INTERACTIONS OF THE MOUNTAIN PINE BEETLE, *DENDROCTONUS PONDEROSAE*, WITH TYPICAL AND ATYPICAL HOST MATERIAL, LODGEPOLE PINE AND INTERIOR HYBRID SPRUCE, IN THE CENTRAL INTERIOR OF BRITISH COLUMBIA

by

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ABSTRACT

Interactions between mountain pine beetles and spruce hosts are rare, but a high frequency of mountain pine beetle attacks on interior hybrid spruce, an atypical host species, have recently been observed within the central interior of British Columbia, an area experiencing a severe outbreak of mountain pine beetle. I first examined the effect of natal history on host selection by females, and settlement patterns of conspecific males. In a second study, I examined the effects of host species and competitors on mountain pine beetle reproduction in a field experiment. The third study examined the association of within-stand beetle population density and pine vs. spruce availability with attraction to traps baited with pine and spruce host material. Using mountain pine beetle as a model organism, this work emphasizes insect host selection and reproductive success, with potential implications for host range expansion in eruptive herbivores.
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1: INTRODUCTION

Bark beetles (Coleoptera: Curculionidae: Scolytinae) comprise the most important group of insect herbivores within conifer ecosystems of western North America (Rudinsky 1962, Furniss and Carolin 1977, Wood 1982b). These phloemophagous insects reside within the sub-cortical tissues of colonized hosts for all but a few days of their life cycles (Rudinsky 1962). Phloem feeding by adults and larvae, coupled with the proliferation of symbiotic fungi that they vector, frequently result in tree mortality, especially among eruptive species (Wood 1982b). Ecologically, bark beetles are important components of forest ecosystems, influencing processes such as stand succession dynamics, nutrient cycling, water quality, physical and genetic stand characteristics, and floral and faunal diversity (Bright 1976, Furniss and Carolin 1977, Wood 1982a, Wood 1982b, Raffa and Berryman 1987, Raffa 1991, Logan and Powell 2001, Safranyik and Carroll 2006, Shore et al. 2006).

The majority of bark beetles are “secondary” species (e.g., *Ips* spp.), generally colonizing senescent, over-mature, or otherwise unthrifty trees. “Primary” species (e.g., several *Dendroctonus* spp. such as *D. brevicomis* LeConte and *D. frontalis* Zimmermann), subsist on weakened hosts during endemic periods (Smith 2008, Smith et al. 2010), but can rapidly increase in population size during favourable conditions and cause severe mortality in otherwise healthy and vigorous forest stands during outbreak conditions. These eruptive species can cause the loss of billions of cubic metres of timber, and millions of dollars in forest product revenues (Wood 1982b, Safranyik and Carroll 2006). Thus, bark beetles can also be extremely important economic pests (Bright 1976, Furniss and Carolin 1977, Wood 1982b)
The mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is an eruptive, primary bark beetle, found throughout northern Mexico, the western United States, and the western provinces of Canada (Safranyik and Carroll 2006). This insect can be one of the most destructive agents of mature *Pinus* spp. trees in western North America (Baker et al. 1971, Bentz et al. 1991, Carroll et al. 2004, Safranyik and Carroll 2006), often killing most healthy hosts within a stand prior to the collapse of an outbreak (Amman and Baker 1972). The mountain pine beetle is a generalist pine-feeding herbivore, colonizing up to 16 native and exotic *Pinus* spp. across its extensive range (Furniss and Schenk 1969, Smith et al. 1981, Cerezke 1995, Safranyik and Carroll 2006). In British Columbia, lodgepole (*Pinus contorta* Douglas ex. Loudon) and ponderosa pine (*P. ponderosa* Douglas ex. Lawson) (Pinales: Pinaceae) are the primary hosts (Safranyik and Carroll 2006).

Host selection by mountain pine beetles occur during the dispersal phase among adults. “Pioneer” females orient to host volatiles at long range (Moeck and Simmons 1991, Saint-Germain et al. 2007), integrate short-range visual and olfactory cues (Pureswaran and Borden 2003, 2005, Campbell and Borden 2006), and make final host selections via tactile cues (Reid 1962) and gustatory host sampling (Hynum and Berryman 1980, Raffa and Berryman 1982). Upon tunnelling into the bark of hosts, females produce pheromones to attract male and female conspecifics to induce a mass-attack that can exhaust host defences (Renwick et al. 1973, Brand et al. 1975, Wood 1982a, Raffa and Berryman 1983, Pureswaran et al. 2000, Pureswaran et al. 2004). If access to a tree is gained, females construct vertical ovipositional galleries within the phloem tissue, and oviposit in niches excavated along the lateral margins of these galleries (Safranyik and Carroll 2006). Resulting larvae tunnel

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perpendicular to the ovipositional gallery while feeding on phloem (Safranyik and Carroll 2006).

Symbiotic fungi vectored by the beetles assist in overcoming host defences (Raffa and Berryman 1983) and offer nutritional benefits to the developing larvae (Six and Paine 1998, Bentz and Six 2006, Bleiker and Six 2007). Larval pupation occurs within the phloem and outer bark of the host. Newly eclosed adults feed on host and fungal tissue prior to emergence, accumulating energy reserves necessary for a dispersal flight (Safranyik and Carroll 2006).

The current epidemic of mountain pine beetles in British Columbia and Alberta, Canada, is distinguished from previous outbreaks by the unprecedented degree of large-scale forest mortality. To date, more than 14 million hectares of lodgepole pine forest have been affected (Westfall and Ebata 2009). Two major factors have contributed to the severity of the current outbreak: host availability and climate change. Under natural environmental conditions, a mosaic of host availability limits the potential for mountain pine beetle population growth on the landscape (Safranyik et al. 1974, Berryman 1982, Safranyik and Carroll 2006). However, wildfire suppression, coupled with a lack of commercial interest in lodgepole pine until the last few decades, has led to large, contiguous stands of mature and over-mature pine across British Columbia (Carroll et al. 2004, Taylor et al. 2006). The widespread availability of such habitats facilitated a rapid increase of mountain pine beetle populations, allowing colonization of large-diameter trees (Raffa and Berryman 1987, Raffa 1991) and resulting in a landscape-level epidemic (Carroll et al. 2004, Aukema et al. 2006, Taylor et al. 2006). Additionally, recent climatic warming trends in British Columbia (Stahl et al. 2006) have reduced mortality to overwintering mountain pine beetle brood (Nealis and
Peters 2008), increasing the rate of population growth and facilitating range expansion of mountain pine beetles beyond historical thermoclimatic barriers (Carroll et al. 2004, Cudmore et al. 2010).

Geographic range expansion has resulted in the invasion of novel habitats within British Columbia, and beyond, to the eastern slopes of the Rocky Mountains in western Alberta (Ono 2003, Nealis and Peters 2008, de la Giroday 2009, Robertson et al. 2009). The potential for the mountain pine beetle to expand its host breadth has also been observed within the recent outbreak. Within Alberta, mountain pine beetles are colonizing jack pine- (P. banksiana Lambert) lodgepole pine hybrids, and threatening pure jack pine hosts (Nealis and Peters 2008). Jack pine may be a suitable host for mountain pine beetles (Furniss and Schenk 1969, Safranyik and Linton 1982, Cerezke 1995). In the central interior region of British Columbia, there have been occurrences of mountain pine beetle attack on Picea spp., particularly interior hybrid spruce (Picea glauca (Moench) Voss x engelmannii Parry ex. Engelmann) (Pinales: Pinaceae), leading to successful brood production and new generation emergence in some instances (Huber et al. 2009). Such behaviour has been previously observed elsewhere, but with limited beetle success (Furniss and Schenk 1969, Smith et al. 1981, Wood 1982b).

This thesis compares the reproductive biology of the mountain pine beetle within an atypical and typical host; interior hybrid spruce and lodgepole pine, respectively – using populations of mountain pine beetles reared from naturally colonized trees of both species. Throughout this thesis, the term “atypical” is used to describe the host-status of interior hybrid spruce due to observations that Picea spp. are only occasionally attacked by mountain

Chapter one examines the effect of developmental host and colonized host species on female mountain pine beetle host acceptance and patterns of male settlement behaviours within both interior hybrid spruce and lodgepole pine hosts in a laboratory setting.

Chapter two studies the effect of female mountain pine beetle developmental host and colonized host species (interior hybrid spruce or lodgepole pine) on the attraction of conspecifics and competitor secondary bark beetle species. Additionally, the reproductive success of female mountain pine beetle within logs of interior hybrid spruce and lodgepole pine is examined. These experiments were conducted under natural environmental conditions within a lodgepole pine stand.

Chapter three investigates mountain pine beetle preference for lodgepole pine logs vs. interior hybrid spruce logs with respect to population density and within-stand availability of typical (i.e., lodgepole pine) and atypical (i.e., interior hybrid spruce) hosts. Data were collected from three regions of central British Columbia comprising 17 plots with varying amounts of available lodgepole pine and interior hybrid spruce.

This thesis is written in chapter format to facilitate the publication of each chapter within professional journals. A small degree of redundancy exists between chapters in order to maintain the independence of each work. Although I served as the principal investigator throughout this research, invaluable collaboration was provided by my supervisor Dr. Brian Aukema and thesis committee members Mr. Robert Hodgkinson, Dr. Staffan Lindgren, and Dr. Dezene Huber. I therefore use plural rather than singular ownership when referring to the research presented within this thesis.
1.1 Literature Cited


lodgepole pine. Natural Resources Canada, Pacific Forestry Centre, Victoria, British Columbia, Canada.


2: EFFECT OF NATAL AND PRESENT HOST ON FEMALE MOUNTAIN PINE BEETLE HOST ACCEPTANCE AND MALE SETTLING BEHAVIOUR IN LODGEPOLE PINE VS. INTERIOR HYBRID SPRUCE.

2.1 Abstract

An immense outbreak of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, currently extends over 14 million hectares of mature pine forests in British Columbia and Alberta, Canada. While lodgepole pine (*Pinus contorta* Douglas ex. Loudon) is considered the insect’s principal host, several instances of attack on interior hybrid spruce (*Picea glauca* (Moench) Voss x *engelmannii* Parry ex. Engelmann) have been noted in areas where the supply of lodgepole pines has been reduced. In some instances, reproduction has been successful. In this study, we 1) examined the effect of larval (i.e., natal) host species on subsequent host selection by female mountain pine beetles, 2) determined whether conspecific developmental host histories among male and female beetles influence male entrance to female nuptial chambers and residency behaviour in galleries within pine and spruce logs, and 3) investigated the effect of host consistency between female natal species and colonized host species on the ovipositional gallery-joining behaviour exhibited by males. Females reared naturally from lodgepole pine and spruce hosts were placed in pine and spruce logs for 24 h before measuring host acceptance or rejection. After 12 h of female ovipositional gallery construction, a single male mountain pine beetle was released onto the log containing both females. Twelve hours following male release onto the logs, the bark was removed to record female host acceptance and male ovipositional gallery choice. Females that had developed in spruce had higher rates of host acceptance of both pine and spruce host species than females that had developed in pine. Additionally, spruce was a more accepted species of host than pine. Males may select females with similar natal
histories, although this trend was not statistically significant. Implications of these findings on the concepts of assortative mating, host adaptation, and population dynamics of the mountain pine beetle are discussed.
2.2 Introduction

Experience with chemical cues associated with host plants during larval and/or adult life stages is often critical to the subsequent selection and acceptance of oviposition and feeding sites in insects (Ehrlich and Raven 1964, Tabashnik et al. 1981, Jaenike 1982, Papaj and Prokopy 1988, Craig et al. 1993). When host plants are abundant, oviposition on host plants with which the adult female has had previous experience can reduce mortality associated with dispersal (Prokopy et al. 1982). In contrast, exploration of nonhost plants may occasionally result in host range expansion, increasing the likelihood of survival when host plants are rare (Courtney and Kibota 1990, Zhang and Liu 2006).

Hopkins' theory of host selection posits that adult insects prefer to feed or oviposit on or within the host species in which they developed as larvae (Hopkins 1917). Though this theory has been well studied, the literature is replete with conflicting results (e.g., Smith and Cornell 1979, Jaenike 1983, Prokopy et al. 1986, Ray 1999, Rojas and Wyatt 1999, Solarz and Newman 2001, Rietdorf and Steidle 2002, Akhtar and Isman 2003). Because many proofs of Hopkins' theory can be attributed to adult conditioning rather than larval experience (Zhang et al. 2007), Jaenike and Grimaldi (1983) proposed the neo-Hopkins host selection principle. This theory states that "exposure of an adult insect will often, though not always, increase the subsequent acceptability of that host as an oviposition site." As with Hopkins' original theory, many studies support this idea (Jaenike and Grimaldi 1983, Papaj and Prokopy 1988, Cunningham et al. 1998, Solarz and Newman 2001, Rietdorf and Steidle 2002, Olsson et al. 2006, Zhang and Liu 2006, Zhang et al. 2007), while others do not, due to either ambiguity (Prokopy et al. 1986, Rojas and Wyatt 1999) or negative results (Tabashnik et al. 1981, Kerpel and Moreira 2005). Factors other than early adult conditioning that may
confound the validity of Hopkins’ principle include genetic differentiation/host race formation (Bush 1969, Feder et al. 1988), environmental pressures (Jaenike 1982), and chemical legacy (Rietdorf and Steidle 2002, Akhtar and Isman 2003).

