured immediately and declined through ordinal day 227 (August 15). Trap captures rose to another peak at ordinal day 254. In 2018, *I. pini* again exhibited initially high trap captures when data collection commenced before declining to low point at ordinal day 227 and again rising. Similar to *I. pini*, *I. grandicollis* were captured throughout the sampling periods. In 2017, *I. grandicollis* captures peaked in the collection period between ordinal days 128 (May 8) and 14 (May 22) with 2,697 beetles captured across all traps. *Ips grandicollis* again peaked in the collection period between ordinal days 206 (July 25) and 227 (August 15) with 353 captures. In 2018, *I. grandicollis* again showed two peaks within our collection period with 2,038 beetles between ordinal days 129 (May 9) and 149 (May 29). *Pityogenes knechteli* (Swaine) trap captures exhibited two peaks: ordinal days 184 (July 3) and 240 (August 28) in 2017 and ordinal days 163 (June 12) and 227 (August 15) in 2018 (Fig. 2.1D).

Bark beetle predators were similarly captured throughout the study (Fig. 2.2). Captures of *T. virescens* showed a single peak with 788 captures between ordinal days 192 (July 11) and 213 (August 1) in 2017 and ordinal days 191 (July 5) and 208 (July 27) in 2018 with 217 captures. Captures of *Platysoma* spp. in 2017 were consistent with a slight peak between ordinal days 192 (July 11) and 213 (August 1) in 2017 with 213 captures before trap catches declined. In 2018, *Platysoma* spp. trap captures slowly increased until they peaked between ordinal days 186 (July 5) and 239 (July 27) with 91 captures. Trap captures of clerid beetles were steady in the beginning of the season and rose to a peak between ordinal days 192 (July 11) and 213 (August 1) in 2017 and ordinal days 186 (July 5) and 239 (July 27) in 2018 with 499 and 530 captures respectively.

Trap Captures by Region

Bark Beetles and Woodborers

Total insect captures by region are shown in Table 2.2. *Dendroctonus ponderosae* were only captured in South Dakota with a total of 503 individuals and a mean capture rate of 21.9 ± 0.9 SE beetles per two week collection period. *Dendroctonus valens* (LeConte) were captured in all three regions with captures in South Dakota approximately double those in Minnesota and South Dakota. Populations of *I. pini* were highest in Minnesota with a mean capture rate of approximately 800 individuals per collection period. This amount was more than double that recorded in both South Dakota and Nebraska where mean capture rates were 288.9 ± 9.2 and 215.4 ± 6.7 per collection period, respectively. Captures of *I. grandicollis* did not differ significantly between regions. South Dakota had mean capture rates of 172.0 ± 3.6 *I. grandicollis* per collection, while Minnesota and Nebraska exhibited mean capture rates of 373.1 ± 13.0

and 326.1 ± 8.7 *I. grandicollis* per collection respectively. *Pityogenes knechteli*, a small twig beetle, was found almost exclusively in South Dakota vs. Minnesota, with a total of 9,502 vs 3 captured in each state respectively, and almost 500 captured in Nebraska.

Hylastes spp. were most commonly found in Nebraska with a mean of 63.7 ± 1.4 insects captured per collection period. However, this amount was not significantly more than capture rates in South Dakota where we caught a mean of 52.3 ± 0.9 individuals per collection period. *Hylastes* spp. captures were significantly lower in Minnesota than both South Dakota and Nebraska where mean capture rates were 9.3 ± 0.9 per collection period. While species of *Hylurgops* were found in all regions in the study, South Dakota capture rates were orders of magnitude higher than those in Minnesota and Nebraska. South Dakota had capture rates of 50.3 ± 2.1 *Hylurgops* spp. captured per biweekly collection as opposed to 1.7 ± 0.1 and 3.8 ± 0.3 *Hylurgops* spp. captured in Minnesota and Nebraska respectively.

Species of *Monochamus* (Dejean) including *M. clamator* (LeConte), *M. carolinensis* (Olivier), *M. titillator* (Fabricius), and *M. scutellatus* (Say) were also found in our pheromone baited traps. The most abundant *Monochamus* spp. captured was *M. clamator*. Most individuals were recorded from the Nebraska site with a mean of 9.3 ± 0.2 individuals caught per collection period. Overall, *Monochamus* spp. were most prevalent in Nebraska averaging of 9.8 ± 0.2 per collection. These counts were significantly higher in Nebraska than both South Dakota 2.5 ± 0.1 and Minnesota 1.0 ± 0.1, respectively. *Monochamus* spp. loads were not significantly different between South Dakota and Minnesota.

Predators

The most common bark beetle predator caught in the pheromone traps were of the family Cleridae (Latreille). Most clerid beetles were captured in South Dakota and Minnesota with respective mean captures of 107.5 ± 2.5 and 152.9 ± 4.2 clerid beetles per biweekly collection. In contrast, less than 100 clerid beetles were captured in Nebraska over two years.

Within the family Cleridae, we found several species that were found almost exclusively in South Dakota. One of these species, *Enoclerus lecontei* (Wolcott), had a mean capture of 10.0 ± 0.5 individuals per collection in South Dakota with no specimens captured in Minnesota or Nebraska. Similarly, a mean of 78.7 ± 2.3 *Enoclerus sphegeus* (Fabricius), another clerid beetle, were captured almost exclusively in South Dakota with only a single individual captured in each of Minnesota and Nebraska. Another group of clerid beetle primarily found in South Dakota were *Phyllobaenus* spp. with a mean of 11.9 ± 0.5 captured per collection period. In contrast, no individuals of *Phyllobaenus* spp. captured in Minnesota and only 0.8 ± 0.1 were captured per collection period captured in Nebraska. Two clerid species were found in the highest numbers in Minnesota. A total of 1270 *Madoniella* spp. (Pic.) and 2159 *Thanasimus dubius* (Fabricius) were captured in Minnesota during the study. In contrast, fewer than 50 of each species were captured in South Dakota and Nebraska over that time.

More *Thanasimus undatulus* (Say) were also found in South Dakota (3.4 ± 0.1) than Minnesota (2.1 ± 0.1) but this result was not statistically significant. *Thanasimus undatulus* was not caught in Nebraska. Only one species of clerid was found in higher numbers in Nebraska than the other regions in the study although the differences were not

statistically significant. *Enoclerus nigripes* (Say) accounted for a majority of clerid beetles captured in Nebraska comprising 71 of 97 total clerid captures. In contrast, 27 and 38 individuals were captured in South Dakota and Minnesota respectively.

Other bark beetle predators included *Platysoma* spp. (Leach) and *Temnoscheila virescens* (Fabricius). Each of these predators was found to be most abundant in South Dakota region but not significantly more than Nebraska. A mean of 71.9 ± 1.7 and 49.2 ± 0.8 *Platysoma* spp. were captured per collection period in South Dakota and Nebraska respectively. In Minnesota, a mean of 14.9 ± 0.4 *Platysoma* spp. individuals were captured per biweekly collection. Captures of *T. virescens* followed the same pattern as *Platysoma* spp. South Dakota exhibited the highest capture rates of 178.2 ± 2.7 *T. virescens* captured in each collection period although this was not significantly higher than Nebraska catch rates of 164.9 ± 2.6 captured per collection. On average, a single *T. virescens* was captured in Minnesota per collection period.

Attraction to Pheromones by Region

Bark beetles and wood-boring insects

Due to ubiquitous region x lure effects (results not shown), responses of insects to lures are reported by region. *Dendroctonus ponderosae,* which was only found in South Dakota, was most attracted to the MPB aggregation pheromone lures over the other treatments (Fig. 2.3A). There was no statistically significant difference found in the capture rates between the two MPB lures ($F_{1, 796} = 0.78$ $P = 0.38$).

The attraction of *D. valens* to the different lures varied among regions (Fig. 2.3B). In South Dakota, MPB+Myr captured the highest number of *D. valens* with 6.2 ± 0.5 beetles per collection period, followed by ipsdienol (6.0 ± 0.6). These two lures attracted

the most *D. valens* in Nebraska as well, although ipsdienol baited traps caught 41.67% more insects that those baited with MPB+Myr. In Minnesota, traps baited with the MPB+Terp lure caught the most *D. valens* with 4.4 ± 0.4 beetles per collection period followed by traps baited with MPB+Myr and ipsdienol which had respective capture rates of 2.3 ± 0.8 and 1.7 ±0.2 individuals per collection period.

Other bark beetles showed very consistent responses to specific pheromones (Fig. 2.4). *Ips pini* was almost exclusively attracted to ipsdienol and *I. grandicollis* was exclusively attracted to ipsenol. Ninety-three-point-seven percent of all *P. knechteli* captures across the three regions were in ipsenol-baited traps.

Hylurgops spp. were highly attracted to ipsdienol pheromone treatments in South Dakota (Fig. 2.5A). *Hylastes* spp. exhibited similar preferences in South Dakota with 32.4% of the total *Hylastes* spp. captured in the region (Fig. 2.5B). *Hylastes* spp. were found in all of the traps but only exhibited statistically significant differences in attraction among treatments in South Dakota.

Predators

Figure 2.6 displays the pheromonal attraction of *Enoclerus sphegeus, Madoniella* spp. and *Thanasimus dubius*, by region. Each of these species were attracted almost exclusively by pheromones of pine engravers- ipsdienol or ipsenol- and in some cases were cross attracted to both. *Enoclerus sphegeus* were primarily attracted to ipsenol over all other treatments with 85.9 % of captures being in ipsenol baited traps (Fig. 2.6A). Ninety-nine percent of *Madoniella* spp. were caught in ipsdienol baited traps and almost exclusively captured in Minnesota (Fig. 2.6B). *Thanasimus dubius* was similarly found

to be most attracted to ipsenol but was also attracted to ipsdienol (Fig. 2.6C). We found no evidence that *T. dubius* was attracted to lures of mountain pine beetle.

In contrast to clerid beetles, *Platysoma* spp. and *T. virescens* demonstrated broad cross-attraction to pheromones of pine engravers and mountain pine beetle. *Platysoma* spp. were similarly consistently attracted to ipsenol and ipsdienol over MPB+Myr, MPB+Terp, and the control treatment (Fig. 2.7A). Ninety-three-point-eight percent of *Platysoma* spp. were captured in traps baited with these two lures. In South Dakota, *T. virescens* was most attracted to traps baited with ipsdienol or ipsenol with a respective mean capture rates of 69.8 ± 4.1 and 56.0 ± 3.2 beetles per collection period (Fig. 2.7B). *Temnoscheila virescens* were also attracted to traps baited with MPB pheromones with host volatiles myrcene or terpinolene, but less so. Approximately 29.3% of *T. virescens* captured in South Dakota were attracted to the latter treatments. In Nebraska the proportions were more heavily biased in favor of the pine engraver pheromone treatments, with 60.4%, 31.3%, and 8.2% of *T. virescens* captured in traps baited with ipsdienol, ipsenol, and MPB lures respectively.

Discussion

Our results showing regional differences in the composition and response profile of the natural enemy complexes existing inside and outside of the native range of mountain pine beetle demonstrate that predator species sharing evolutionary history with mountain pine beetle recognize and respond to its associated semiochemicals as an indicator of potential prey. In contrast, natural enemies in ranges allopatric to mountain pine beetle are unlikely to recognize or be attracted to blends of mountain pine beetle aggregation and host volatiles. These data suggest that direct potential impacts of native

natural enemies on mountain pine beetle in the Great Lakes region would likely be minor if the mountain pine beetle were to be inadvertently introduced and establish (Pfammatter et al., 2015).

We noted strong geographic variation in the abundance and pheromone response profiles of several natural enemies present in all three regions, which likely reflects varying degrees of prey specialization. For example, *Platysoma* spp. and *Temnoscheila virescens*, both predators of bark beetles (DeLeon, 1934; Aukema et al., 2000), were present in all three regions but exhibited disparate responses to lures. Overall, highest numbers were captured at traps baited with the pheromones of pine engravers, ipsenol or ipsdienol, over the other treatments in each region. In Nebraska, however, *Platysoma* spp. also showed moderate attraction to the mountain pine beetle pheromone lure with myrcene (Fig. 2.7A) even though this area was devoid of mountain pine beetle (Costello and Schaupp, 2011). It is possible these predators were responding to the host volatiles myrcene and terpinolene and not the pheromones of mountain pine beetles deployed with each bait, although previous additions of myrcene and terpinolene to pheromones of mountain pine beetle did not increase trap captures of *Platysoma* spp. in Wisconsin (Pfammatter et al., 2015), another area without mountain pine beetle. It is also possible that *Platysoma* spp. showed increased responses to myrcene in Nebraska and because the phloem of ponderosa pine contains significant quantities of myrcene. *Platysoma* spp. in Nebraska have regionally adapted to associate this host volatile bark beetle prey (Rosenberger et al., 2017b). A third, but not mutually exclusive reason for geographic variation in the response of *Platysoma* spp. may be cryptic species differences within the genus that we did not identify.

Thanasimus dubius is another generalist bark beetle predator that feeds on mountain pine beetle and spruce beetle (*Dendroctonus rufipennis* Kirby) where their ranges overlap (DeLeon, 1934; Bentz and Munson, 2000). While *T. dubius* was attracted to the pheromones of *Ips* spp. (Fig. 2.6C; Erbilgin and Raffa 2001), we could not find evidence of weak attraction of *T. dubius* (or any other clerid) to pheromones of mountain pine beetle, in contrast to the results of Pfammatter and colleagues (2015). It is possible that strong preferential responses of clerids such as *T. dubius* to pheromones of pine engraver beetles in our choice assay obscured any responses of *T. dubius* to semiochemicals associated with mountain pine beetle (Aukema et al., 2000). Pfammatter and colleagues (2015) used pheromone of pine engravers as a positive control adjacent to their behavioral choice assays to verify presence of such prey instead of as a treatment incorporated within the choice assay per our protocol.

All of the abundant predators – clerids, *Platysoma* spp., and *T. virescens* – peaked in abundance approximately one month before peak flight of mountain pine beetle in South Dakota (Fig. 2.1A, 2). It is currently unknown when mountain pine beetle might emerge in the Great Lakes region if it were to arrive. Previous work suggests the possibility of faster larval development in some novel species of pine (Rosenberger et al., 2018) and therefore possible earlier adult emergence for beetles infesting these trees. More modelling must be done to determine the potential seasonality of mountain pine beetle in the Great Lakes region as it is impossible to study without introducing it to the Great Lakes region (Bentz et al., 2010; Pfammatter et al., 2015).

Aside from several predators, we noted several other bark beetles that could be potential competitors with mountain pine beetle (Rankin and Borden, 1991; Lindgren and

Raffa 2013). *Ips pini* and *I. grandicollis* were both captured in similar numbers in all three regions, with each insect exhibiting strong fidelity to their respective species-specific aggregation pheromones of ipsdienol and ipsenol. *Pityogenes knechteli* was only captured in high numbers in South Dakota and displayed significant cross-attraction to ipsdienol, the aggregation pheromone of *I. pini* (Poland and Borden 1994a). *Pityogenes knechteli*'s ability to co-infest trees with *I. pini* may enhance the negative effects on mountain pine beetle reproduction *I. pini* imposes (Rankin and Borden, 1991; Poland and Borden, 1994b). Moreover, production of aggregation pheromones by non-aggressive species of bark beetles that co-infest trees with mountain pine beetle, incurs potential predation to *D. ponderosae* by natural enemies not usually attracted to its semiochemicals (Boone et al., 2008).

The lower stem and root-colonizing insects *D. valens*, *Hylurgops* spp. and *Hylastes* spp. were captured in all three regions and were broadly attracted to all types of lures associated with upper stem-infesting bark beetles (Fig. 2.3B, 4). As these genera generally infest the base of trees, it is unlikely they would compete with mountain pine beetles that infest tree boles. Because each of these lower stem and root-colonizing species flies earlier than mountain pine beetle (Fig. 2.1; Erbilgin and Raffa, 2002), it is possible that these bark beetles would actually facilitate endemic populations of mountain pine beetle (Smith et al., 2011; Boone et al., 2011).

Similar to geographically disparate responses noted among *Platysoma* spp., the preference of *D. valens* for lures containing terpinolene vs. myrcene in Minnesota may reflect longstanding geographic separation from western species of pine, such as ponderosa pine that exhibit significantly higher concentrations of myrcene within their

phloem than eastern species of pine such as red, eastern white, and scots pine (Rosenberger et al., 2017b). Stronger responses to myrcene in South Dakota and Nebraska support this hypothesis (Fig. 2.3B). Previous research on semiochemical responses of geographically separated populations of *D. valens* has uncovered a conserved attraction to the monoterpene (+)-3-carene among geographically separated populations in North America and invasive populations in China. These populations also elicited variable rates of attraction to other single semiochemicals and blends of those semiochemicals (Erbilgin et al., 2007) however. This finding is consistent with other species of bark beetles like *I. pini* that have geographically variable semiochemical "dialects" (Miller et al., 1997; Aukema et al., 2000; Erbilgin et al., 2007).

We observed no significant difference in mountain pine beetle trap captures between the two mountain pine beetle lures in its native range of South Dakota (Fig. 2.3A). Attraction of mountain pine beetle to host semiochemicals, myrcene and terpinolene, changes with local population densities (Klutsch et al., 2020). High population densities are more attracted to terpinolene and myrcene than *trans*-verbenol, but at low population densities, mountain pine beetles are more attracted to the aggregation pheromone over the host volatiles (Klutsch et al., 2020). Our study was conducted during a period of endemic population levels as defined by Safranyik and Carroll (2006). Our results indicate that both pheromones perform relatively equally when trapping mountain pine beetle at endemic levels.

Species of *Monochamus* are known to be competitors and facultative predators of bark beetles and can contribute to mortality when co-infesting a tree (Schenk and Benjamin, 1969; Schoeller et al., 2012). We captured significantly higher numbers of

Monochamus spp. in the Nebraska national forest than in either South Dakota or Minnesota but, conversely, did not capture any mountain pine beetle despite established populations being documented on the western edge of the state (Costello and Schaupp, 2011). At endemic levels, mountain pine beetle exists with a suite of co-colonizing subcortical herbivores (Safranyik and Carroll, 2006; Smith et al., 2011), and destruction of phloem resources by *Monochamus* spp. over a one-year generation time could be a significant barrier to establishment in "stepping-stone" forests that exist throughout Nebraska. More research is needed comparing our study sites to the forests of western Nebraska to understand why mountain pine beetle has not expanded past these initial established populations.

Further research is needed to understand how the response of natural enemies to chemical cues might change as population levels rise, similar to mountain pine beetle (Klutsch et al., 2020), and how interspecific variation in host tree chemistry may influence natural enemy responses. (Erbilgin and Raffa, 2000). Our results suggest that natural enemies within the Great Lakes region at present are naïve to the presence of mountain pine beetle and therefore are not highly attracted to its associated semiochemicals as an indication of prey. It is unknown if and on what time scale these natural enemies might adapt to recognize the aggregation pheromones of mountain pine beetle after a potential introduction. If mountain pine beetle were to invade the Great Lakes region, however, it would not only interact directly with natural enemies. It is possible that indirect interaction with native defoliators such as jack pine budworm (*Choristoneura pinus* Freeman) may alter live host susceptibility with induced defenses that studies using logs of eastern pine species are unable to capture (Colgan and Erbilgin,

2011; Rosenberger et al., 2017b). Other areas of further research include dispersal capabilities of natural enemies that could move with the expanding range of *D. ponderosae* and how their potential life cycles may synchronize or decouple in new climatic conditions.