Not only can developmental history affect oviposition, but larval development or recent adult feeding experience can also influence mate selection among insects. The assessment of a potential mate’s developmental history may be advantageous for several reasons. First, sequestration of plant-derived metabolites can be used as visual (Geuder et al. 1997, Knüttel and Fiedler 2001) and chemical (Amano et al. 1999, Shelly et al. 2007) stimuli to promote mate recognition. Plant compounds may also be used as precursors of pheromones that are essential to mate attraction (Nishida and Fukami 1990, Landolt and Phillips 1997, Amano et al. 1999). Second, different host plant species can influence insect fecundity and fertility (Langor 1989, Langor et al. 1990, Awmack and Leather 2002). Third, host compounds can provide potential mates with desirable qualities such as chemical defence that may protect offspring through direct, vertical transmission (Dussourd et al. 1988), or indirectly by deterring adult predation (Nishida and Fukami 1990, Amano et al. 1999, Labeyrie et al. 2003).

One group of insects that are intimately associated with their hosts are the bark beetles (Coleoptera: Curculionidae: Scolytinae). The entire life-cycle of these insects, usually with the exception of a brief dispersal flight period among adults, is spent under the bark and within the tissues of host trees (Wood 1982). Because of this close insect-host relationship, host chemistry is arguably the most important phenotypic trait influencing bark beetle success. Insects orient to their hosts via olfactory detection of compounds present within the air-stream (Chapman 1962, Atkins 1966, Bennett and Borden 1971, Moeck and

To resist attack, trees employ a complex defensive strategy involving constitutive and induced resin defences (Raffa and Berryman 1983). Conifer resin contains concentrated terpenoid-based compounds (Trapp and Croteau 2001, Huber et al. 2004) that are toxic to the invading beetles and the fungi they vector (Berryman and Ashraf 1970, Reid and Gates 1970, Wood 1982, Safranyik and Linton 1983, Amman et al. 1985, Seybold et al. 2006). Boring into host tissue, however, beetles can convert defensive, terpenoid compounds of the host into aggregation pheromones (Byers 1981, Byers and Wood 1981, Wood 1982a, Byers and Birgersson 1990, Seybold et al. 2006). The efficacy of these pheromones is often synergized by host kairomones (Borden et al. 1983, Conn et al. 1983, Borden et al. 1987, Camacho et al. 1998). If successful entry to the host is gained, mating, oviposition, and larval development all occur within the subcortical environment.

Because of their intimate associations with trees, bark beetles are important agents of forest disturbance (Wood 1982). For example, the mountain pine beetle, Dendroctonus ponderosae Hopkins, is arguably the most ecologically and economically important phloecophagous herbivore in western North America due to its eruptive nature and frequent outbreaks (Furniss and Carolin 1977). The distribution of the mountain pine beetle ranges from the pine forests of British Columbia and Alberta through the western United States to northern Mexico (Safranyik and Carroll 2006). In this insect, females are the host-selecting sex. Although lodgepole (Pinus contorta Douglas ex. Loudon) and ponderosa pine (P. ponderosa Douglas ex. Lawson) (Pinales: Pinaceae) are the most commonly utilized hosts,
mountain pine beetles will reproduce within virtually any native or introduced pine species throughout its range (Furniss and Carolin 1977, Wood 1982b, Safranyik and Carroll 2006). The largest recorded mountain pine beetle outbreak is currently occurring in British Columbia and Alberta and has resulted in extensive mortality of over 14 million hectares of pine forest since 1999 (Aukema et al. 2006, Westfall and Ebata 2009).

Within the central interior region of British Columbia, mountain pine beetles have recently been observed attacking interior hybrid spruce (*Picea glauca* (Moench) Voss x *engelmannii* Parry ex. Engelmann) (Pinales: Pinaceae). Observations of spruce attack by the mountain pine beetle have been reported occasionally within the published literature (Furniss and Schenk 1969, Smith et al. 1981, Unger 1993) and may occur during the peak stages of epidemics (Wood 1982). Successful production of brood, however, appears to be an exceedingly rare event (Furniss and Schenk 1969), although some reproduction of mountain pine beetles in live, standing spruce, has been documented during the current outbreak in British Columbia (Huber et al. 2009).

Few studies have examined the influence of natal species on the host selection behaviour of the mountain pine beetle. Studies by Richmond (1933) and Langor and Spence (1991) did not find evidence to support the theory that mountain pine beetle females preferentially colonize hosts that are conspecific to their natal species, in contrast to a study by Baker et al. (1971) in lodgepole and whitebark (*P. albicaulis* Engelmann) pine stands. Furniss and Schenk (1969) reported repeated attacks on Norway spruce (*Picea abies* Karsten) over three successive years, while Smith et al. (1981) noted that Norway spruce on their study site were heavily attacked, while nearby lodgepole and other pine species were largely
ignored. These latter observations are consistent with the suggestion that the mountain pine beetle may be influenced by larval experience during the selection of new hosts.

In the current study, we took advantage of the declining availability of susceptible lodgepole pine in the terminal phases of the mountain pine beetle outbreak in British Columbia, Canada to investigate host selection behaviour of mountain pine beetles in lodgepole pine and interior hybrid spruce using cut logs in laboratory bioassays. The objectives of this study were to: 1) examine the effect of larval (i.e., natal) host species on subsequent selection of hosts by female mountain pine beetles, and 2) determine whether natal host consistency among male and female beetles influences male entrance to female ovipositional galleries and residency behaviour in lodgepole pine and interior hybrid spruce logs, and 3) investigate the effect of host consistency between female natal species and colonized host species on the ovipositional gallery-joining behaviour exhibited by males.

2.3 Materials and Methods

2.3.1 Study Materials

Interior hybrid spruce (hereafter referred to as “spruce”) and lodgepole pines (“pine”) of similar height, diameter at breast height (dbh; 1.3 m), and growing conditions were harvested from a stand judged to be free of outbreaking mountain pine beetle populations near Crassier Creek, British Columbia (55°38‘00"N, 122°15‘00"W). The stand where trees were harvested was located in the SBSwk2 biogeoclimatic zone, (an ecosystem classification system used in British Columbia; Meidinger and Pojar 1991). Historically, mountain pine beetle activity in the area surrounding Crassier Creek has been low (Safranyik et al. 1974), and incipient beetle eruptions from dispersal events were scattered infrequently across the
landscape at the time of tree harvest (de la Giroday et al. 2010). After transport to the laboratory, the ends of the logs were sealed with paraffin wax to reduce desiccation. Logs were stored outdoors under tarpaulins to avoid colonization by secondary bark beetles (e.g., *Ips* spp. and others) and wood borers (e.g., Coleoptera: Cerambycidae and Buprestidae).

To obtain insects for bioassays, pines colonized by the mountain pine beetle under natural field conditions were harvested from Crassier Creek, British Columbia, while spruce colonized by mountain pine beetles was obtained from Prince George, British Columbia, Canada (N 53°53'00" W 122°48'00"). After falling, the spruce and pines were split into slabs, keeping the outer bark intact. The slabs, containing teneral mountain pine beetle adults, were placed in emergence containers in the laboratory at 22°C on a 24:0 h light:dark photoperiod. Insects were collected daily from transparent collecting jars that contained moist Kimwipes® to provide a substrate for walking. Following collection, insects were separated by sex and maintained in plastic containers at 5°C. Insects in storage were provided moistened Kimwipes® and spruce or pine phloem as described in Safranyik (1976), who found that beetles can generally be maintained in this manner for up to two weeks without apparent detrimental impacts to their health.

2.3.2 Experimental Design

Sections of the non-colonized spruce and pine trees were cut into 20 logs 15 cm in length for each species. Each log was then split lengthwise to yield two equal halves ("half-logs"). All cut surfaces were coated with paraffin wax to reduce desiccation. In total, 40 spruce and 40 pine half-logs were prepared in this manner.
Two female mountain pine beetles, one reared from spruce and one reared from pine, were introduced into each half-log (Fig. 2.1). Starter holes 3 mm in diameter were drilled through the outer bark into the phloem layer at one end of the half-log. A single female was then gently inserted into each hole using forceps. Females that did not enter the phloem via a starter hole at the time of introduction were not used. In each half-log, the two females were placed as far apart as possible, and a minimum of 3 cm from the wood/bark edge interface to allow vertical ovipositional gallery excavation while minimizing encounters with potentially desiccated phloem. Aluminum screening was stapled over the starter holes to reduce female abandonment during the initial stages of ovipositional gallery construction. The screening was fixed such that a female could exit the starter hole, but not escape. The left or right side position for the female from pine or spruce within each half-log was determined randomly. Only apparently vigorous females less than or equal to 7 d old were used in the experiments. The half-logs with each pair of females were stored vertically in ventilated plastic containers at room temperature (22°C) with the females located at the bottom of the half-logs.

After 12 h of female ovipositional gallery construction, the aluminum screening was removed and a single male mountain pine beetle was released at a central position on the bark surface, equidistant from both females. Only apparently vigorous males less than or equal to 7 d old were used. Of the 40 spruce half-logs, 20 received a male reared from spruce and 20 received a male reared from pine. Males were distributed in an identical manner on the 40 pine half-logs, such that the final distribution was a male reared from either a spruce or pine choosing between two females – one reared from spruce and one reared from pine – on each of 20 half-logs of pine and spruce (Fig. 2.1). After male release onto the bark,
the half-logs were again stored vertically in ventilated plastic containers at 22°C for an additional 12 h.

After 12 h, the bark was removed from each log to record female host acceptance and male mate choice. Female host acceptance was defined as presence within an ovipositional gallery after a 24 h exposure period to the given host. Female host rejection was defined as an abandonment of the ovipositional gallery. Males were recorded to have chosen a female if the ovipositional gallery contained the male. The body sizes of male and female beetles recovered from the logs were measured using the width of the pronotum at the widest point.

2.3.3 Statistical Analysis

Host acceptance by females was examined first from a female natal-species perspective (i.e., do females preferentially accept hosts that are conspecific vs. heterospecific to their natal host?) and secondly from a host-species perspective (i.e., do females prefer spruce vs. pine hosts regardless of natal species?). Data were analysed using a generalized linear mixed effects model incorporating female host acceptance/rejection as the binomial response variable, female natal species and host species as fixed effects, and half-log (replicate) as a random effect.

Because few males were found in ovipositional galleries at the end of the experiment (see Results), we did not analyse natal or present host species effects with respect to male mate choice. We did, however, test the hypothesis that males join females based on body size, using two analyses. First, the relationship between male and female beetles in galleries was examined using regression analysis. The second test was restricted to data in which males had a choice of two females reared from different natal species present within the same
host. An ANOVA ($\alpha = 0.05$) was used to examine whether the mean pronotal width of females joined by males was significantly different than those of females who did not have males residing in their nuptial chambers at the time of experiment completion. All statistical analyses were performed using R (R Development Core Team, 2009).

2.4 Results

Females reared from spruce hosts had higher rates of host acceptance of both pine and spruce, relative to females that were reared from pine ($Z = 2.484, P = 0.0130$). In addition, females reared from either host species were more likely to accept spruce than pine ($Z = 3.003, P = 0.0027$) (Fig. 2.2). Overall, however, females reared from pine or spruce rarely accepted the same piece of host material, as at least one female from each pair frequently abandoned the half-log (45 of 80 half-logs in total).

Of the 25 males that were found with a female in a nuptial chamber when the bark was peeled, 17 had been reared from spruce (Table 2.1). Of these 17 from spruce, 12 were found in galleries with females reared from spruce, regardless of the species of bioassay half-logs. On the spruce half-logs, seven males joined females reared from spruce, vs. four males joining females reared from pine. Additionally, on pine half-logs, five spruce-reared males joined females reared from spruce while only one male joined a pine-reared female.

Body sizes of male and female beetles did not appear to influence male joining behaviour. In cases where males had the option of joining a single female in her nuptial chamber or foregoing the possibility, there was no correlation between male body size and the body size of the selected females ($F_{1,11} = 1.02, P = 0.33$). The body sizes of females that were found with males in their nuptial chambers or ovipositional galleries when the bark was...
peeled were not significantly different than females that were not joined by males in bioassays where two females were present within a host simultaneously ($F_{1,18} = 0.25, P = 0.63$).

2.5 Discussion

Our results that female mountain pine beetles reared from spruce had higher rates of host acceptance than those reared from pine is consistent with the theory that the frequency of genotypes promoting host discrimination declines with increasing population size (Raffa and Berryman 1983, Bigger and Fox 1997, Wallin and Raffa 2002, 2004). During mountain pine beetle outbreaks, the most suitable hosts are preferentially colonized early in the infestation and are thus not available to beetles in the later phases of the infestation (Amman 1972, Safranyik and Carroll 2006). Hence, beetles colonizing spruce may be a symptom of declining host specificity exhibited by the highest density populations at the centre of the outbreak. The progeny of such beetles may be genetically or environmentally predisposed to attack non-hosts, as has been seen during past outbreaks (Wood 1982, Unger 1993). The use of a broader host range in increasing and/or large herbivore populations may be ecologically adaptive by granting herbivores access to a more consistent supply of resources both spatially and temporally (Bigger and Fox 1997). For example, diamondback moths *Plutella xylostella* L. (Lepidoptera: Plutellidae) sampled from high-density populations were less discriminating during ovipositional host selection relative to moths from low-density populations (Bigger and Fox 1997).

Ideally, sympatric collection of mountain pine beetles would have occurred from both host species. However, this was not possible due to the scarcity of colonized pines remaining...
in the centre of the outbreak where we obtained colonized spruce, and the rarity of successful spruce colonization on the northern leading edges of the outbreak where the primary host supply of lodgepole pine had not yet been exhausted (Furniss and Schenk 1969, Furniss and Carolin 1977, Wood 1982, Aukema et al. 2006, Huber et al. 2009). Because the host types for rearing insects were thus necessarily confounded with location and population density, we are not able to properly evaluate the effects of spruce and pine on host acceptance behaviour and exclude alternate hypotheses such as those involving population density (e.g., see Chapter 4). Previous work with *D. rufipennis* (Kirby) in spruce, however, has demonstrated that development within well-defended hosts can elevate rates of host entrance relative to individuals from weaker hosts (Wallin and Raffa 2004). A decline in discrimination with increased population density and possible increased aggressive tree-killing behaviour by progeny reared from spruce are consistent with the female colonization behaviour noted in this study.

Even though spruce is not normally a host of the mountain pine beetle, aspects of host chemistry may have promoted its acceptance and colonization by female mountain pine beetles. For instance, β-phellandrene significantly influences host landing (Hynam and Berryman 1980) and ovipositional gallery initiation (Raffa and Berryman 1982), while δ-3-carene, α-pinene, β-pinene, terpinolene, and limonene stimulate ovipositional gallery initiation and sustained construction activities (Raffa and Berryman 1982). Bole resins of interior hybrid spruce contain greater quantities of δ-3-carene, α-pinene, and β-pinene relative to lodgepole pine, although the latter contains more limonene and approximately eight-fold more β-phellandrene (Pureswaran et al. 2004). These compounds interact in a complex manner to promote host acceptance behaviour in mountain pine beetles. Spruce
tissues may contain a more efficacious blend of chemicals that promote host acceptance
behaviour in mountain pine beetles relative to pine tissues. Chemical attraction to atypical
hosts is known to occur in other systems, such as the apple fruit moth, *Argyresthia conjugella*
Zeller, (Lepidoptera: Argyresthiidae). During periods of coincident low host availability and
high insect populations, this insect becomes attracted to the fruit of the non-host species
domestic apple (*Malus domestica* Borkh., (Rosaceae)) as a result of chemical similarities
with the fruit of the host plant, rowan, *Sorbus aucuparia* L., (Rosaceae) (Bengtsson et al.
2006).

Phloem properties such as moisture and nutrient content may also have differed
between host types and affected colonization and reproductive behaviour (Amman 1972,
example, is positively correlated with beetle size (Amman and Pace 1976). Care was taken,
however, to harvest host material from similar sites, visually assessing host vigour and
processing experimental material in identical manners to minimize differences in tree and/or
phloem characteristics. We did not find evidence of host species-specific size differences in
the female progeny of the parent generation based on measurements of pronotal widths
(FRM unpublished data, Huber et al. 2009; but see Safranyik and Linton, 1983, who found
larger progeny emerging from pine vs. spruce in laboratory rearing). Past studies on
mountain pine beetle from other hosts in the genus *Pinus* have shown that beetle size-host
species relationships are inconsistent (Amman 1982, Sturgeon and Mitton 1986, Langor and
Spence 1991). The similar sizes of beetles emerging from spruce and pine in our study
suggests that lipid content did not vary between beetles emerging from different host types,
as beetle size is positively correlated with lipid content (Graf 2009). Lipid content may also affect rates of host acceptance in bark beetles (Wallin and Raffa 2004).

Our results of the males found in ovipositional galleries with female beetles, though small in numbers, does not support the phenomenon of assortative mating in this eruptive herbivore. Although host and geographic effects are necessarily confounded regarding the insects used in this study, the long distance dispersal of mountain pine beetle within the present outbreak would likely preclude any assortative mating due to geographical isolation (Safranyik and Linton 1982, Aukema et al. 2006, de la Giroday 2009, Westfall and Ebata 2009). Significant genetic variation has been reported at local (Sturgeon and Mitton 1986) and landscape-level scales among mountain pine beetle (Stock and Guenther 1979, Langor and Spence 1991, Anderson et al. 1993, Kelley et al. 1999, Six et al. 1999, Kelley et al. 2000, Mock et al. 2007, Bartell 2008), although many studies have sampled endemic populations with higher probabilities of experiencing genetic divergence due to random drift and environmental effects relative to epidemic populations (Langor and Spence 1991, Mock et al. 2007, Cook et al. 2008). While earlier studies have found evidence of host-associated genetic divergence in mountain pine beetles (Stock et al. 1979, Stock and Amman 1980, Sturgeon and Mitton 1986), more recent studies have not (Langor and Spence 1991, Kelley et al. 1999, Kelley et al. 2000, Mock et al. 2007).

The biggest barrier to host-specific assortative mating is likely the host susceptibility of the spruce. Among eruptive species of bark beetles, successful beetle reproduction is often dependant upon the death of all, or part, of the host tree (Wood 1982). Host susceptibility to bark beetle attack and suitability for bark beetle reproduction are the two most critical factors governing bark beetle reproduction (Raffa and Berryman 1983).
study, as well as others (Furniss and Schenk 1969, Smith et al. 1981, Safranyik and Linton 1983, Huber et al. 2009) suggests that all potential pine and spruce hosts within a forest stand are located on a continuum of susceptibility and suitability. Potential hosts can be extremely susceptible and suitable or unsusceptible and unsuitable, or any combination thereof. The constitutive and induced defensive capacity of spruce generally renders the tree highly unsusceptible to mountain pine beetle colonization (Furniss and Schenk 1969, Smith et al. 1981, Safranyik and Linton 1983, Unger 1993). These hosts may be quite suitable, however, for brood development if host constitutive and induced defences can be overcome (Huber et al. 2009).

2.6 Acknowledgements

We extend our gratitude to Mr. Brian Pate of West Fraser Timber Co. Ltd. for acquisition of pine and spruce research material from tenured land at Crassier Creek. We also thank Mr. Sven Holmkvist for providing spruce colonized by mountain pine beetle. We thank Mr. Gareth Hopkins and Ms. Hollie Moore (University of Northern British Columbia) for excellent laboratory assistance. Funding for this project was provided by the Canadian Forest Service and NSERC Discovery to BHA.
2.7 Figure Captions

Fig. 2.1. Experimental design for the examination of natal host and host species effects on host acceptance by female mountain pine beetles, and the joining behaviour of male mountain pine beetles with females in their ovipositional galleries. Beetles denoted with an “S” and those denoted by the letter “P”, are individuals reared from interior hybrid spruce and lodgepole pine hosts, respectively. Logs denoted “S” and “P” are interior hybrid spruce and lodgepole pine, respectively. The “X” on the logs indicates the point of male release.

Fig. 2.2. Percentage (+ SE) of female mountain pine beetle acceptance of interior hybrid spruce and lodgepole pine hosts by females reared from interior hybrid spruce and lodgepole pine hosts. Host acceptance was defined as the presence of a female within an ovipositional gallery 24 h after introduction to a pine or spruce log. N = 40 females per treatment.
- Fig. 2.1 -

S = interior hybrid spruce  P = lodgepole pine
Fig. 2.2

Female Natal Species ($P = 0.0130$)

- Pine
- Spruce

Log Species ($P = 0.0027$)

% (+ SE) of females present in ovipositional galleries
Table 2.1. Settlement patterns of single male mountain pine beetles placed on logs of pine and spruce containing two females reared from pine and spruce.

<table>
<thead>
<tr>
<th>male Natal host type</th>
<th>female Spruce</th>
<th>female Pine</th>
<th>none</th>
<th>unaccounted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce Spruce</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Pine Spruce</td>
<td>3</td>
<td>3</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Spruce Pine</td>
<td>5</td>
<td>1</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Pine Pine</td>
<td>0</td>
<td>2</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>

* Spruce and Pine denote interior hybrid spruce (Picea glauca (Moench) Voss x engelmannii Parry ex. Engelmann) and lodgepole pine (Pinus contorta Douglas ex. Loudon), respectively.

† Instances where males were not found within the ovipositional gallery of a female.

‡ Males not recovered during the deconstruction of the bioassay.
2.8 Literature Cited


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3: EFFECTS OF HOST SPECIES AND COMPETITORS ON MOUNTAIN PINE BEETLE REPRODUCTION IN INTERIOR HYBRID SPRUCE VS. LODGEPOLE PINE.

3.1 Abstract

Insect population dynamics depend upon the reproductive success of individuals, which is frequently linked to associations with host plants. Expanding host breadth may cause mortality from maladaptive chemistry of a new host, while at the same time providing escape in space or time from natural enemies. Bark beetles typically reproduce within the confines of the stems of mature trees, and may suffer high mortality from plant defences, inter- and intra-specific competition, and predation. A current mountain pine beetle epidemic \((Dendroctonus ponderosae)\) in British Columbia and Alberta, Canada now extends over 14 million hectares of lodgepole pine \((Pinus contorta)\) forest. In some areas, mountain pine beetles are attacking interior hybrid spruce \((Picea glauca)\) with increasing frequency, in some cases leading to successful colonization and reproduction. Using populations of mountain pine beetle reared from interior hybrid spruce and lodgepole pine hosts, we examined the effect of female natal species and colonized host species on the ability to attract mates and reproduce in logs deployed as a choice assay in a field setting. Additionally, we examined whether the arrival and reproduction of competitor bark beetles such as \(Ips\) spp. affected mountain pine beetle brood production. The developmental history of female pioneers did not influence ovipositional gallery establishment or ovipositional gallery length, the number of larval galleries, number of pupal chambers, or brood production. Although the female pioneer beetles reared from pine and spruce appeared to be functionally equivalent, the overall reproduction in spruce, while successful, was significantly lower than in pine. This
reduction occurred despite the fact that spruce was almost entirely free of competing secondary beetles, while species such as *Ips pini* were commonly found in the lodgepole pine logs. Hence, the availability of enemy-free space within spruce logs did not greatly benefit the reproduction of mountain pine beetles in interior hybrid spruce, a rarely-utilized atypical host.
3.2 Introduction


In some instances, polyphagy on novel hosts can facilitate range expansion of herbivores (Stastny et al. 2006). In western Canada, for example, there is heightened concern of the potential range expansion of mountain pine beetle (Dendroctonus ponderosae Hopkins) (Coleoptera: Curculionidae: Scolytinae) through a lodgepole pine (Pinus contorta Douglas ex. Loudon) - jack pine (P. banksiana Lambert) (Pinales: Pinaceae) hybrid zone into
novel jack pine hosts that stretch across the boreal forest of Canada (Cerezke 1995, Logan
and Powell 2001, Nealis and Peters 2008). Colonizing plant hybrids may facilitate the
evolutionary and physiological processes of switching to a novel host (Floate and Whitham
1993, Pilson 1999). Moreover, Huber et al. (2009) recently documented the reproduction of
mountain pine beetle in interior hybrid spruce (Picea glauca (Moench) Voss x engelmannii
Parry ex. Engelmann) (Pinales: Pinaceae), causing some concern as exposure to novel and
atypical hosts can pre-adapt insects to other novel hosts (Gould 1979, Jaenike 1983).

Not only can the utilization of novel hosts facilitate geographical range expansion,
but such hosts can provide a measure of enemy-free space. Enemy-free space has been
defined (Jeffries and Lawton 1984) as “… [a way] of living that [reduces or eliminates] a
species’ vulnerability to one or more species of natural enemies.” In fact, the influence of
pressure exerted by natural enemies on the diet breadth of phytophagous insects (Price et al.
occasionally been argued to supercede the importance of plant chemistry (Bernays and
Graham 1988). A plant may be suitable for use by an insect but remain excluded from the
diet due to reduced fitness imposed by natural enemies (Barbosa 1988, Denno et al. 1990).
Paradoxically, reductions in natural enemy risk can also select for the inclusion of sub-
optimal hosts into an insect’s diet (Mira and Bernays 2002). Reductions in the enemy
assemblages of herbivorous insects among alternate hosts may be sufficiently common (Ball
necessary to consider interactions with predatory or competitive species when studying
herbivore interactions among novel and/or alternate host species (Gratton and Welter 1999,
Mira and Bernays 2002, Poff et al. 2002, Singer et al. 2004). Although insects may experience physiological complications within new hosts (Bush 1969, Kelley and Farrell 1998, Gratton and Welte 1999), reductions in predation and/or competition can offset initial increases in mortality and provide a stable selection period sufficient to allow the insect population to adapt to the new host plant (Berlocher and Feder 2002).