Table 2.1 Components within each lure treatment used and the relative release rate of each compound.

Pheromone Lure	Component	Release Rate
Ipsenol	cis-verbenol	300 µ/day
	ipsenol	0.6-0.8 mg/day
Ipsdienol	Ipsdienol	150-250 µ/day
MPB+Myr	exo-brevicomin	120 µ/day
	myrcene	170 mg/day
	transverbenol	1.2 mg/day
MPB+Terp	exo-brevicomin	120 µ/day
	terpinolene	170 mg/day
	transverbenol	1.2 mg/day

Table 2.2 Summary of trap captures of each insect group in each region in the collection periods of 2017-2018 (6 plots of 5 Lindgren funnel traps per year; *n* = 30 traps/region/year). Summary statistics reflect species comparison between regions. Means followed by the same lowercase letter indicate no significant difference in abundance between regions within row.

Species	Minnesota			Nebraska			South Dakota					
	Mean	SE	Total	Mean	SE	Total	Mean	SE	Total	*F*	df	*P*
A. Bark and wood boring beetles												
Dendroctonus ponderosae	0 a	0	0	0 a	0	0	21.87 b	0.85	503	24.54	2, 15.2	<0.0001
Dendroctonus valens	8.74 a	0.41	201	7.00 a	0.22	161	17.57 b	0.39	404	8.55	2, 15.1	<0.0001
Hylastes spp.	9.26 b	0.21	213	63.65 a	1.19	1,464	52.30 a	1.7	1,203	18.72	2, 32.9	<0.0001
Hylurgops spp.	1.65 a	0.11	40	3.83 b	0.3	90	50.26 b	2.08	1,200	25.09	2, 15.0	<0.0001
Ips grandicollis	373.09 a	12.98	8,581	326.09 a	8.69	7,500	172.04 a	3.62	3,957	3.73	2, 14.9	0.1003
Ips pini	799.39 b	24.7	18,386	215.35 a	6.66	4,953	288.87 a	9.16	6,644	9.49	2, 15.0	0.0486
Monochamus clamator	0.30 a	0.03	7	9.30 b	0.23	214	2.35 a	0.08	54	19.53	2, 33.1	<0.0001
Monochamus spp.	0.96 a	0.05	22	9.83 b	0.24	226	2.48 a	0.08	57	16.27	2, 33.1	<0.0001
Pityogenes knechteli	0.13 a	0.01	3	21.00 a	0.94	483	413.13 b	17.19	9,502	88.29	2, 33.9	<0.0001
B. Predators												
Cleridae	152.87 a	4.16	3,516	4.22 b	0.14	97	107.48 a	2.48	2,472	27.41	2, 15.1	<0.0001
Enoclerus lecontei	0 a	0	0	0 a	0	0	9.96 b	0.48	229	13.38	2, 13.8	<0.0001
Enoclerus nigripes	1.65 a	0.14	38	3.09 b	0.13	71	1.17 a	0.1	27	5.89	2, 14.7	0.1323
Enoclerus sphegeus	0.04 a	0.01	1	0.04 a	0.01	1	78.65 b	2.27	1,809	25.38	2, 15.1	<0.0001
Madoniella spp.	55.22 b	3.3	1,270	0.17 a	0.07	4	1.96 ab	0.1	45	3.68	2, 15.1	0.04937
Phyllobaenus spp.	0 a	0	0	0.83 a	0.05	19	11.87 b	0.5	273	27.11	2, 15.2	<0.0001
Platysoma spp.	14.91 a	0.36	343	49.22 b	0.76	1,132	71.91 b	1.7	1,654	32.27	2, 15.0	<0.0001
Temnoscheila virescens	1.00 a	0.09	23	164.91 b	2.64	3,793	178.17 b	2.67	4,098	41.02	2, 14.9	<0.0001
Thanasimus dubius	93.87 b	2.57	2,159	0.09 a	0.02	2	0.43 a	0.05	10	62.90	2, 15.2	<0.0001
Thanasimus undatulus	2.09 b	0.11	48	0 a	0	0	3.43 b	0.1	79	6.72	2, 15.1	<0.0001

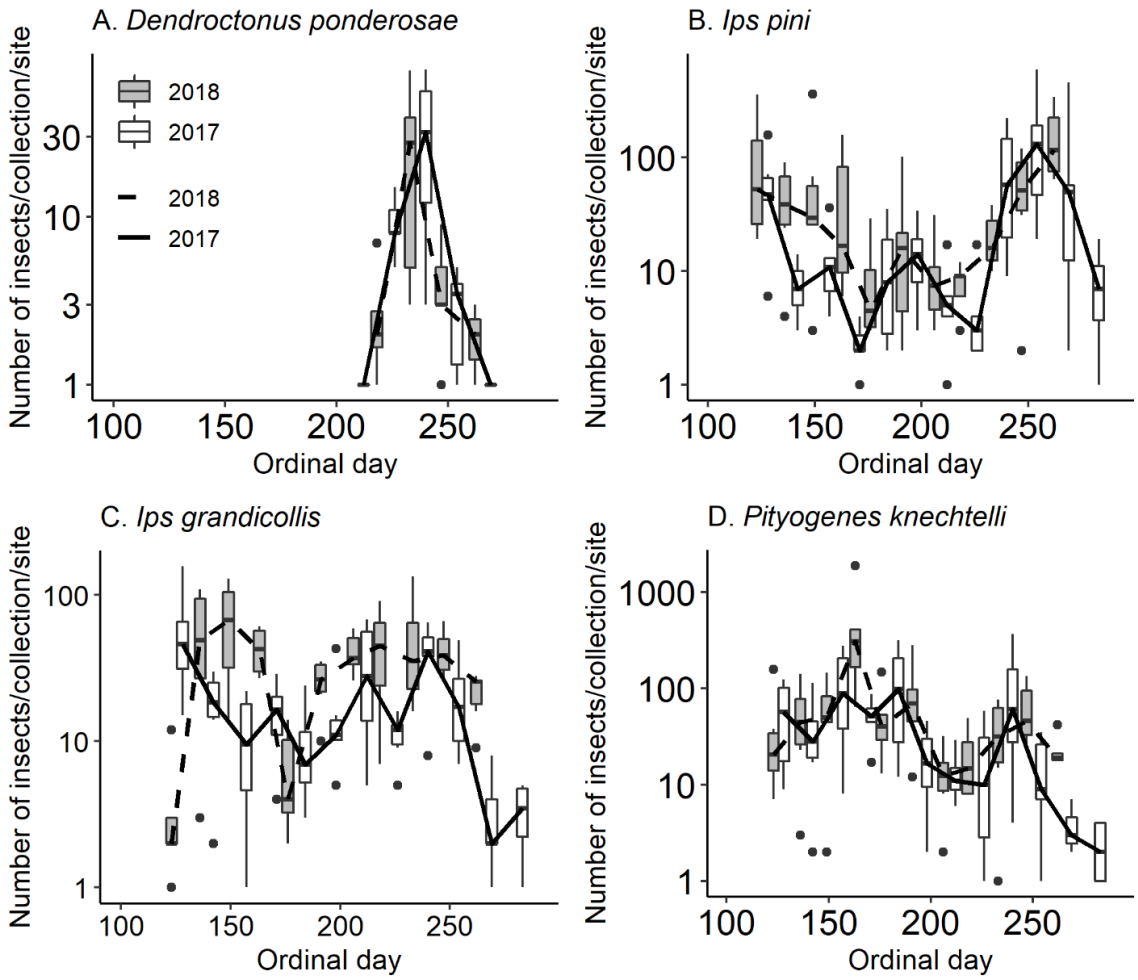

Figure 2.1 Box and whisker plots of number of bark beetles (A) *Dendroctonus ponderosae, (B) Ips pini, (C) Ips grandicollis,* and (D) *Pityogenes knechteli* in each 14-day sample period per site captured in summers 2017 (solid line) and 2018 (dashed line). Variation within funnel trap captures at each collection period is displayed in box and whisker plots for 2017 (white) and 2018 (shaded). The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

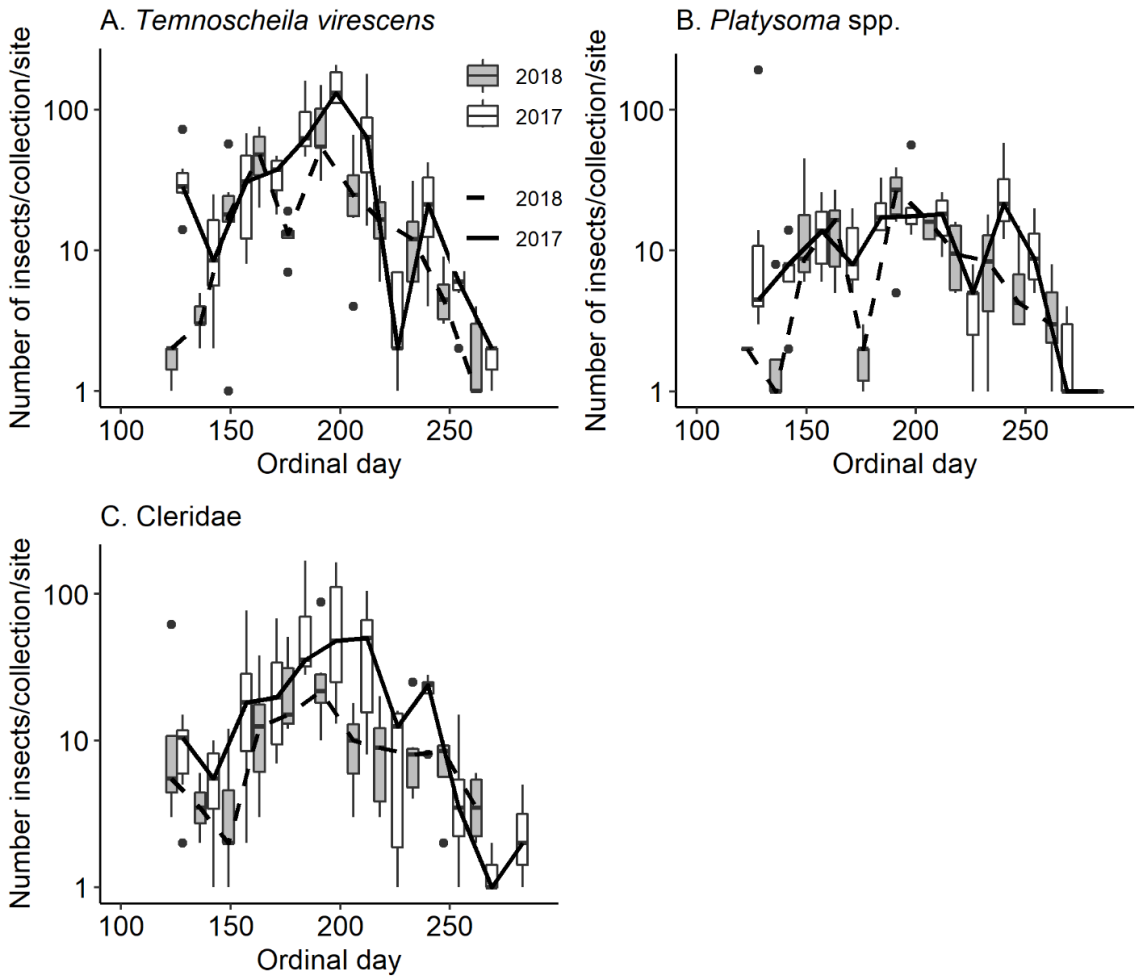

Figure 2.2 Box and whisker plots of number of bark beetle predators (A) *Temnoscheila virescens*, (B) *Platysoma* spp., and (C) Cleridae captured in each 14-day sample period per site captured in summers 2017 (solid line) and 2018 (dashed line). Variation within funnel trap captures at each collection period is displayed in box and whisker plots for 2017 (white) and 2018 (shaded). The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

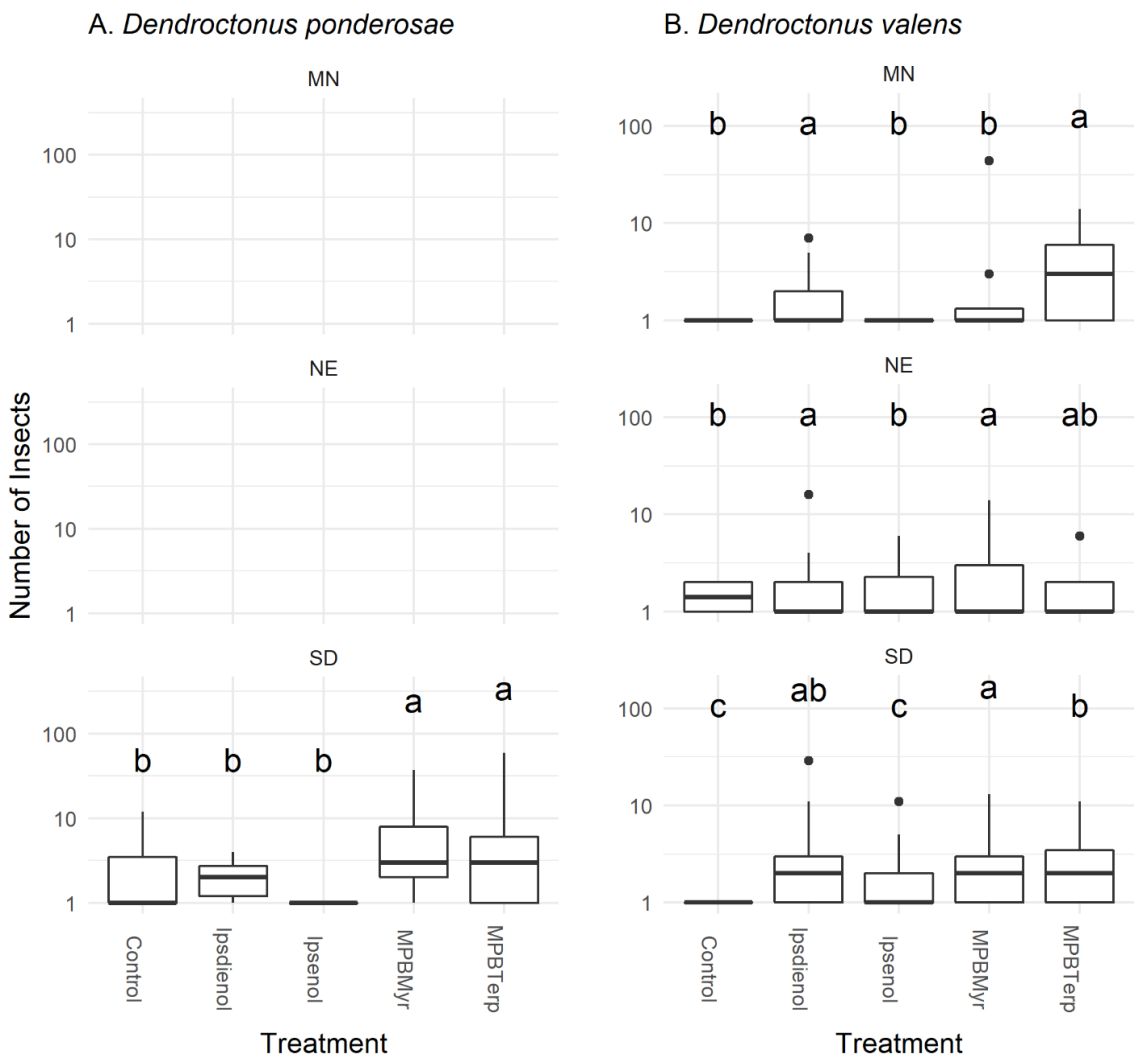

Figure 2.3 Boxplots displaying the total trap captures of (A) *Dendroctonus ponderosae* and (B) *Dendroctonus valens* in Minnesota (top), Nebraska (middle), and South Dakota (bottom) by lure treatment in the collection periods of 2017 and 2018. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

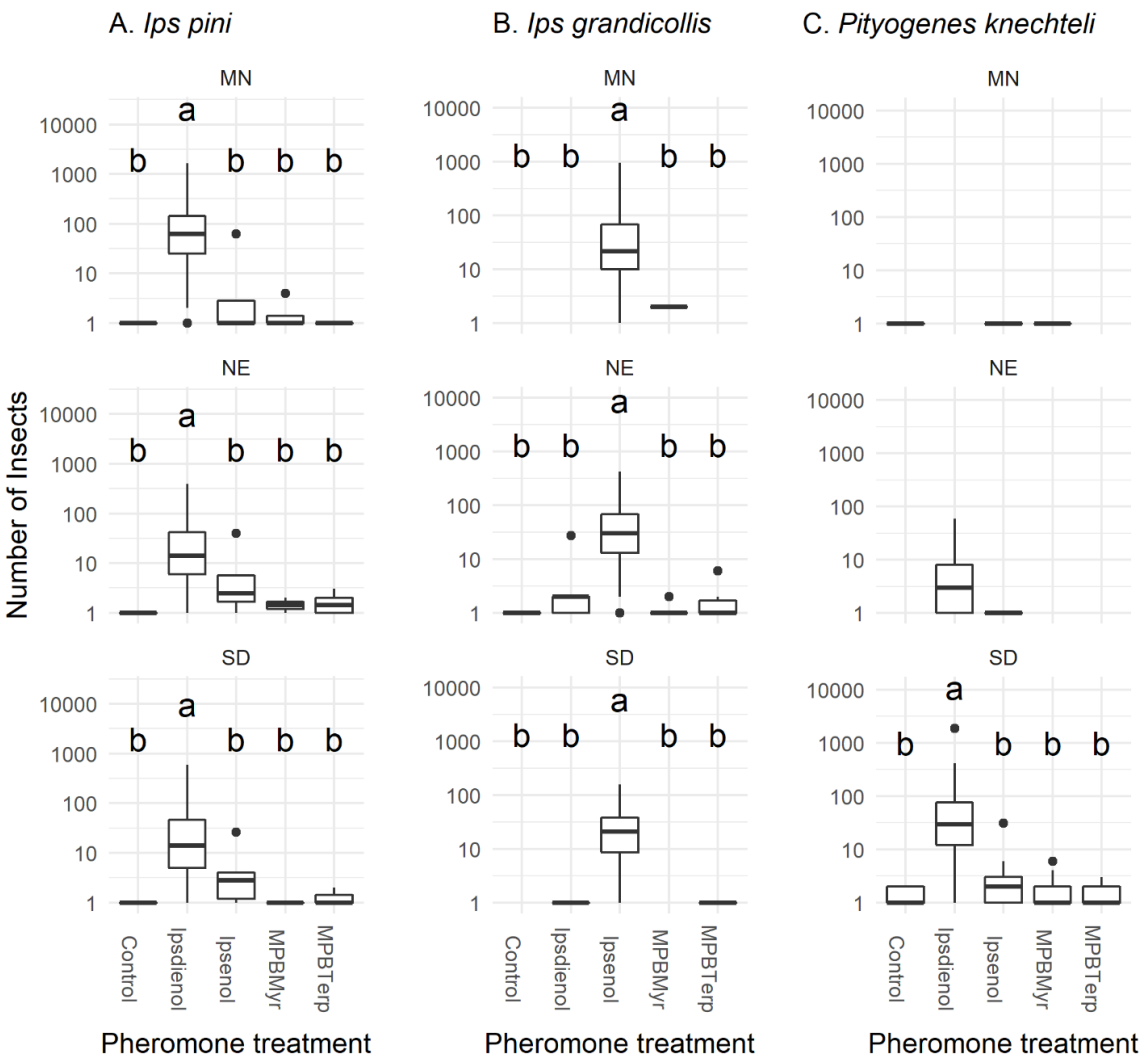

Figure 2.4 Boxplots displaying the total trap captures of total trap captures of non-aggressive bark beetles (A) *Ips pini* and (B) *Ips grandicollis*, and (C) *Pityogenes knechteli* in Minnesota (top), Nebraska (middle), and South Dakota (bottom) by pheromone treatment. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