The current study takes advantage of a large outbreak of mountain pine beetle in western Canada (Kurz et al. 2008, Raffa et al. 2008) to examine questions of novel or atypical host use in an eruptive herbivore. Although the primary host of the mountain pine beetle in British Columbia is considered to be lodgepole pine, it will attack most species of pines, potentially facilitating novel range expansions (Safranyik and Carroll 2006, Nealis and Peters 2008). This study examines sympatric reproduction of mountain pine beetles in lodgepole pine and an atypical host, interior hybrid spruce (hereafter referred to as “pine” and “spruce”, respectively). The objectives of this study were to examine the effects of natal host and colonized host species on the reproductive potential of mountain pine beetles to determine the extent of successful reproductive behaviour in usual and atypical hosts in a field experiment using cut logs. We test whether reproduction in an atypical host might confer reproductive advantages of natural enemy-free space.

3.3 Materials and Methods

3.3.1 Experimental Setup

Female mountain pine beetles from both spruce and pine were obtained from naturally-colonized material. Mountain pine beetle-colonized spruce and pines were harvested near Prince George (N 53°53’00” W 122°48’00””) and Crassier Creek (55°38’00”N,
Colonized material was transported to the laboratory and placed in rearing chambers. After emergence, beetles were provided with phloem and stored at 5°C in an environmental chamber. Only vigorous females, assessed by a visual inspection of activity, less than or equal to 7 days old were selected for use in experiments.

Uninfested spruce and pines for the bioassays were harvested near Crassier Creek, British Columbia. The sites where tree harvest occurred were located in the SBSwk2 biogeoclimatic zone, a land classification system used in British Columbia (Meidinger and Pojar 1991). Spruce and pine trees of similar height, growing conditions, and diameter at breast height (dbh) were selected from the same geographic area to reduce qualitative and quantitative chemical variation in resin quality (Pureswaran et al. 2004) due to environmental or geographic factors. Trees were cut in stands that were free of epidemic beetle activity, and stands in the area were just beginning to exhibit mortality due to local immigration events from the provincial epidemic (Aukema et al. 2006). Trees were felled and cut into 2.5 m logs. After transport to the laboratory, the ends of the 2.5 m logs were sealed with paraffin wax to reduce desiccation. Logs were stored under sealed tarps to avoid colonization by secondary bark beetles such as *Ips* spp. and/or longhorned beetles or wood borers (Coleoptera: Cerambycidae and Buprestidae). Within 2 days, the 2.5 m logs were sectioned into smaller experimental logs 40 cm in length with average diameters of 21.1 cm (± 0.4 SE) and 21.9 cm (± 0.5 SE) for spruce and pine, respectively. After sectioning, the cut ends of the experimental logs were sealed with paraffin wax to reduce desiccation.

Our study consisted of four treatments in a 2 x 2 present host x natal host factorial design. Two treatments consisted of spruce and pine logs that had been colonized in the
laboratory with female mountain pine beetles reared from spruce. The remaining two treatments consisted of spruce and pine logs colonized with female mountain pine beetles reared from pine. Five females were inserted equidistantly around the base of each log at a distance of 5 cm from the cut and sealed edge. The females were inserted via starter-holes 3 mm in diameter drilled through the outer bark into the interface with the phloem layer. A single female was then gently inserted into each hole using forceps. A small piece of aluminum screening was used to cover the gallery entrance to reduce female abandonment while tunnelling. The screening was fixed such that a female could exit the starter hole, but not escape the log. Inserted females were monitored over a 24 h period for host acceptance and ovipositional gallery construction, judged by visible frass production at the gallery opening. Screening was not removed until transport of logs to the field site had occurred.

Following the 24 h monitoring period, logs were transported to the field site. The logs were set upon metal stakes 2 m in height. A 12-funnel Lindgren trap (Lindgren 1983) was hung from the log via a 30 cm length of wood fastened to the top of each log. This trap is particularly effective for capturing larger-bodied insects. Smaller insects were captured using a 12.5 x 20 cm section of vinyl screening coated with Tanglefoot® insect adhesive (The Tanglefoot Company, Grand Rapids, MI). These sticky screens were fastened on the surface of each log using black push-pins.

The experiment was deployed in a randomized complete block design within a lodgepole pine stand near Chief Lake, British Columbia, Canada (N 54°21’00” W 122°59’00”). The treatments in each block included four artificially infested logs (two spruce and two pine, with one of each species infested with females emerged from pine, and the other from spruce) as well as two uninfested control logs (one spruce and one pine log)
Each log was randomly positioned at one of six equidistantly-spaced points within a circular pattern of radius 3.5 m. Each log served as the attractant for its associated pair of traps (one Lindgren trap and one sticky screen). In total, 12 blocks of six trap pairs each were deployed 14 - 16 July, 2007.

Insects were collected from both funnel traps and sticky screens at 5 d intervals over the predominant flight period for mountain pine beetle from field deployment until 16 August, 2007. Upon collection, insects were stored at -20°C until identified. On 16 August, all logs were transported to the laboratory and placed in rearing containers. Insects were collected from the rearing containers every 5 d from 22 August until 29 November, after which no further emergence occurred. Collected specimens were stored at -20°C until identified. Bark was removed from the experimental logs during April 2008 to examine colonization patterns, as different species of bark beetles leave distinct patterns of reproductive galleries etched in the wood (Wood 1982b).

3.3.2 Statistical Analysis

We used 2-factor ANOVAs to analyse the effects of natal species of pioneer females, and host species on mountain pine beetle attraction measured as the number of beetles captured in the Lindgren trap and the sticky screen. We evaluated two response variables to measure the latter; total attraction to each treatment, and attraction per colonizing female, as some females abandoned the logs after transport to the field (see Results). Analyses were conducted in a mixed-effects framework with natal and host species as fixed effects and block as a random effect. A subset of analyses excluded the control logs to evaluate the effects of logs containing female pioneer insects on insect attraction. We also tested whether
the tree from which the log had been cut, or the log’s diameter, influenced attraction. As these variables did not have any significant effect, results are not presented. Finally, the effect of the number of successful female galleries within each log on the attraction of mountain pine beetle was tested.

Upon peeling the bark off the logs after all insects had emerged, we recorded the number of female ovipositional galleries established per log, total ovipositional gallery length per log, ovipositional gallery length per female, number of larval galleries per female, and the number of pupal chambers per female. The number of offspring produced per female was determined by dividing the number of emergent brood per log by the number of established ovipositional galleries per log. The number of offspring produced per log, standardized by phloem surface area, was also tested. Additionally, the number of ovipositional gallery abandonments by females per log was measured (i.e., galleries less than 2 cm in length). All recorded data pertain only to female mountain pine beetles that were manually inserted into the experimental logs within the laboratory. Such females are hereafter referred to as ‘pioneer’ beetles. We used a similar mixed effects framework for each analysis, with fixed effects of natal species of female beetles and log species, and block as a random effect. To examine enemy-free space, we also examined how the number of mountain pine beetle pupal chambers and emerging progeny within each treatment varied with respect to the number of parental galleries constructed by the secondary bark beetles Ips pini (Say), Pseudips mexicanus (Hopkins), and Orthotomicus latidens (LeConte) (Coleoptera: Curculionidae: Scolytinae). Substantial trap catches of I. pini in the field allowed statistical analyses to be performed for this species individually. However, due to
low catch numbers, *P. mexicanus* and *O. latidens* were pooled with *I. pini* data for analysis of a “competitor complex.” The three species were pooled for all emergence data as well.

Similar to analyses of capture of mountain pine beetle, the data for *I. pini* or the competitor complex were analysed with respect to natal species of female mountain pine beetle and log species with block as a random effect. Again, we also tested whether the tree from which the log had been cut or the log’s diameter influenced attraction. Because these did not, results are not presented.

In all analyses data transformations such as square-root(y) were utilized as necessary to satisfy model assumptions of normal distribution of residuals and equal variances. All statistical analyses were performed using R (R Development Core Team, 2009).

### 3.4 Results

#### 3.4.1 Attraction, colonization, and reproduction of mountain pine beetles

Mountain pine beetles were differentially attracted to the logs deployed in the field ($F_{3,55} = 3.45, P = 0.0088$). This effect could be attributed to the attraction of beetles to the colonized vs. uncolonized (control) logs, as within the former, there were no differences in the number of mountain pine beetles captured with respect to natal species of the inserted female colonizers ($F_{1,33} = 0.23, P = 0.64$) or host species ($F_{1,33} = 0.32, P = 0.57$) (Fig. 3.2A).

On average, spruce logs containing spruce- and pine-reared females attracted the most mountain pine beetles with $55.0 \pm 31.1$ and $33.9 \pm 9.6$ (means ± SE), respectively, per 5 d period. Pine logs attracted an average of $29.8 \pm 6.5$ and $32.5 \pm 7.2$, for spruce- and pine-reared females, respectively. Control spruce and pine logs attracted only $17.3 \pm 3.9$ and $22.5 \pm 3.5$ mountain pine beetles over the same time periods.
Once deployed to the field, female beetles inserted in the laboratory did not always continue tunnelling in the log. The number of abandonments by pioneer females was greatest in spruce hosts ($F_{1,33} = 5.35, P = 0.0271$) by females of either natal species ($F_{1,33} = 0.98, P = 0.33$). Pine-and spruce-reared females averaged $1.3 \pm 0.5$ and $1.6 \pm 0.5$ abandonments per spruce host, respectively, while females averaged $0.3 \pm 0.2$ and $0.8 \pm 0.3$ abandonments per pine host. Despite the occasional abandonment, the number of established ovipositional galleries per m$^2$ in colonized logs did not vary by treatment ($F_{3,33} = 0.54, P = 0.66$). Similar to the number of beetles attracted to the logs, the number of ovipositional galleries established by female pioneers was neither influenced by host species ($F_{1,33} = 1.03, P = 0.32$), or the natal species of the inserted females ($F_{1,33} = 0.035, P = 0.85$). Colonized pine logs had an average of $14.5 (\pm 0.9)$ and $15.3 (\pm 1.2)$ ovipositional galleries per m$^2$ for pine- and spruce-reared females, respectively, while colonized spruce logs averaged $14.0 (\pm 2.1)$ and $12.6 (\pm 1.7)$ ovipositional galleries per m$^2$ for pine- and spruce-reared females, respectively. On a per-female-basis, female pioneers reared from either natal host species within spruce logs attracted approximately $2x$ greater numbers of mountain pine beetles than females with conspecific natal species within pine logs ($F_{1,32} = 5.33, P = 0.0276$). Each pine- and spruce-reared female within spruce logs attracted an average of $12.1 (\pm 3.2)$ and $16.1 (\pm 8.1)$ mountain pine beetle per 5 d period, respectively. Pine- and spruce-reared females in pine logs attracted an average of $6.4 (\pm 1.5)$ and $7.9 (\pm 2.0)$ mountain pine beetles, respectively. Mountain pine beetle attraction per female pioneer was not affected by the females’ natal host species ($F_{1,32} = 0.27, P = 0.60$).

The total length of female ovipositional gallery excavated per log did not differ across treatments ($F_{3,33} = 1.92, P = 0.15$). On a per female basis, ovipositional gallery
lengths in pine logs averaged 15.8 cm (± 2.8), and 13.1 cm (± 2.0) for pine- and spruce-reared females, respectively. In spruce logs, per female ovipositional gallery length averaged 14.0 cm (± 5.1), and 11.0 cm (± 2.2) for pine- and spruce-reared females, respectively. These lengths were similar with respect to female natal host species ($F_{1,32} = 0.09, P = 0.76$) and log species ($F_{1,32} = 1.75, P = 0.20$). The standard deviation in average ovipositional gallery length per pioneer female was also similar with respect to female natal species and log species, as neither factor influenced this measurement ($F_{1,32} = 0.04, P = 0.85$ and $F_{1,32} = 1.39, P = 0.25$, respectively).

Ovipositional behaviour and larval performance was similar in both hosts, regardless of the host of origin of the female pioneers. The mean number of larval galleries per pioneer did not vary with female natal species ($F_{1,32} = 0.47, P = 0.50$) or the species of log colonized ($F_{1,32} = 0.620, P = 0.44$) (Fig. 3.2B). Similarly, the mean number of pupal chambers counted per female was not influenced by natal species ($F_{1,32} = 0.53, P = 0.47$) or log species ($F_{1,32} = 2.72, P = 0.11$). On average, there were $13.6 ± 2.9$ and $8.9 ± 2.6$ larval galleries per female for pine- and spruce-reared individuals within pine logs vs. $14.8 ± 8.4$ and $9.6 ± 3.7$ larval galleries per pine- and spruce-reared female, respectively, within spruce logs. The number of pupal chambers associated with the ovipositional galleries were approximately one-third the numbers of larval galleries, ranging from two to six in spruce and pine hosts, irrespective of female natal host (Fig. 3.2C).