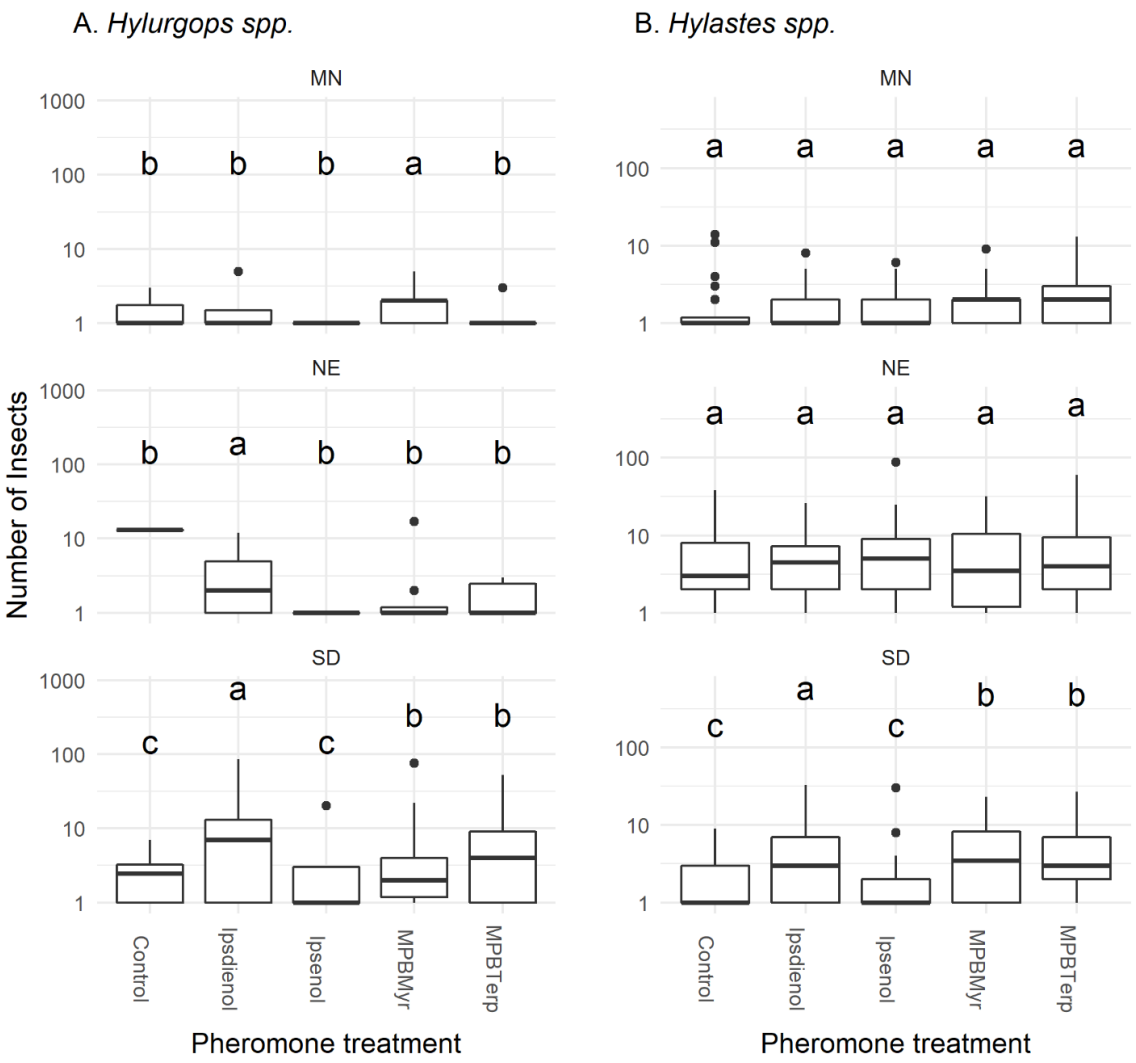

Figure 2.5 Boxplots displaying the total trap captures of lower-stem infesting insects (A) *Hylurgops* spp. and (B) *Hylastes* spp. (right) in Minnesota (top), Nebraska (middle), and South Dakota (bottom) by pheromone treatment. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

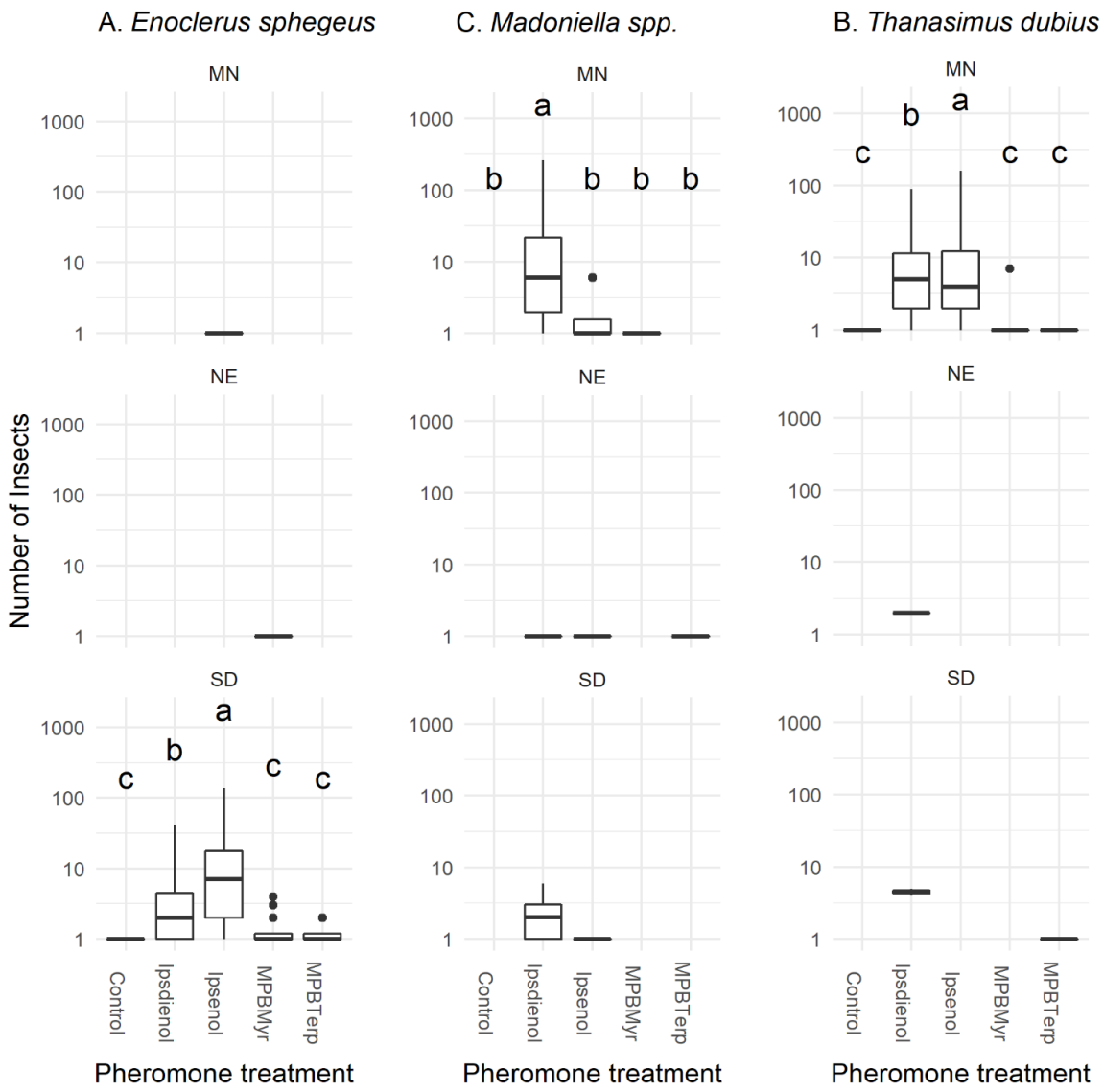

Figure 2.6 Boxplots displaying the total trap captures of predators (A) *Enoclerus sphegeus*, (B) *Madoniella* spp., and (C) *Thanasimus dubius* in Minnesota (top), Nebraska (middle), and South Dakota (bottom) by pheromone treatment. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