Although the establishment and colonization dynamics of female mountain pine beetles were similar among natal hosts and log species, there were differences in reproductive output. The number of brood produced per mountain pine beetle female was higher in pine vs. spruce ($F_{1,32} = 7.95, P = 0.0493$). Brood production in pine logs containing
pine- and spruce-reared females averaged 5.2 (± 1.6) and 3.5 (± 0.9) offspring, respectively. Brood production in spruce logs was less than half of that observed in pine logs, averaging 1.9 (± 0.9) and 1.3 (± 0.6) offspring for pine- and spruce-reared females, respectively (Fig. 3.2D). There were no effects of female natal species on the number of progeny, however ($F_{1,32} = 0.18$, $P = 0.67$). Results did not change when emergence results were standardized by log (i.e., phloem) surface area. Host species ($F_{1,33} = 9.84$, $P = 0.0036$), but not maternal natal species ($F_{1,33} = 1.75$, $P = 0.20$), influenced brood production.

**3.4.2 Effects of competitors on the emergence of mountain pine beetles**

More *I. pini* were captured in traps baited with pine logs vs. spruce ($F_{1,33} = 7.31$, $P = 0.0107$), averaging 10.4 ± 3.9 for pine compared to only 1.7 ± 0.3 for spruce, pooled across treatments, respectively. Natal species of female mountain pine beetle colonizers did not affect trap captures ($F_{1,33} = 0.94$, $P = 0.34$). A similar pattern was noted when all of the competitors (*I. pini, P. mexicanus*, and *O. tomitus*) were pooled. The attraction of the competitor complex was not related to natal species of female mountain pine beetle colonizers ($F_{1,33} = 1.13$, $P = 0.30$), but was related to host species ($F_{1,33} = 25.89$, $P < 0.0001$), with pine being more attractive. Traps baited with pine logs captured between 5.6 – 16.7x more individuals than spruce logs (Fig. 3.3A). *Ips pini*, *O. latidens*, and *P. mexicanus* accounted for 74, 20, and 6% of captured competitors ($n = 165$) within pine logs colonized by pine-reared female mountain pine beetles, respectively. Pine logs colonized by spruce-reared female mountain pine beetles attracted 266 competitors, of which, *I. pini, O. latidens*, and *P. mexicanus* accounted for 61, 19, and 20%, respectively. The attraction of the competitor species to spruce logs was extremely low compared to pine. Traps baited with
spruce logs colonized by pine-reared female mountain pine beetles captured 29 competitors consisting of 25 *I. pini*, one *O. latidens*, and three *P. mexicanus*, while traps baited with spruce logs colonized by spruce-reared female mountain pine beetles captured 14 *I. pini* and only one *O. latidens*.

The density of ovipositional galleries constructed by competitor species was greatest within the colonized pine logs compared to spruce (*F*$_{1,33}$ = 46.20, *P* < 0.0001). Pine logs containing female mountain pine beetles reared from pine or spruce had an average of 44.2 (± 7.6) and 50.0 (± 13.4) competitor galleries per m$^2$, respectively. The density of competitor galleries was much lower in spruce logs, averaging only 7.8 (± 5.1) and 3.4 (± 1.2) galleries per m$^2$ for logs containing pine- and spruce-reared female mountain pine beetles, respectively. Gallery density within treatment logs was not influenced by the natal species of the female mountain pine beetle colonizers (*F*$_{1,33}$ = 0.17, *P* = 0.68).

Patterns of emergence for the competitor complex from mountain pine beetle-colonized logs were similar to those observed for attraction. Emergence success of these competitors was highly influenced by host species (*F*$_{1,33}$ = 29.28, *P* < 0.0001), but not natal species of mountain pine beetle colonizers (*F*$_{1,33}$ = 0.47, *P* = 0.50). The average number of competitors of all species emerging per 5 d period from pine logs containing female mountain pine beetles reared from pine and spruce was 30.6 (±12.1) and 38.8 (± 18.3), respectively. Of all competitors, *Ips pini* was the most numerous species to emerge, followed by *P. mexicanus*, and *O. latidens*. Numbers were highly variable, however. In pine logs colonized by pine-reared female mountain pine beetles, *I. pini, P. mexicanus* and *O. latidens* accounted for 69%, 29%, and 2%, respectively, of the 367 beetles total. Within pine logs colonized by spruce-reared female mountain pine beetles, *I. pini* emergence dropped to 54%
of total species emergence, while *P. mexicanus* rose considerably to account for 44% and *O. latidens* constituted the final 2% (*n* = 466 beetles).

In contrast, competitor emergence from spruce logs was extremely low, with only one *I. pini* and nine *P. mexicanus* emerging from logs colonized by pine-reared female mountain pine beetles. We did not observe successful *O. latidens* emergence from this treatment, and no competitors emerged from any of the spruce logs colonized by spruce-reared female mountain pine beetles (Fig. 3.3B).

Emergence of *I. pini, P. mexicanus,* and *O. latidens* from the logs did not affect emergence of mountain pine beetle brood (*F*$_{1,35}$ = 0.02, *P* = 0.89). Similarly, there was no association between the number of combined *I. pini, P. mexicanus,* and *O. latidens* ovipositional galleries and brood emergence of mountain pine beetles (*F*$_{1,35}$ = 63.99, *P* = 0.95).

### 3.5 Discussion

In this study, the availability of enemy-free space within an atypical host did not necessarily benefit the reproduction of this eruptive herbivore. Indeed, there was no effect of competitors such as *I. pini, O. latidens,* and *P. mexicanus* on the emergence of mountain pine beetles in this study although previous studies have demonstrated both negative (Rankin and Borden 1991) and positive (Smith 2008) effects of *I. pini* and *P. mexicanus* on the brood success of mountain pine beetles, respectively. The lack of any host-mediated reductions in mortality due to interspecific competition may be surprising, given that each of these species are solely, or predominately, in the case of *I. pini,* pine specialists (Furniss and Carolin 1977). Although *Ips perturbatus* (Eichhoff) and *Ips tridens* (Mannerheim) are common in
the study region (Furniss and Carolin 1977), we did not capture any in this experiment, likely due to the rarity of live, standing, *Picea* spp. hosts and/or logging slash within the study area and surrounding forest. All of these species utilize semiochemical-mediated resource partitioning (Hunt and Borden 1987) and may be phenologically separated in time (Koopmans 2010), resulting in either avoidance of occupied hosts or optimized spacing of beetles within the available phloem. Interactions between *P. mexicanus*, *O. latidens*, and mountain pine beetles are likely minimized via temporal separation of dispersal flight and host selection activities. However, due to the multi-voltinism and multiple flight periods of *I. pini* (Wood 1982b) it is likely that competitive interactions between *I. pini* and mountain pine beetles occur under some circumstances. Competition is an important mortality factor in bark beetle systems (Anderbrant et al. 1985, Bergvinson and Borden 1991, Rankin and Borden 1991, Boone et al. 2008, Huber et al. 2009), such that reduced intraspecific competition can result in increased reproduction in standing, live, atypical hosts (Huber et al. 2009).

The similar attraction of mountain pine beetles to each of the four treatments suggests that the behavioural activity of mountain pine beetle pheromone is maintained across pine and spruce hosts. Host phytochemistry is an important determinant of host selection in phytophagous insects (Kühnle and Müller 2009) and the ubiquitous terpenoid-based chemistry throughout the Pinaceae (Huber et al. 2004) may evoke reproductive behaviour in mountain pine beetles across a broad host spectrum. Females with developmental histories in spruce must manufacture – either via host tissue-derived precursors (Byers 1981, Wood 1982a, Hunt et al. 1986, Seybold et al. 2006) or possibly via de novo synthesis (Hager and Teale 1996, Hall et al. 2002, Seybold and Tittiger 2003, Keeling et al. 2004) – pheromones.
that are functionally equivalent to those produced by females with developmental histories in
lodgepole pine. Pheromone consistency among hosts is advantageous for a pine-feeding
generalist herbivore to maintain intraspecific functionality (Raffa et al. 2007). Moreover, as
the developmental history of mountain pine beetle in this study did not influence
ovipositional gallery establishment, ovipositional gallery length, number of larval galleries,
number of pupal chambers, or brood emergence on a per-female-basis within either host,
female mountain pine beetles with spruce developmental histories may have equal fitness to
beetles reared from pine.

While host species did not influence attraction on a per log basis, the approximate
two-fold increase in response to females within spruce logs on a per female basis suggests
that subtle variations in pheromone production (Birgersson et al. 1984, Hunt et al. 1986,
Birgersson et al. 1988, Miller et al. 1989, Pureswaran et al. 2000, Pureswaran and Borden
2003), possibly combined with variability of host-derived chemical pheromone precursors
and/or attractants (Byers 1981, Birgersson et al. 1984, Miller and Borden 2000, Erbilgin et al.
responsible for this effect, as total ovipositional gallery lengths were similar. For example,
quantitative differences in spruce and pine resin (Pureswaran et al. 2004) may yield different
levels of pheromone precursors that influence pheromone production. Such differences in
pheromone attractiveness have been noted previously with pine engravers tunnelling within
various hosts (Erbilgin and Raffa 2000, Aukema et al. 2004), and are thought to provide
some measure of enemy-free space from competitors and predators.

It is important to note that measures of oviposition among Scolytids, e.g., initiation of
ovipositional galleries or total lengths of such galleries excavated, do not necessarily reflect
equal host suitability (Amman 1982, Safranyik and Linton 1982, 1983, Cerezke 1995). For example, in a laboratory assay, *D. ponderosae, D. pseudotsugae* Hopkins, and *D. rufipennis* (Kirby) exhibited the greatest establishment rate of ovipositional galleries on logs of sub-alpine fir (*Abies lasiocarpa* (Hooker)) among four tested host and non-host species. Complete gallery failure by all three species ultimately occurred, however (Safranyik and Linton 1983). Larval survivorship is highly dependant on host suitability for insect herbivores, particularly when utilizing regular vs. occasional hosts (Larsson and Strong 1992, Brown et al. 1995, Larsson and Ekbom 1995, Awmack and Leather 2002, Xue and Yang 2007). Similar larval survival and subsequent pupation within each host species during this experiment supports the hypothesis that spruce may be an equally suitable host as pine (Chapter 1, Huber et al. 2009), although inferring equal host quality from egg and larval survival (Reid and Gates 1970) within our study must be made with caution because egg niches could not be counted within spruce logs due to reduced etching of the sapwood by tunnelling beetles.

Reproductive complications for mountain pine beetles within spruce hosts in this study did not occur until pupation. Delayed deleterious host effects on mountain pine beetles infesting spruce could imply that spruce adequately supports the basic nutritional requirements for mountain pine beetle larvae, but lacks the resources necessary for larval metamorphosis. Fungi, for example, (Bentz and Six 2006, Adams and Six 2007), are an important source of nutrition to developing bark beetle larvae (Baker and Norris 1968, Coppedge et al. 1995, Ayers et al. 2000, Bentz and Six 2006, Bleiker and Six 2007) and can influence brood development and emergence (Barras 1973, Six and Paine 1998, Kopper et al. 2004). Zhang and Schlyter (2004) suggest that symbiotic fungi of coniferophagous bark
beetles may not survive within non-hosts. While the fungal associates of the mountain pine beetle, *Grosmannia clavigera* (Robinson-Jeffrey and Davidson) Zipfel, de Beer and Wingfield and *Ophiostoma montium* (Rumbold) von Arx, are able to naturally colonize spruce phloem (Bleiker 2009), the extent of colonization has not been quantified. *Grosmannia clavigera* and *O. montium* growth is dependant upon physical (Ballard et al. 1982, 1984, Bleiker and Six 2009a), chemical (Paine and Hanlon 1994), and nutritional (Bleiker and Six 2009b) characteristics within the phloem. Moreover, beneficial bacteria that prevent the growth of antagonistic fungi (Cardoza et al. 2006, Scott et al. 2009) may be reduced within alternate hosts (Shifrine and Phaff 1956), resulting in reduced larval fitness. An alternative, but not mutually exclusive, explanation for reduced realized insect fecundity (Awmack and Leather 2002) arising during larval/pupal development in spruce may be chronic, sub-lethal effects to mountain pine beetle larvae from spruce resin.

In contrast to our study, Huber et al. (2009) report that mountain pine beetles that colonized standing, live, spruce hosts in central British Columbia produced more progeny per female than conspecifics colonizing nearby pine. The discrepancy may reflect the use of logs rather than intact hosts for reproductive studies of mountain pine beetles. It is possible, for example, that degradation of pine phloem occurs at a slower rate than spruce phloem after being cut. Higher phloem quality in pine logs throughout our study would certainly benefit bark beetle brood production (Amman 1972, Amman and Pace 1976, Reid and Robb 1999, Redmer et al. 2001), perhaps by facilitating the proliferation of fungal and microbial associates that condition the host and benefit reproduction (Bentz and Six 2006, Cardoza et al. 2006, Bleiker and Six 2007).
Our results demonstrate that although brood production within spruce may be significantly lower than within pine, the host may function as a source rather than a sink and insect population replacement may be possible if the insects are able to colonize susceptible spruce (Huber et al. 2009). Differential reproduction of mountain pine beetles among different host species (e.g., Langor 1989) as well as the apparent interspecific functionality of progeny, could extend outbreaks in certain instances. The spatial separation of sub-populations using different host species (Tavormina 1982, Jaenike and Grimaldi 1983, Feder et al. 1988, Kelley et al. 2000, Berlocher and Feder 2002) or sub-species can cause genetic divergence. Such separation may have caused the genetic divergence of populations of *Dendroctonus brevicomis* in California and Colorado (Kelley et al. 1999). The current work, however, does not provide evidence that similar separation might be occurring in mountain pine beetles in the central interior of British Columbia.

### 3.6 Acknowledgements

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3.7 Figure Captions

Fig. 3.1. Experimental design for the examination of natal host and host species effects on the reproductive potential of female mountain pine beetles in interior hybrid spruce and lodgepole pine logs. Beetles denoted with an “S” and those denoted by the letter “P”, are individuals reared from interior hybrid spruce and lodgepole pine hosts, respectively. Logs denoted “S” and “P” are interior hybrid spruce and lodgepole pine, respectively. Control logs did not contain manually inserted female mountain pine beetles.

Fig. 3.2. Effect of natal host of origin and present host on the reproduction of mountain pine beetle. Diagonal lines and dotting represent logs seeded with female mountain pine beetles (n = 5) reared from pine and spruce natal hosts, respectively. N = 12 replicates per log- and female mountain pine beetle natal-species combination. (A) Mean (+ SE) number of mountain pine beetles attracted to each treatment per 5 d period. (B) Mean (+ SE) number of larval galleries per manually inserted female mountain pine beetle per treatment. (C) Mean (+ SE) number of pupal chambers per manually inserted female mountain pine beetle per treatment. (D) Mean (+ SE) number of brood produced per manually inserted female mountain pine beetle per treatment.

Fig. 3.3. Effect of natal host of origin and present host on the attraction and reproduction of competitors of mountain pine beetle. Diagonal lines and dotting represent logs seeded with female mountain pine beetles (n = 5) reared from pine and spruce natal hosts, respectively. N = 12 replicates per log- and female mountain pine beetle natal-species combination. (A) Mean (+ SE) pooled attraction per treatment per 5 d period by the secondary bark beetle.
species *Ips pini* (Say), *Pseudips mexicanus* (Hopkins), and *Orthotomicus latidens* (LeConte).

(B) Mean (+ SE) pooled emergence per treatment per 5 d period by the secondary bark beetle species complex *Ips pini* (Say), *Pseudips mexicanus* (Hopkins), and *Orthotomicus latidens* (LeConte).
Fig. 3.1 - Female MPB natal species

Log species

No females inserted

S = interior hybrid spruce  P = lodgepole pine

N = 12  12  12  12  12  12  12

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Fig. 3.2 -

Female Natal Species (P = 0.54)
- Pine
- Spruce

Log Species (P = 0.57)
- Pine
- Spruce

Mean (+ SE) number of mountain pine beetle attracted / treatment

Log Species (P = 0.10)
- Pine
- Spruce

Mean (+ SE) number of pupal chambers / female mountain pine beetle

Female Natal Species (P = 0.85)
- Pine
- Spruce

Log Species (P = 0.0493)
- Pine
- Spruce

Mean (+ SE) number of prophy / female mountain pine beetle

- Fig. 3.2 -

Female Natal Species (P = 0.91)
- Pine
- Spruce

Log Species (P = 0.10)
- Pine
- Spruce

Mean (+ SE) number of pupal chambers / female mountain pine beetle

Female Natal Species (P = 0.85)
- Pine
- Spruce

Log Species (P = 0.0493)
- Pine
- Spruce

Mean (+ SE) number of prophy / female mountain pine beetle

- Fig. 3.2 -

Female Natal Species (P = 0.57)
- Pine
- Spruce

Log Species (P = 0.10)
- Pine
- Spruce

Mean (+ SE) number of pupal chambers / female mountain pine beetle

Female Natal Species (P = 0.85)
- Pine
- Spruce

Log Species (P = 0.0493)
- Pine
- Spruce

Mean (+ SE) number of prophy / female mountain pine beetle

- Fig. 3.2 -

Female Natal Species (P = 0.50)
- Pine
- Spruce

Log Species (P = 0.44)
- Pine
- Spruce

Mean (+ SE) number of larval galleries / female mountain pine beetle

Log Species (P = 0.10)
- Pine
- Spruce

Mean (+ SE) number of pupal chambers / female mountain pine beetle

Log Species (P = 0.0493)
- Pine
- Spruce

Mean (+ SE) number of prophy / female mountain pine beetle

- Fig. 3.2 -

Female Natal Species (P = 0.91)
- Pine
- Spruce

Log Species (P = 0.10)
- Pine
- Spruce

Mean (+ SE) number of pupal chambers / female mountain pine beetle

Female Natal Species (P = 0.85)
- Pine
- Spruce

Log Species (P = 0.0493)
- Pine
- Spruce

Mean (+ SE) number of prophy / female mountain pine beetle
Fig. 3.3 -

- A -

Mean (+ SE) number of secondary competitors attracted / 5 d

Female Natal Species ($P = 0.30$)

- Pine
- Spruce

Log Species ($P < 0.0001$)

- B -

Mean (+ SE) number of emergent secondary competitors / 5 d

Female Natal Species ($P = 0.50$)

- Pine
- Spruce

Log Species ($P < 0.0001$)

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3.8 Literature Cited


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concentrations on the response of *Ips pini* (Coleoptera: Scolytidae) to its aggregation
pheromone: implications for pest management and ecology of bark beetles.
Agricultural and Forest Entomology 5:269-274.

Erbilgin, N. and K. F. Raffa. 2000. Effects of host tree species on attractiveness of tunneling
pine engravers, *Ips pini*, to conspecifics and insect predators. Journal of Chemical
Ecology 26:823-840.

host races of the apple maggot fly *Rhagoletis pomonella*. Nature 336:61-64.

Floate, K., D. and T. Whitham, G. 1993. The "hybrid bridge" hypothesis: host shifting via

Furniss, R. L. and V. M. Carolin. 1977. Western forest insects. Miscellaneous publication
No. 1339 US Department of Agriculture.

Gould, F. 1979. Rapid host range evolution in a population of the phytophagous mite


natural enemies for *Chrysomela lapponica* in pioneering a new host plant. Ecological
Entomology 29:584-593.


oviposition by *Ips calligraphus* (Coleoptera: Scolytidae) on relation to slash pine

Hager, B. J. and S. A. Teale. 1996. The genetic control of pheromone production and

Hall, G. M., C. Tittiger, G. L. Andrews, G. S. Mastick, M. Kuenzli, X. Luo, S. J. Seybold,
monoterpenoid pheromone component ipsdienol *de novo*. Naturwissenschaften 89:79-
83.

Biology, host specificity tests, and risk assessment of the sawfly *Heteroperreyia
hubrichi*, a potential biological control agent of *Schinus terebinthifolius* in Hawaii.
BioControl 48:461-476.


4: ATTRACTION OF AN ERUPTIVE HERBIVORE TO TYPICAL AND ATYPICAL SYMPATRIC HOSTS AS A FUNCTION OF INSECT POPULATION DENSITY AND HOST AVAILABILITY: SELECTION OF LODGEPOLE PINES VS. INTERIOR HYBRID SPRUCE BY MOUNTAIN PINE BEETLES.

4.1 Abstract

Host plant distribution and availability are important factors in determining patterns of host-use for phytophagous insects. When preferred host species are rare within the landscape, host preference hierarchies may be subject to modification as host-searching adults increasingly accept less preferable plant species. An unprecedented outbreak of mountain pine beetle (*Dendroctonus ponderosae* Hopkins) in British Columbia and Alberta, Canada, has caused severe losses of lodgepole pine (*Pinus contorta* Douglas ex. Loudon) over 14 million hectares of forest. Lodgepole pine is the preferred host species of mountain pine beetle in British Columbia, but there has been a recent increase in the occurrence of mountain pine beetle attack on interior hybrid spruce (*Picea glauca* (Moench) Voss x *engelmannii* Parry ex. Engelmann) in areas of the province that have experienced the most severe lodgepole pine mortality. In this study, we test the hypothesis that host specificity declines with increasing population pressure and decreasing host availability. Using regression analysis and model evaluation with Akaike’s Information Criterion scores, we examined the attraction of mountain pine beetle to pine and spruce logs within 17 stands across central British Columbia as a function of within-stand insect population pressure and pine vs. spruce availability in the immediate local habitat. The importance of these factors on mountain pine beetle host selection was determined under both simulated “pioneering” and mass-attack conditions (i.e., with and without synthetic pheromone baits added to the logs). Our results indicate that pioneering mountain pine beetle are highly discriminating towards
the preferred host, lodgepole pine, and are not influenced by the availability of spruce within a stand. However, when logs were baited, mountain pine beetle displayed evidence of preferring the species of host that was most prevalent within a stand. Even after accounting for this signal, the insects shift their landing behaviour toward pine hosts with increasing population densities. This suggests that host specialization may actually be enhanced at high insect densities. The consequences of such a behavioural shift in host preference on the population dynamics of this eruptive herbivore under both endemic and outbreak conditions are discussed.
4.2 Introduction


Likewise, habitat selection is a critical aspect of insect success since individual fitness is closely linked to the type of host with which an insect becomes associated (Gould 1979, Gratton and Welter 1999, Awmack and Leather 2002, Mira and Bernays 2002, Xue and Yang 2007). Plants have evolved elaborate means of defence in order to resist, deter, or interrupt phytophagy and reduce detrimental effects of insect feeding (Raffa 1991, Herms...

The phloemophagous larvae of bark beetles (Coleoptera: Curculionidae: Scolytinae) are cryptic and reside within the toxic subcortical tissues of only a single host. Bark beetles, therefore, represent a group of insects in which host selection is extremely important (Wood 1982b). The highly developed resin defence system of conifers dictates that most species of bark beetles attempt colonization in hosts that are weakened, senescent, or otherwise compromised (Wood 1982b). Due to the inherent risk of conifer colonization, many species of bark beetles utilize aggregation pheromones to collectively overwhelm host defences and find mates while engaging in host procurement (Wood 1982a, Wood 1982b, Raffa and Berryman 1983). "Pioneering" bark beetles, or those that initiate attack on a host rather than responding to pheromones, may have an advantage whenever many potential hosts are available because they have the opportunity to select a susceptible host that is most suitable for colonization (Birgersson et al. 1988).

A major mountain pine beetle, Dendroctonus ponderosae Hopkins, epidemic within British Columbia and Alberta, Canada currently extends over 14 million hectares of lodgepole pine (Pinus contorta Douglas ex. Loudon) (Pinales: Pinaceae) forest (Westfall and
Ebata 2009). Within British Columbia, lodgepole pine serves as the principal host for mountain pine beetle (Safranyik and Carroll 2006). Due to the unprecedented severity of the current mountain pine beetle outbreak, many forest stands have experienced almost complete mortality of lodgepole pine (Westfall and Ebata 2009). In areas of the province that have experienced some of the most severe lodgepole pine mortality, such as the central interior region, there has been a recent increase in the occurrence of mountain pine beetle attack on interior hybrid spruce (*Picea glauca* (Moench) Voss x *engelmannii* Parry ex. Engelmann) (Pinales: Pinaceae) (Huber et al. 2009). Although most interior hybrid spruce resist colonization attempts by mountain pine beetles, tree mortality and successful insect reproduction has occurred in certain cases (McKee and Aukema [unpublished], Huber et al. 2009). The use of *Picea* spp. hosts by mountain pine beetles appears to be a rare occurrence in natural settings and opportunities for detailed study have been limited (Furniss and Schenk 1969, Smith et al. 1981, Wood 1982b, Unger 1993, Huber et al. 2009). Wood (1982b) and Huber et al. (2009) both report this behaviour occurring during mountain pine beetle epidemics; however, this may not always be the case (Smith et al. 1980, Furniss & Schenk 1969). The causal mechanisms of such behaviour may include physiological stress to host spruce trees (Smith et al. 1981), proximity to colonized *Pinus* spp. hosts (Unger 1993), and declining host availability at high population densities (Huber et al. 2009).

The objective of this study was to test the hypothesis that host specificity declines with increasing population pressure and decreasing host availability in an eruptive herbivore (Raffa and Berryman 1983). We use the mountain pine beetle as the model organism and examine attraction to interior hybrid spruce and lodgepole pine logs deployed as a choice
4.3 Materials and Methods

A field experiment was conducted from 16 July - 26 August 2008, in the central interior of British Columbia, Canada. Seventeen research plots were selected and established in three areas. At the time of study, the area surrounding Crassier Creek (N 55°38'00", W 122°15'00"), MacKenzie (N 55°10'48", W 123°4'48"), and Chief Lake (N 54°21'00" W 122°59'00") were experiencing incipient-epidemic, epidemic, and post-epidemic mountain pine beetle population phases (Safranyik and Carroll 2006), respectively.