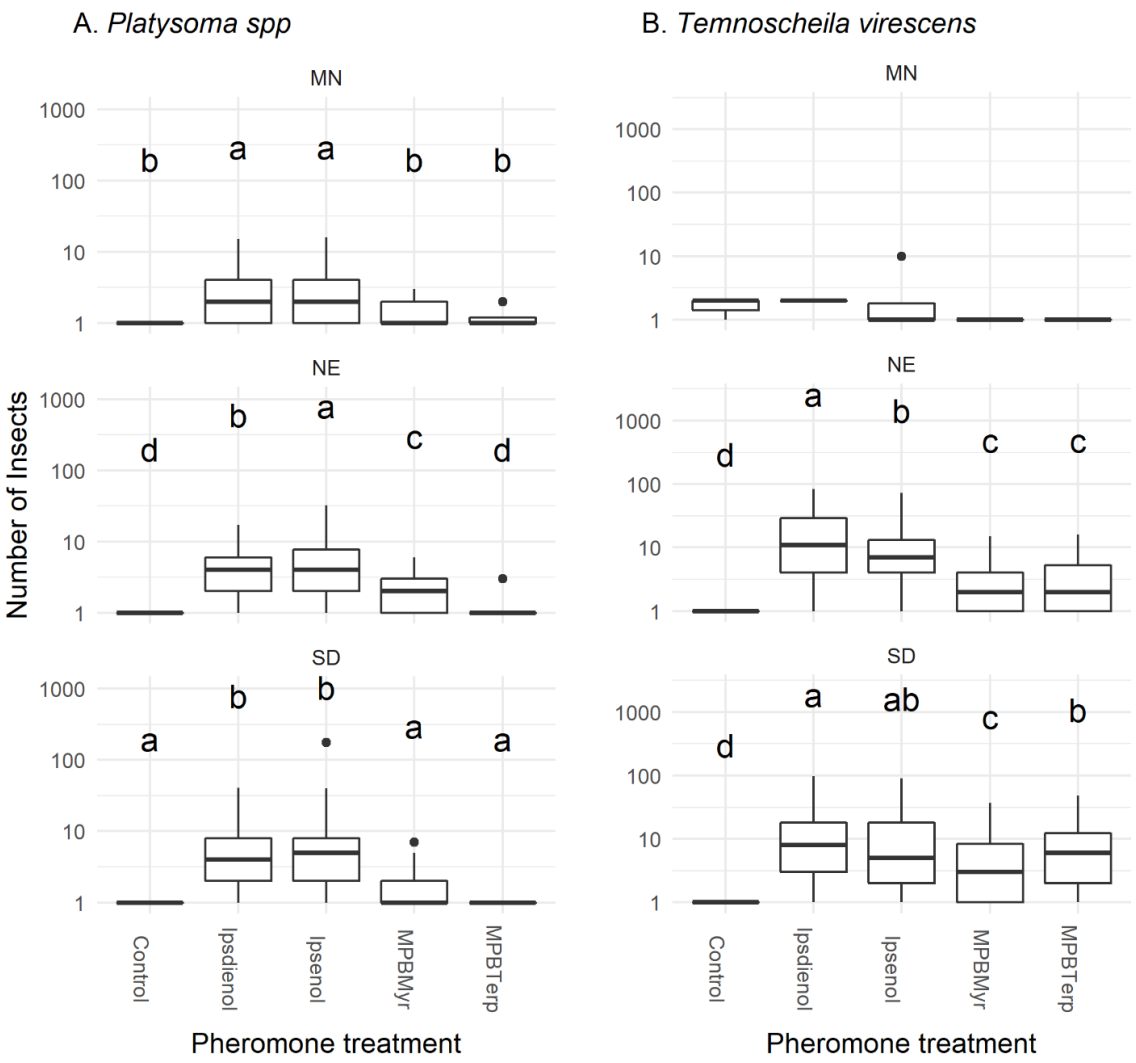

Figure 2.7 Boxplots displaying the total trap captures of total trap captures of predators (A) *Platysoma* spp. and (B) *Temnoscheila virescens* in Minnesota (top), Nebraska (middle), and South Dakota (bottom) by pheromone treatment. The upper whisker indicates the maximum value as the third quartile added to 1.5 times the interquartile range. The lower whisker represents the minimum value as the first quartile minus 1.5 times the interquartile range. The middle line of each box and whisker plot represents the median of the data set. Dots represent data points beyond plus or minus 1.5 times the interquartile range.

Thesis Conclusions

Chapter 1

1) **Colonization and reproduction of *I. grandicollis*, a bark beetle native to the Great Lakes region and potential interspecific competitor with mountain pine beetle, was inhibited by the presence of mountain pine beetle aggregation pheromones and host volatiles.** *Ips grandicollis* constructed fewer galleries and produced fewer progeny when a log was baited with mountain pine beetle lure, even in the presence of their own aggregation pheromone.
2) ***Monochamus* spp. may compete more with *I. grandicollis* than mountain pine beetle if the latter were to arrive in the Great Lakes region.** *Monochamus* spp. preferentially colonized logs baited with the *I. grandicollis* pheromone lure, suggesting that they may not be a major source of mortality to mountain pine beetle.

Chapter 2

1) **There is no difference in catch rate of mountain pine beetle using lures containing terpinolene vs. myrcene.** As such, either lure may be sufficient for continued monitoring of mountain pine beetle as a threat to the Great Lakes region.
2) **Minnesota, Nebraska, and South Dakota each have a unique suite of natural enemies of and competitors with bark beetles.** The species composition in each region differ in variety and abundance.
3) **Natural enemies that co-evolved with mountain pine beetle are more likely to be attracted to its associated semiochemicals.** Natural enemies captured in the

native range of mountain pine beetle were kairomonally attracted to its pheromone lures. Natural enemies in the non-native range of mountain pine beetle were not highly attracted to the mountain pine beetle lures. Therefore, it is unlikely that the latter would compete with or predate on mountain pine beetle if it were to arrive in the Great Lakes region.

4) **Populations of some predators and competitors were found to be attracted to host volatiles associated with co-evolved trees.** *Dendroctonus valens* in Minnesota was attracted to the mountain pine beetle lure containing terpinolene but not the one with myrcene. However, *D. valens* in South Dakota was attracted to both lures. Likewise, *Platysoma* spp. in South Dakota were preferentially attracted to lures containing myrcene. Host tree species in Minnesota contain lower levels of myrcene in their phloem tissue than those in South Dakota. Species of natural enemies attracted to host volatiles characteristic of a region's primary tree increases the likelihood of colonizing the same trees as beetle prey. However, colonizing bark beetles such as *D. valens* that infest the lower stem may facilitate mountain pine beetle infestation rather than compete.

Bibliography

Allison. J.D., Morewood, W.D., Borden, J.H., Hein, K.E., Wilson, I.M. 2003. Differential bio-activity of *Ips* and *Dendroctonus* (Coleptera: Scolytidae) pheromone components for *Monochamus clamator* and *M. scutellatus* (Coleoptera: Cerambycidae). Environ. Entomol. 32(1): 23-30

Amman, G.D. 1984. Mountain pine beetle (Coleoptera: Scolytidae) mortality in three types of infestations. Environ. Entomol. 13:184-191

Aukema, B.H. Raffa, K.F. 2002. Relative effects of exophytic predation, endophytic predation and intraspecific competition on a subcortical herbivore: consequences to the reproduction of *Ips pini* and *Thanasimus dubius*. Oecologia 133:483-491

Aukema, B.H., Carroll, A.L., Zheng, Y., Zhu, J., Raffa, K.F., Moore, R.D., Stahl, K., Taylor, S.W. 2008. Movement of outbreak populations of mountain pine beetle: influences of spatiotemporal patterns and climate. Ecography 31:348-358

Aukema, B.H., Carroll, A.L., Zhu, J., Raffa, K.F., Sickley, T.A., Taylor, S.W. 2006. Landscape level analysis of mountain pine beetle in British Columbia, Canada: spatiotemporal development and spatial synchrony within the present outbreak. Ecography 29(3): 427-441

Aukema, B.H., Dahlsten, D.L., Raffa, K.F. 2000. Improved population monitoring of bark beetles and predators by incorporating disparate behavioral responses to Semiochemicals. Environ. Entomol. 29(3): 618-629

Aukema, J.E., McCullough, D.G., Holle, B.V., Liebhold, A.M., Britton, K., Frankel, S.J. 2010. Historical accumulations of nonindigenous forest pests in the continental United States. Bioscience 60(11): 886-897

Aukema, B.H., McKee, F.R., Wytrykush, D.L., Carroll, A.L. 2016. Population dynamics and epidemiology of four species of *Dendroctonus* (Coleoptera:Curculionidae): 100 years since J.M. Swaine. Can. Entomol. 148: 82-110

Ayres, B.D., Ayres, M.P., Abrahamson, M.D., Teale, S.A. 2001. Resource partitioning and overlap in three sympatric species of *Ips* bark beetles (Coleoptera:Scolytidae). Oecologia 128: 443-453

Bentz, B.J., Munson, A.S. 2000. Spruce beetle population suppression in northern Utah. West. J Appl. For. 15(3): 122-128

Bentz, B.J., Regniere, J., Fettig, C.J., Hansen, E.M., Hayes, J.L., Hicke, J.A., Kelsey, R.G., Negron, J.F., Seybold, S.J. 2010. Climate change and bark beetles of the western United States and Canada: direct and indirect effects. Bioscience 60(8): 602-613

Beudert, B., Bassler, C., Thorn, S., Noss, R., Schroder, B., Dieffenbach-Fries, H., Foullois, N., Muller, J. 2014. Bark beetles increase biodiversity while maintaining drinking water quality. Conserv. Lett. 8(4): 272-281.

Birch, M.C., Wood, D.L., 1975. Mutual inhibition of the attractant pheromone response by two species of *Ips* (Coleoptera: Scolytidae). J. Chem. Ecol. 1(1):101-113

Bleiker, K.P., O'Brien, M.R., Smith, G.D., Carroll, A.L. 2014. Characterisation of attacks made by the mountain pine beetle (Coleoptera: Curculionidae) during its endemic population phase. Can. Entomol. 146(3):271-284

Boone, C.K., Aukema, B.H., Bohlmann, J., Carroll, A.L., Raffa, K.F. 2011. Efficacy of tree defense physiology varies with bark beetle population density: a basis for positive feedback in eruptive species. Can. J. For. Res. 41:1174-1188

Boone, C. K., Six, D. L., & Raffa, K. F. 2008. The enemy of my enemy is still my enemy: Competitors add to predator load of a tree-killing bark beetle. Agricultural and Forest Entomology, 10(4):411–421

Borden, J. H. 1982. Aggregation pheromones. In: Mitton, J. B.; Sturgeon, K. B., eds. Bark beetles in North American forests. Austin, TX: University of Texas Press: 74-139.