During site selection, forest stands were inspected for the amount of lodgepole pine (hereafter, “pine”) relative to interior hybrid spruce (hereafter, “spruce”) and other tree species, with the goal of establishing plots exhibiting a gradient of available pine among the forest sites. Detailed plot assessments categorized stand structure regarding tree size (diameter at breast height (dbh)), species, stand density, and the proportion of suitable pine hosts. Plots were assessed by describing all tree species (dbh ≥ 1cm) within six 5 m-radius subplots, located 75 m from plot centre along transects radiating at 0, 60, 120, 180, 240, and 300 degrees, respectively. Attempts were made to standardize plots on the basis of stand density, tree size, and age, selecting sites with trees considered “suitable” for mountain pine beetles (i.e., generally pine ≥ 60 years of age and ≥ 8 cm dbh; Furniss and Carolin 1977, Safranyik and Carroll 2006, but see Maclauchlan et al. 2007). The relative availability of pine or spruce within a plot was calculated as the green pine or spruce basal area (m²) belonging to trees with dbh ≥ 8 cm, expressed as a percentage of the total basal area of all
live pines, spruce and non-host trees with dbh ≥ 8 cm. Stands had a minimum size of 5 hectares with sufficient width and breadth to reduce the possibility of edge effects, e.g., from adjacent harvested areas. All pines were examined for entrance holes, boring dust or other signs of beetle colonization – as well as for crown fading, which indicates imminent tree death. Any trees with any such symptoms were excluded from site characterizations as suitable hosts, as mountain pine beetles usually exhaust the resource of a tree within one generation and must kill new trees to reproduce when at outbreak levels (Safranyik and Carroll 2006).

Within each of the 17 plots, six 12-unit funnel traps (Lindgren 1983) were set up to capture mountain pine beetles. Each trap had one of six treatments: a log of pine, a log of spruce, a log of pine with a mountain pine beetle tree-bait (Contech, Inc., Delta, BC), a log of spruce with a tree-bait, a “blank” funnel trap, and a funnel trap with a tree-bait. The tree-baits consisted of a trans-verbenol bubble-cap and exo-brevicomin flex-lure. The treatments were chosen such that logs of spruce and pines measured the relative attraction by mountain pine beetles to each tree species, logs with baits measured mountain pine beetle attraction to each tree species under simulated mass attack, blank funnel traps measured relative mountain pine beetle population density among sites, and the baited funnel traps determined that the tree baits were effective (via comparisons with the blank funnel traps).

The logs for the treatments were cut from six spruce and six pines harvested near Crassier Creek, British Columbia. The sites where tree harvest occurred were located in the SBSwk2 biogeoclimatic zone (i.e., an ecosystem classification scheme used in British Columbia; Meidinger and Pojar 1991). The logs were 40 cm in length with a mean (± SE) diameter of 18.5 (± 0.3) and 19.14 (± 0.2) for spruce, and pine, respectively. The trees were...
selected based on similar growing and environmental conditions, apparent health, overall size, and dbh. All trees were visually inspected to be free of any bark beetles or stem boring insects prior to cutting. Immediately after cutting, and returning to the laboratory, log ends were sealed with boiling paraffin wax to reduce desiccation. Logs were stored at 5°C for 2 d until deployment to the field.

The traps were deployed in the field on 11-12 July, 2008. In each of the 17 plots, six traps, one of each treatment were arranged equidistantly in a circular pattern (radius = 25 m) with 25 m spacing between adjacent traps. Treatments were randomly assigned to each position. Treatments with a pine or spruce log were set upon a metal stake 2 m above the ground. A free-hanging Lindgren trap was fastened to a 30 cm length of wood attached to the top of each log. For the funnel trap control treatments without logs, Lindgren traps were attached directly to “L” shaped metal poles to allow the traps to hang freely. Care was taken to ensure that the height of the Lindgren funnel traps was consistent. Tablets of Ortho Home Defense Max™ (active ingredient: 19.2% Dichlorvos) were inserted into all trap collecting cups to prevent destruction of captured bark beetles by insect predators. Trap collections occurred every 5 d from 16 July to 26 August, 2008. Treatment positions within plots were randomized following each collection. Specimens were stored at -20°C until identification.

The variation in the number of mountain pine beetles captured among the six treatments was analysed using ANOVA. Specific means comparisons were performed using linear contrasts where significant treatment differences existed. As well, the effects of log diameter, stand density, and tree of origin on the attraction of mountain pine beetles was tested using regression and ANOVA models as quality control checks for experimental protocol (i.e., uniform characteristics of experimental treatments to allow the isolation of
signals of interest such as attraction to tree species). One data point for the controls at one collection period (26 July 2008) was discarded due to mass attack of a neighbouring, standing live pine and the associated “switching” behaviour (Safranyik and Carroll 2006), which happened to shift colonization focus to the trap. Analytical assumptions of normality of errors and homoscedasticity were examined by visual inspection of residual plots. When necessary, data were log(y+1) transformed to fulfill these assumptions.

To test the hypothesis that preferential attraction of beetles to pine vs. spruce changes with population density and/or habitat characteristics, we analysed the number of insects captured in pine vs. spruce traps as a function of the number of insects captured in the blank controls and the percentage of available pine in the stand using logistic regression. The number of insects captured in the blank control traps served as a surrogate for background insect population density in all analyses. Analyses were conducted using both explanatory variables separately, as well as together, and models were compared using Akaike’s Information Criterion (Akaike 1973). Models with lowest AIC values are judged to fit the best. Analyses were done separately for the logs with and without tree-baits, as baiting the logs to simulate mass attacks changed the distribution of insects arriving at pine vs. spruce (see Results). All data analysis was performed using R (R Core Development Team 2009).

4.4 Results

The 17 stands selected had open understories with shrubs ≤ 0.5 m and small (≤ 3 m in height) saplings dispersed throughout the stand. The percentage basal area of available pine ranged from 12 – 100% of all live trees present. Percentage basal area of available spruce ranged from 0 – 53%. The average stand density was 1533 ±102 (mean ± SE) stems per ha.
(dbh ≥ 8 cm). Neither log diameter, tree of origin, or stand density influenced patterns of mountain pine beetle attraction to baited or non-baited pine and spruce logs with varying availabilities of potential pine or spruce hosts (P > 0.05 in all cases).

The number of mountain pine beetles captured in the Lindgren traps varied among treatments (F_{5,80} = 29.6, P < 0.0001), with the highest catch in traps baited with a pine log and pheromone bait. The mean number of mountain pine beetles attracted to non-baited pine and non-baited spruce logs was similar with 9.4 (± 3.4) and 4.9 (± 1.0) individuals caught, respectively, per 5 d collection period (Z = -0.504, P = 0.62) (Fig. 4.1). Within tree-species, the addition of pheromone baits significantly increased mountain pine beetle attraction to both pine (Z = 9.396, P < 0.0001) and spruce (Z = 6.159, P < 0.0001) logs. Pheromone-baited pine logs attracted significantly more mountain pine beetles than baited spruce logs, averaging (± SE) 356.5 (± 157.1) and 115.0 (± 62.0) insects respectively, per 5 d collection period (Z = -3.740, P = 0.0002).

In traps without pheromone baits, approximately 65.7% of the insects were attracted to traps baited with pine logs vs. spruce logs [P (pine vs. spruce) = e^{0.6501} / 1 + e^{0.6501}, Z = 4.80, P < 0.0001]. The addition of baits to both of the logs to simulate mass attack increased the proportion of insects arriving at pine logs as opposed to spruce logs by approximately 10%, to 75.6% [P (pine vs. spruce) = e^{0.6501+0.4813x} / 1 + e^{0.6501+0.4813x}, where x = 1 if baited, Z = 3.49, P = 0.0005]. Because the distribution of insects changes between pine and spruce logs in the presence of baits, results of the analyses examining the association between population density, surrounding pine availability in the forest, and the likelihood of choosing pine relative to spruce are displayed separately for baited and non-baited logs in Table 4.1.
In tests simulating pioneer host selection (i.e., non-baited conditions), we found that the likelihood that insects were captured in traps associated with pine vs. spruce host material was positively related to the percentage of pine available in the surrounding forest (positive coefficient of “best model” regression, AIC = 90.11, Table 4.1A). The availability of spruce in the surrounding stand did not affect the propensity of beetles to land in traps associated with pine or spruce logs.

We found significant evidence that mountain pine beetles are preferentially attracted to pine vs. spruce with increasing population densities, as the regression coefficient for population density is consistently and significantly positive when the local pine/spruce habitat variables are taken into account for analysis with pheromone-baited host material (Table 4.1B). The spruce coefficients, however, reveal an opposite trend, that higher proportions of beetles will move away from pine-baited traps (to spruce-baited traps) in the presence of increasing spruce in the stand (negative coefficients for spruce variable in Table 4.1B). The pine and spruce habitat variables are almost identical in the best fitting model (Table 4.1 bottom row; pine $1.04 \times 10^{-2}$ vs. spruce $1.02 \times 10^{-2}$; $t_{13} = 0.03$, $P = 0.97$). That is, the best model indicates that mountain pine beetles are prone to similarly associate with the potential host (pine or spruce) that is more common within a stand even though the insects will select pine preferentially with increasing population densities.

4.5 Discussion

Our results do not support the hypothesis that host discrimination decreases with increasing population pressure, as higher numbers of beetles were captured in pine-baited traps relative to spruce-baited traps as insect populations increased for this pine-specializing
herbivore. Raffa & Berryman (1983), for example, propose that the frequency of host
discriminating genotypes among mountain pine beetles varies inversely with population size.
At endemic levels, beetles must exhibit a relatively high level of discrimination to colonize
weakened hosts which may be ephemeral in space and time. Such selectivity is not as critical
in outbreak stages as mass attacks facilitated by aggregation pheromones reduce selective
pressures exerted by the chemical defences of host trees. While such changes in gene
frequencies may explain the spread to less-favoured species during the final stages of an
epidemic (Wood 1982b, Raffa and Berryman 1983), our results suggest that species fidelity
may actually strengthen as an outbreak progresses. Although discrimination among pines of
varying defensive capacities may be less critical as populations build (Raffa and Berryman
1983), strengthening host fidelity to pines vs. non-hosts or atypical host species would act as
a key source of positive feedback in an eruptive system once a key transition threshold is
breached. This would ensure resources to facilitate procurement of the largest and most
vigorous trees with thicker phloem that produces the most beetle offspring (Amman 1972,
Within stands that are composed mainly of pine hosts, spillover into less-preferred hosts,
such as spruce (Huber et al. 2009), may occur when insect populations are at extremely high
levels concomitant with decreasing preferred host availability within the stand.

Increased proportions of insects captured in traps baited with pine relative to spruce
logs when coupled with attractant pheromone baits may be due to the synergistic activity
between specific monoterpenes in the host tissues and the trans-verbenol and exo-brevicomin
in the pheromone baits. The monoterpene myrcene is the most active synergist for the
pheromones trans-verbenol and exo-brevicomin (Borden et al. 1983, Conn et al. 1983,
Pureswaran and Borden 2005). Bole tissue of lodgepole pine contains significantly higher quantities of myrcene relative to the bole tissue of interior hybrid spruce (Pureswaran et al. 2004). Moreover, the aggregative effect of exo-brevicomin varies with the species of host colonized by mountain pine beetles (Borden et al. 1987) and therefore may not be as effective when paired with interior hybrid spruce.

Selection of baited pines vs. spruce based on the most prevalent species in the surrounding stand (Table 4.1B) suggest that host selection strategies can be moderated by the availability of hosts within the surrounding area, with pine and spruce having almost identical effects (Table 4.1B). These results suggest that under epidemic conditions this insect may exhibit an elective host selection strategy based on resource availability. This is perhaps surprising, as bark beetles exhibit a necessarily brief dispersal and host selection period (Wood 1982b). As such, the potential for environmental interactions to result in learned behavioural traits may be somewhat limited (Courtney and Forsberg 1988, Papaj and Prokopy 1989), especially for a species such as mountain pine beetle. To our knowledge, there are no comparative studies regarding this phenomenon in bark beetles, although similar host selection strategies have been observed in species of Lepidoptera (Capinera 1985, Courtney and Forsberg 1988, Singer et al. 1989, Chew and Courtney 1991, Kuussaari et al. 2000, Singer and Stireman 2001).

While patterns of host use may vary with degree of connectivity of surrounding habitat patches and the type of host use occurring in those surrounding patches (e.g., Chew and Courtney 1991, Kuussaari et al. 2000), it is difficult to attribute local host selection behaviour to intrinsic differences within insect populations as we cannot know the origins of the insects among the sites. Although plots within each of the three regions in this study
were separated by $\geq 1$ km, Aeolian dispersal events may transport mountain pine beetle
distances of tens or hundreds of kilometres (Jackson et al. 2008, de la Giroday 2009,
Robertson et al. 2009). Beetles immigrating from surrounding non-assessed areas could have
displayed behaviours developed in areas with different host constraints and interactions
(Chew and Courtney 1991, Singer and Parmesan 1993). Other studies with Lepidopterans
exhibiting similarly high dispersal capacities, however, have demonstrated altered host-use in
response to the local densities of preferred and secondary host species (Courtney and

Although host properties are integrated into flight and host orientation by bark beetles
(Shepherd 1966, Campbell and Borden 2005, 2006a, 2006b) and changing proportions of
insects captured in pine and spruce-baited traps relative to species abundance likely reflect
host-searching behaviour, we note a few important limitations to our study. First, the use of
captured adult individuals in examining patterns of beetle attraction may not reflect ultimate
host choices for this insect. For example, there exists much empirical support for a random
landing hypothesis during host selection (Hynum and Berryman 1980, Moeck et al. 1981,
Raffa and Berryman 1982, Pureswaran and Borden 2003, 2005, Saint-Germain et al. 2007),
with host selection decisions made following gustatory sampling of host tissue (Raffa and
Berryman 1982, Pureswaran and Borden 2003, 2005). In the present study, however,
captured individuals may not have had the opportunity to sample the log associated with the
trap prior to being removed from the population. As such, examining adult colonization
effort (in this system, for example, pitch tubes), or alternately, egg or larval distributions
amongst host species within each forest may have been a more reliable means of inferring
ultimate adult host selection preferences (Rausher et al. 1981, Courtney and Forsberg 1988,
Chew and Courtney 1991, Kuussaari et al. 2000, Singer and Stireman 2001). Second, the capacity for mountain pine beetle to continue production of aggregation pheromone s during host colonization efforts is related to the ability of a host to maintain an induced resinosis response (Raffa and Berryman 1983). The use of harvested logs in our study precludes the interaction of mountain pine beetle with actively induced defensive responses of the host and thus may have altered host searching dynamics. Finally, the use of basal area measurements to characterize a stand's host availability for phloegophagous herbivores may have limitations, as dense stands with small diameter trees may appear numerically similar to those with low densities of large trees, and susceptibility of either will depend on insect population density (Björklund and Lindgren 2009, Boone et al. 2010). However, the exclusion of "unsuitable" (small diameter and/or dead) hosts in our stand characterizations may have improved such metrics.

4.6 Acknowledgements

We extend our gratitude to Mr. Brian Pate of West Fraser Timber Co. Ltd. For acquisition of pine and spruce research material, and for providing research sites on tenured land at Crassier Creek, and to Mr. Les Dillabaugh of Lakeland Mills for providing research sites at Chief Lake. In addition we thank Brent Sinclair of Canfor Corporation (MacKenzie) and Judi van der Maaten (municipality office of MacKenzie) for securing research sites in the MacKenzie district. We also thank Mary Mitchell (BC Ministry of Forests & Range) for her assistance in locating suitable research sites in the MacKenzie area. We thank Ms. Talya Truant and Mr. Jordan Koopmans (University of Northern British Columbia) for their hard
work, and excellent assistance under a variety of field conditions. Funding for this project was provided by the Canadian Forest Service and NSERC Discovery to BHA.
4.7 Figure Captions

Fig. 4.1. Mean (+ SE) catch of mountain pine beetle at six different treatments in 17 plots across central British Columbia, Canada, 16 July 2008 to 26 August 2008. For each treatment, means were calculated across 17 plots using a 5 d collection cycle. In all treatments, beetles were captured using 12-unit Lindgren funnel trap. Mountain pine beetle tree-baits consisted of a trans-verbolen bubble cap and an exo-brevicomin flex lure.
Table 4.1. Effects of mountain pine beetle population density, percent available pine, and percent available spruce on the likelihood of beetles being captured in traps with a pine vs. spruce log. Logistic regression equations are to be read across a row (e.g., likelihood of attraction to pine vs. spruce, both non-baited, as a function of population density, can be read as \[ y = 0.59 + 0.00152x \], where \( x \) is the number of mountain pine beetle captured in a blank control trap / 5 d period and \( y \) is subsequently back-transformed (\( e^y / (1 + e^y) \)) to obtain the probability of attraction to pine).

<table>
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<th>% Pine Available Estimate (SE)</th>
<th>% Spruce Available Estimate (SE)</th>
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* Lowest AIC values represent models with best fits within a subgroup of models.
4.8 Literature Cited


Hopkins (Coleoptera: Scolytidae), to five semiochemicals in British Columbia lodgepole pine forests. Canadian Journal of Forest Research 17:118-128.


5: RESEARCH CONCLUSIONS AND IMPLICATIONS

The recent mountain pine beetle epidemic in British Columbia has altered the forest landscape in a manner that has never before been witnessed in recorded history. The severity of pine mortality within central British Columbia has caused public concern and curiosity regarding the potential for the mountain pine beetle to become an important agent of mortality for remaining conifer species, specifically interior hybrid spruce (FRM, personal observation). The potential for subsets of the present mountain pine beetle population to cause sustained large-scale mortality of spruce hosts may be dependant upon at least three criterion; the development of spruce- rather than pine-associated preferences regarding selection and colonization of host material; the ability to reproduce within spruce hosts at levels sufficient to maintain beetle populations at densities required for mass-attack on healthy hosts; and the presence of environmental conditions conducive to promoting the utilization of spruce hosts. The potential of such factors to promote spruce attack by mountain pine beetles are discussed with respect to the results obtained in our study, in addition to relevant information present within the published literature.

The formation of host-adapted sub-populations of insects encountering novel hosts has been documented among some insect species (Bush 1969, Feder et al. 1988, Prokopy et al. 1988, Carroll and Boyd 1992, Craig et al. 1993, Brown et al. 1995, Barron 2001, Eubanks et al. 2003). Perhaps the most well-studied and notable of such occurrences are the derived and ancestral “host races” of the Rhagoletis flies (Diptera: Tephritidae) (Bush 1969, Feder et al. 1988, Prokopy et al. 1988). Host race formation in insects may occur when reduced transfer of genetic material between sub-populations utilizing different host species, e.g., through preferential mating, is combined with the preferential use of a given host over many
insect generations (Bush 1969). It has been suggested that speciation within the bark beetle genus *Dendroctonus* may have occurred historically as portions of populations attacked novel hosts during insect outbreaks, subsequently developing preferences and adaptations to such novel hosts, which led to the formation of host races and ultimately speciation events (Kelley and Farrell 1998). The current outbreak of mountain pine beetles within British Columbia, combined with the notable instances of interior hybrid spruce attack by this insect (Huber et al. 2009) may reflect past conditions that were conducive to host race formation among *Dendroctonus* spp. bark beetles.

It appears, however, that the conditions necessary for genetic divergence among spruce- and pine-associated populations of mountain pine beetles are unfavourable for the development of similar host races in central British Columbia at this time, as there is insufficient evidence to support preferential mate selection by males of females with conspecific natal host histories. As such, the potential for amplification of host-associated differences among populations of beetles from different natal hosts may not exist, providing such mate selection behaviour continues consistently across time. The longest reported use of a *Picea* host, (*Picea abies* (L.)) in consecutive years by mountain pine beetles was only three insect generations (Furniss and Schenk 1969). Such short-lived host associations are unlikely to greatly influence the genetic complement of the beetles, nor possibly the propensity for preferred host associations. Additionally, our results demonstrate that female mountain pine beetles reared from either a spruce or a pine host did not preferentially accept the host species matching their natal host, but preferred spruce logs relative to pine logs regardless of natal host. Without absolute fidelity between developmental and colonized host species in a portion of the breeding population, the probability of mountain pine beetle sub-
populations diverging as a result of host-associated differences seems unlikely. Although Hopkins (1917) states adult mountain pine beetles prefer to colonize the host species which they developed in, the evidence for this theory is inconclusive based on more recent studies (Richmond 1933, Baker et al. 1971, Langor and Spence 1991). Evidence of host effects on genetic variability within mountain pine beetles and other *Dendroctonus* spp. populations is likewise highly inconclusive (Stock and Guenther 1979, Stock et al. 1979, Stock and Amman 1980, Stock et al. 1984, Sturgeon and Mitton 1986, Langor and Spence 1991, Kelley et al. 1999, Kelley et al. 2000, Mock et al. 2007). Given the evidence provided within our study and within the published literature, it seems improbable that the mountain pine beetle will become host-adapted to interior hybrid spruce within central British Columbia.

The reproductive success of mountain pine beetles within spruce hosts appears to be a function of the interaction between host suitability (i.e., nutritional potential) and host susceptibility (i.e., efficacy of host defence system) (Raffa and Berryman 1983). In this context, spruce hosts may be suitable for mountain pine beetle reproduction, but often have defence systems that are not easily overcome in healthy individuals. Available evidence suggests that mountain pine beetle survival in spruce is possible given appropriate ecological conditions, such as physiological stress of the host (Smith et al. 1981), high beetle population densities (Wood 1982, Huber et al. 2009), proximity to attacked pine hosts (Unger 1993), or for reasons not entirely apparent (Furniss and Schenk 1969).

Manually inserted female colonizers abandoned interior hybrid spruce logs significantly more often than lodgepole pine logs. This suggests that the internal environment of interior hybrid spruce may be less suitable for mountain pine beetles. Under natural conditions, Huber et al. (2009) reported that the colonization density of mountain
pine beetles on standing interior hybrid spruce was significantly lower than that observed on nearby lodgepole pine. Similarly, Smith et al. (1981) reported poor gallery initiation on *P. abies* by mountain pine beetles. Fewer female colonizers may result in a subdued aggregation pheromone signal, fewer recruits, and, ultimately, a lower probability of host mortality and successful mountain pine beetle reproduction.

On a per female basis, however, we found that recruitment of conspecifics by female mountain pine beetles in spruce logs was significantly higher (*ca.* two-fold) than for females in pine. Because of this, fewer females within spruce logs attracted similar numbers of conspecifics as the more numerous females within pine logs, resulting in equal attraction on a per-log basis. The influence of host species on attraction of conspecifics has been demonstrated in the pine engraver system as well (Erbilgin and Raffa 2000, Erbilgin et al. 2003). The increased attraction of mountain pine beetles to females tunnelling in the field indicate that spruce tissue may be a superior substrate for mountain pine beetle pheromone production, or perhaps may contain compounds that synergize the pheromone signal in some superior manner.

Despite the similar overall attraction of colonized spruce and pine material, it appears that mountain pine beetle colonization efforts are severely hampered by the resin defence system of spruce. Successful reproductive efforts by mountain pine beetle in spruce are likely to continue to be rare events under natural conditions (Furniss and Schenk 1969, Smith et al. 1981, FRM personal observation). Even though many aspects of mountain pine beetle reproduction, such as per-female gallery length, were similar among interior hybrid spruce and lodgepole pine logs, brood production within spruce logs was significantly lower than within pine logs, averaging two offspring or less, regardless of natal host. These numbers...
approach the number of offspring required to sustain a mountain pine beetle population (Safranyik and Carroll 2006), suggesting that while it may be possible for an outbreak of mountain pine beetles to be prolonged by the use of spruce hosts (Furniss and Schenk 1969, Safranyik and Linton 1983, Huber et al. 2009), the overall lack of success of mountain pine beetle attack on spruce means that the degree to which an outbreak may be extended mould be limited. As previously stated, Furniss and Schenk (1969) witnessed sustained mountain pine beetle activity within standing Norway spruce over three years. Huber et al. (2009) noted that adult mountain pine beetle produced more brood per adult pair within standing interior hybrid spruce than did their pine-infesting counterparts. In other cases, however, such colonization attempts often fail outright (Smith et al. 1981, Huber et al. 2009).

There is an increase in the level of host discrimination with higher population densities of mountain pine beetles, predisposing the insects to be more attracted to pine vs. spruce hosts. At the same time, however, attraction to pine or spruce logs within forest stands in central British Columbia was positively correlated with the amount of pine and spruce in the surrounding stand. Therefore, in areas where mountain pine beetle populations are declining, available spruce within stands experiencing extreme mortality of lodgepole pine may be at an increased risk of being targeting for attack by dispersing mountain pine beetles as a result of a decline in pine host availability. This may be a new result for a bark beetle system, although some Lepidopteran species have demonstrated changes in host selection behaviour based on preferred host availability (Capinera 1985, Rausher 1985, Courtney and Forsberg 1988, Singer et al. 1989, Chew and Courtney 1991, Kuussaari et al. 2000, Singer and Stireman 2001).
Forest managers can expect some spruce mortality to occur in mixed pine-spruce stands that have been heavily attacked by the mountain pine beetle. However, due to the unpredictability of mountain pine beetle reproduction within interior hybrid spruce in British Columbia, forest health managers do not need to be concerned with this phenomenon occurring on a large scale. However, it would be prudent to manage instances of attack and colonization in order to minimize the potential for beetle spread into localized areas containing trees of aesthetic value, for example, on residential or municipal properties. For example, mountain pine beetles killed many of the mature spruce in a mixed pine-spruce stand campground at Lac Le Jeune near Kamloops, BC in 2006 (Maclauchlan et al. 2006). However, widespread spruce mortality in most stands seems unlikely to occur provided the remaining spruce are in good health and remain vigorous. Thus, it is not recommended, based on current information, that forest health managers expend a great deal of resources monitoring the spruce within lodgepole pine stands killed by mountain pine beetles.
5.4 Literature Cited