Borden, J.H., Devlin, D.R., Miller, D.R. 1992. Synomones of two sympatric species deter attack by the pine engraver, *Ips pini* (Coleoptera: Scolytidae). Can. J. For. Res 22:523-524

Burke, J.L., Carroll, A.L. 2016. 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. For. Ecol. Manag. 359:59-64

Byers, J.A. 1989. Behavioral mechanisms in reducing competition in bark beetles. Holarctic. Ecol. 12(4):466-476

Byers, J.A. 1989. Chemical ecology of bark beetles. Experientia. 45:271-283.

Byers, J.A. Wood, D.L. 1980. Interspecific inhibition of the response of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus*, to their pheromones in the field. J. Chem. Ecol. 6(1):149-164

Cale, J.A., Muskens, M., Najar, A., Ishangulyyeva, G., Hussain, A., Kanekar, S.S., Klutsch, J.G., Taft, S., Erbilgin. 2017. Rapid monoterpene induction promotes the susceptibility of a novel host pine to mountain pine beetle colonization but not to beetle-vectored fungi. Tree Physiol. 37(12): 1597-1610

Carroll, A. L., S. W. Taylor, J. Régnière, and L. Safranyik. 2004. Effects of climate and climate change on the mountain pine beetle. Proc. of the mountain pine beetle symposium: Challenges and solutions, Canadian For. Serv., Pacific Forestry Centre, Information Rep. BC-X-399. 223-232.

Conn, J.E., Borden, J.H., Scott, B.E., Friskie, L.M., Pierce Jr., H.D., Oehlschlager, A.C. 1983. Semiochemicals for the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae) in British Columbia: field trapping studies. Can. J. For. Res. 13(2): 320-324

Cooke, B.J., Carroll, A.L. 2017. Predicting the risk of mountain pine beetle spread to eastern pine forests: Considering uncertainty in uncertain times. For. Ecol. Manag. 396: 11-25

Cole, W.E. 1981. Some risks and causes of mortality in mountain pine beetle populations: a long-term analysis. Res. Popul. Ecol. 23:116-144

Colgan, L.J., Erbilgin, N. 2011. Tree-mediated interactions between the jack pine budworm and a mountain pine beetle fungal associate. Ecol. Entomol. 36(4):425-434

Corbett, L.J., Whithey, P. Lantz, V.A., Ochuodho, T.O. 2016. The economic impact of mountain pine beetle infestation in British Columbia: provincial estimates from a CGE analysis. Forestry. 89(1):100-105

Costello, S., Schaupp, W. 2011. First Nebraska state collection record of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae). The Coleopterists Bulletin 65(1):21-23

Cudmore, T.J., Bjorklund, N., Carroll., A.L., Lindgren, B.S. 2010. Climate change and range expansion of an aggressive bark beetle: evidence of higher beetle reproduction in naïve host tree populations. J. Appl. Ecol. 47: 1036-1043

Cullingham, C. I., Cooke, J.E.K., Dang, S., Davis, C.S., Cooke, B.J., Coltman, D.W., 2011. Mountain pine beetle host-range expansion threatens the boreal forest. Mol. Ecol. 20: 2157-2171

De la Giroday, H.M.C., Carroll, A.L., Aukema, B.H., 2012. Breach of the northern Rocky Mountain geoclimatic barrier: initiation of range expansion by the mountain pine beetle. J. Biogeogr. 39(6): 1112-1123

DeLeon, D. 1934. An annotated list of the parasites, predators, and other associated fauna of the mountain pine beetle in western white pine and lodgepole pine. Ibid. 66: 51-61

Dodds K.J., de Groot P. 2012. Sirex, surveys and management: Challenges of having *Sirex noctilio* in North America. In: Slippers B., de Groot P., Wingfield M. (eds) The Sirex Woodwasp and its Fungal Symbiont. Springer, Dordrecht. Pp 265-286 https://doi.org/10.1007/978-94-007-1960-6_19

Dodds, K.J., Graber, C. Stephen, F.M. 2001. Facultative intraguild predation by larval Cerambycidae (Coleoptera) on bark beetle larvae (Coleoptera: Scolytidae). Environ. Entomol. 30(1): 17-22

Drooz, A.T. (1985) Insects of Eastern Forests. USDA Forest Service Miscellaneous Publication No. 1426. US Government Printing Office, Washington, DC

Embree, D. G. 1971. The Biological Control of the Winter Moth in Eastern Canada by Introduced Parasites. In C. B. Huffaker (Ed.), Biological Control: Proceedings of an AAAS Symposium on Biological Control, held at Boston, Massachusetts December 30--31, 1969 (pp. 217–226). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4615-6531-4_9

Erbilgin, N., Raffa, K.F. 2000. Effects of host tree species on attractiveness of tunneling pine engravers *Ips pini,* to conspecifics and insect predators. J. Chem. Ecol. 26(4):823-840

Erbilgin, N., & Raffa, K. F. 2001. Modulation of predator attraction to pheromones of two prey species by stereochemistry of plant volatiles. Oecologia, 127(3):444–453. https://doi.org/10.1007/s004420000606

Erbilgin, N., Raffa, K.F. 2002. Association of declining red pine stands with reduced populations of bark beetle predators, seasonal increases in root colonizing insects, incidence of root pathogens. For. Ecol. Manag. 164:221-236

Erbilgin, N., Mori, S.R., Sun, J.H., Stein, J.D., Owen, D.R., Merrill, L.D., Campos Bolanos, R., Raffa, K.F., Montiel, Mendez Montiel, T., Wood, D.L., Gillette, N.E. 2007. Response to host volatiles by native and introduced populations of *Dendroctonus valens* (Coleoptera: Curculionidae, (Scolytinae) in North America and China. J. Chem. Ecol. 33:131-146

Erbilgin, N., Nordhein, E.V., Aukema, B.H., Raffa, K.F. 2002. Population dynamics of *Ips pini* and *Ips grandicollis* in red pine plantations in Wisconsin: within- and

between- year associations with predators, competitors and habitat quality. Environ. Entomol. 31(6): 1043-1051

Fettig, C.J., Gibson, K.E., Munson, A.S., Negron, J.F. 2014. Cultural practices for prevention and mitigation of mountain pine beetle infestations. For. Sci. 60(3):450-463

Forest Service BC. 2013. Facts About B.C.'s Mountain Pine Beetle. https://www.for.gov.bc.ca/hfp/mountain_pine_beetle/Updated-Beetle-Facts_April2013.pdf

Gandhi, K. J. K., & Herms, D. A. 2010. Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. Biol. Invasions, 12(2):389–405. https://doi.org/10.1007/s10530-009-9627-9

Haugen, D. A. 1990. Control procedures for Sirex noctilio in the Green Triangle: Review from detection to severe outbreak (1977–1987). Aust. For. 53:1:24-32 DOI: https://doi.org/10.1080/00049158.1990.10676057

Herms D.A., McCullough D.G. 2014. Emerald ash borer invasion of North America: history, biology, ecology, impacts, and management. Annu. Rev. Entomol. 59:13–30

Hicke, J.A., Allen, C.D., Desai, A.R., Dietze, M.C., Hall, R. J. 2012. Effects of biotic disturbances on forest carbon cycling in the United States and Canada. Glob. Chang. Biol. 18: 7-34

Hicke, J.A., Logan, J.A., Powell, J. Ojima, D.S. 2006. Changing temperatures influence suitability for modeled mountain pine beetle (*Dendroctonus ponderosae*) outbreaks in the western United States. J. Geophys. Res. 111, G02019, DOI:10.1029/2005JG000101.

Hoebeke, E. R., Haugen, D. A., & Haack, R. A. 2005. *Sirex noctilio*: Discovery of a Palearctic Siricid Woodwasp in New York. Newsl. Mich. Entomol. Soc. 50(1-2):24-25

Hothorn, T., Bretz, F., Westfall, P. 2008. Simultaneous inference in general parametric models. Biom. J. 50(3):346-363

Hunt, D.W.A., Borden, J.H. 1988. Response of mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and pine engraver, *Ips pini* (Say) to ipsdienol in southwestern British Columbia. J. Chem. Ecol. 14(1):277-293

Iede, E.T., Penteado, S.R.C., Schaitza, E.G. 1998. *Sirex noctilio* problem in Brazil: detection, evaluation, and control. Proceeding of a conference training in the control of *Sirex noctilio* by the use of natural enemies 45-52

Klutsch, J.G., Shamoun, S.F., Erbilgin, N. 2017. Drought stress leads to systemic induced susceptibility to a nectrotrophic fungus associated with mountain pine beetle in *Pinus banksiana* seedlings. PLOSOne 12(12): e0189203

Kurz, W.A., Dymond, C.C., Stinson, G. Rampley, G.J., E.T., Neislon, A.L.Carroll, T., Ebata, L. Safranyik. 2008. Mountain pine beetle and forest carbon feedback to climate change. Nature 452: 987-990

Lanstchner, M.V., Aukema, B.H., Corley, J.C. 2019. Droughts drive outbreak dynamics of an invasive forest insect on an exotic host. For. Ecol. Manag. 433:762-770

Liebhold A.M, MacDonald W.L, Bergdahl D, Mastro V.C. 1995. Invasion by exotic forest pests: a threat to forest ecosystems. Forest Science Monograph, 41 (2):1–49

Lindgren, B.S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). Can. Entomol. 115(3):299-302

Lindgren, B.S., Raffa, K.F. 2013. Evolution of tree killing in bark beetles (Coleoptera: Curculionidae): trade-offs between the maddening crowds and a sticky situation. Can. Entomol. 145: 471-495

Lombardero, M.J., Ayres, M.P., Ayres, B.D. 2006. Effects of fire and mechanical wounding on *Pinus resinosa* resin defenses, beetle attacks, and pathogens. For. Ecol. Manag. 225: 349-358

Lusebrink, I., Erbilgin, N., Evenden, M.L. 2013. The lodgepole x jack pine hybrid zone in Alberta, Canada: a steppingstone for the mountain pine beetle on its journey east across the boreal forest? J. Chem. Ecol. 39: 1209-1220

McGugan, B.M., and H.C. Coppe. 1962. A review of the biological control attempts against insects and weeds in Canada. Part II. Biological control of forest insects 1910-1958. P. 35-216. Commonw. Agric. Bur, Farnham Royal, England

Mikkelson, K.M., Bearup, L.A., Maxwell, R.M., Stednick, J.D., McCray, J.E., Sharp, J. O. 2013. Bark beetle infestation impacts on nutrient cycling, water quality and interdependent hydrological effects. Bio. Geo. Chem 115: 1-21

Miller, D.R., Borden, J.H., Lindgren, B.S. 1995. Verbenone: dose-dependent interruption of pheromone-based attraction of three sympatric species of pine bark beetles (Coleoptera: Scolytidae). Environ. Entomol. 24(3): 692-696

Miller, D.R., Gibson, K.E., Raffa, K.F., Seybold, S.J., Teale, S.A., Wood, D.L. 1997. Geographic variation in response of pine engraver, *Ips pini*, and associated species to pheromone, lanierone. J. Chem. Ecol. 23(8): 2013-2031

Morin R.S., Liebhold A.M., Pugh S.A., Crocker S.J. 2017. Regional assessment of emerald ash borer, *Agrilus planipennis*, impacts in forests of the Eastern United States. Biol. Invasions 19:703–711

Nuttall M.J. 1989. *Sirex noctilio* F., sirex wood wasp (Hymenoptera: Siricidae). Review of Biological control of invertebrate pests and weeds in New Zealand from 1874 to 1986. 299-306

Paine, T.D., Birch, M.C., Svihra, P. 1981. Niche breadth and resource partitioning by four sympatric species of bark beetles (Coleoptera: Scolytidae). Oecologia. 48:1-6

Pfammatter, J.A., Krause, A., Raffa, K.F. 2015. Evaluating predators and competitors in Wisconsin red pine forests for attraction to mountain pine beetle pheromones for anticipators biological control. Environ. Entomol. 44(4): 1161-1171

Poland, T.M., Borden, J.H. 1994a. Semiochemical-based communication in interspecific interactions between *Ips pini* and *Pityogenes knechteli* (Swaine) (Coleoptera: Scolytidae) in lodgepole pine. Can. Entomol. 126:269-276

Poland, T.M., Borden, J.H. 1994b. Attack dynamics of *Ips pini* (Say) and *Pityogenes knechteli* (Swaine) (Col., Scolytidae) in windthrown lodgepole pine trees. J. Appl. Entomol. 155(1-5):434-443

Raffa, K.F., Berryman, A.A. 1983. Physiological aspects of lodgepole pine wound responses to a fungal symbiont of the mountain pine beetle, *Dendroctonus ponderosae* (Coleptera:Scolytidae). Can. Entomol. 155(7):723-734

Raffa, K.F., Aukema, B.H., Bentz, B.J., Carroll, A.L., Hicke, J.A., Turner, M.G., Romme, W.H. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: The dynamics of bark beetle eruptions. BioSci. 58(6): 501-517

Rankin, L.J., Borden, J.H. 1991. Competitive interactions between the mountain pine beetle and the pine engraver in lodgepole pine. Can. J. For. Res. 21(7): 1029-1036

Reinikainen, M.R., Nagel, L., Pike, C., Olesiak, R., Cuomo, G. 2015. Cloquet forestry center management plan. Staff Paper Series No. 244 Department of Forest Resources.

Rosenberger, D.W., Aukema, B.H., Venette, R.C. 2017a. Cold tolerance of mountain pine beetle among novel eastern pines: a potential for trade-offs in an invaded range? For. Ecol. Manag. 400: 28-37

Rosenberger, D.W., Venette, R.C., Maddox, M.P., Aukema, B.H. 2017b. Colonization behaviors of mountain pine beetle on novel hosts: Implications for range expansion into northeastern North America. PLoSOne. 12(5): e0176269. https://doi.org/10.137/journal.pone.0176269

Rosenberger, D.W., Venette, R.C., Aukema, B.H. 2018. Development of an aggressive bark beetle on novel hosts: Implications for outbreaks in an invaded range. J. Appl. Ecol. 55: 1526-1537

R Core Team. 2020. R: A language and environment for statistical computing. R foundation for statistical computing. Vienna, Austria. URL https://www.R-project.org

Ryan, R. B. 1990. Evaluation of Biological Control: Introduced Parasites of Larch Casebearer (Lepidoptera: Coleophoridae) in Oregon. Environ. Entomol. 19(6):1873-1881

Safranyik, L.; Shrimpton, D.M.; Whitney, H.S. 1975. An interpretation of the interaction between lodgepole pine, the mountain pine beetle, and its associated blue stain fungi in western Canada. in D.M. Baumgartner, editor. Management of Lodgepole Pine Ecosystems Symposium Proceedings, October 9-11, 1973, Pullman, Washington, USA. Washington State University Coop. Extension Service, Pullman, WA. Pages 406-428

Safranyik, L. 1978. Effects of climate and weather of mountain pine beetle populations. Proceedings of symposium on theory and practice of mountain pine beetle management in lodgepole pine forests. 77-84.

Safranyik, L., Carroll, A.L. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests, pp. 3-66. *In* L. Safranyik and B. Wilson (eds), The mountain pine beetle: a synthesis of its biology, management and impacts on lodgepole pine. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC, Canada.

Safranyik, L., Carroll, A.L. Regniere, J., Langor, D.W. 2010. Potential range expansion of mountain pine beetle into the boreal forest of North America. Can. Entomol. 142(5): 415-442

Sambaraju, K.R., Carroll., A.L., Zhu, J., Stahl, K. Moore, R.D., Aukema, B.H. 2012. Climate change could alter the distribution of mountain pine beetle outbreaks in western Canada. Ecography 35(3): 211-223

Scheller, R.M., Mladenoff, D.J. 2005. A spatially interactive simulation of climate change, harvesting, wind, and tree species migration and projected changes to forest composition and biomass in northern Wisconsin, USA. Glob. Change Biol. 11: 307-321

Schenk, J.A., Benjamin, D.M. 1969. Notes on the biology of *Ips pini* in central Wisconsin jack pine forests. Ann. Etomol. Soc. Am. 62(3): 480-485

Schlyter, F., Anderbrandt, O. 1993. Competition and niche separation between two bark beetles: existence and mechanisms. Oikos 68:437-447

Schneider, C.A., Rasband, W.S., Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods. 9(7): 671-675

Schoeller, E.N., Husseneder, C., Allison, J.D. 2012. Molecular evidence of facultative intraguild predation by *Monochamus titillator* larvae (Coleoptera: Cerambycidae) on member of the southern pine beetle guild. Naturwissenschaften. 99: 913-924

Small, M.J., Small, C.J., Dreyer, G.D. 2005. Changes in a hemlock-dominated forest following woolly adelgid infestation in southern New England. J. Torrey Bot. Soc. 132:458–470

Smith, G.D., Carroll, A.L., Lindgren, B.S. 2011. Facilitation in bark beetles: endemic mountain pine beetle gets a helping hand. Agr. Forest Entomol. 13: 37-43

Stephen, F.M. 2011. Southern Pine Beetle Competitors. In: Coulson, R.N.; Klepzig, K.D. 2011. Southern Pine Beetle II. Gen. Tech. Rep. SRS-140. Asheville, NC: U.S. Department of Agriculture Forest Service, Southern Research Station. Pp.183-198

Straw, N.A., Williams, D.T., Kulinich, O., Gninenko, Y.I. 2013. Distribution, impact and rate of spread of emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae) in the Moscow region of Russia. Forestry 86:515–52

Svihra, P., Paine T.D., Birch, M.C. 1980 Interspecific olfactory communications in southern pine beetle. Naturwissenschaften 67: 518-520.

Symonds, M.R.E., Elgar, M.A. 2004. Species overlap, speciation and the evolution of aggregation pheromones in bark beetles. Ecol. Lett. 7: 202-212

Taylor, S.W., Carroll, A.L. 2003. Disturbance, forest age, and mountain pine beetle outbreak dynamics in BC: A historical perspective. Natural Resources Canada, Canadian Forest Serve, Pacific Forestry Centre.

Tribe, G.D., & Cillié, J.J. 2004. The spread of *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) in South African pine plantations and the introduction and establishment of its biological control agents. African Entomology. 12(1):9-17

Wallin, K.F., Raffa, K.F. 2004. Feedback between individual host selection behavior and population dynamics in an eruptive herbivore. Ecol. Monogr. 74(1): 101-116

Walters, B.F., Woodall, C.W., Piva, R.J., Hatfield, M.A., Domke, G.M., Haugen, D.E. 2013. Forests of the Black Hills National Forest 2011. Resour. Bull. NRS-83. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. Pp. 36

Windmuller-Campione, M.A. 2018. Assessing the future susceptibility of mountain pine beetle (*Dendroctonus ponderosae*) in the Great Lakes Region using forest composition and structural attributes. Can. J. For. Res. 48:451-459

Witanachchi, J.P., Morgan, F.D. 1981. Behaviour of the bark beetle, *Ips grandicollis* during host selection. Physiol. Entomol 6: 219-223

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera:Scolytidae), a taxonomic monograph. Great Basin Naturalist Memoirs 6, Provo, UT.