Maintenance Of Endemic *Dendroctonus ponderosae* Populations Through Interactions With A Secondary Bark Beetle, *Pseudips mexicanus* 

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### ABSTRACT

Endemic *Dendroctonus ponderosae* are often found inhabiting weakened lodgepole pine (*Pinus contorta* var. *latifolia*) in western Canada previously attacked by the little known bark beetle *Pseudips mexicanus*. Interactions between the two species have not been elucidated, so three studies were conducted. The first examined the life history of *P. mexicanus*, describing developmental characteristics. The second explored the interaction between the two species and found that *D. ponderosae* attacking ephemeral hosts previously occupied by *P. mexicanus* do so in greater densities with earlier offspring emergence when compared to hosts *D. ponderosae* attacked alone. *P. mexicanus* may alter host chemistry, allowing *D. ponderosae* to be more successful when compared to trees *D. ponderosae* attacked alone. The third study investigated the attraction of *D. ponderosae* to *P. mexicanus*-produced volatiles, revealing no response by *D. ponderosae*. The scope of this work contributes to the understanding of bark beetle ecology in the lodgepole pine ecosystem.

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#### CHAPTER 1

#### GENERAL INTRODUCTION

Competition is generally accepted as the driving force in ecological theory (Kaplan and Denno 2007), as numerous decades of research have focused on it as the principal mechanism of interaction between species. Interspecific competition is commonly described as either interference (where one species reduces the other's ability to obtain resources through direct interaction) or exploitation (depression of both species when using a shared resource) (Gotelli 2001). Facilitation, on the other hand, is a positive interaction, where at least one of the species benefits without harming the other (Bruno et al. 2003). Competition has been the most commonly reported interaction, but recently plant and marine invertebrate studies have begun to show that facilitation is much more common than suspected (Callaway 1995, Stachowicz 2001). In insects, facilitation as a mode of interaction has not been identified regularly, however; recent work has suggested that interactions between phytophagous insects do not fit the general pattern of competition and that facilitation should be examined more rigorously (Kaplan and Denno 2007). Positive interactions have not been studied extensively in the bark beetles (Coleoptera: Curculionidae: Scolytinae), but some research has suggested that such interactions do occur (Amman and Schmitz 1988, Carroll et al. 2006).

To assess the implications of interactions between species, basic biological information is required for all involved. Although a vast number of organisms have been identified and named, life history descriptions are often lacking. This is especially true in the insect world, and can be seen in the bark beetles associated with lodgepole pine trees (*Pinus contorta* var. *latifolia* Engelm.). More than 50 species of bark beetles associated with lodgepole pine have

been identified in Canada, but not all have life history information available (Bright 1976). Life history studies examine traits such as attack dynamics, mating preference (i.e., polygyny versus monogyny), offspring behaviour, development rate and voltinism. Once established, these can be used to explain and possibly predict behaviours when interspecific encounters occur.

Bark beetles are integral to healthy forest ecosystems, contributing to the degradation of senescent or recently dead woody plant material, and they are often categorized by their host preference. Apart from the mycophagous ambrosia beetles, all species in the Scolytinae are primarily phytophagous, and a large proportion of them, the bark beetles, feed subcortically on conifers in the phloem region (Wood 1982). The majority of bark beetle species are 'secondary' (Wood 1982) and attack weakened or dying trees almost exclusively (Doliner and Borden 1984), with some species able to kill living trees when populations build up (Thomas 1961). 'Primary' beetles are the first species to arrive at, and successfully infest, healthy living hosts (Doliner and Borden 1984), but may have to subsist in weakened trees when populations are low. Primary beetles have the ability to increase rapidly in numbers during periods of host stress and to become severe pests (Wood 1982, Safranyik and Carroll 2006).

Initial host selection by bark beetles is conducted by so-called pioneer beetles that often orient to host volatiles, a behaviour termed primary attraction (Person 1931). Primary attraction has been studied extensively, e.g. for *Dendroctonus ponderosae* Hopkins (the mountain pine beetle). For this species it is generally accepted that pioneer females orient to host volatiles at long distances (Gara et al. 1984), recognize silhouettes and volatiles at short distances (Moeck and Simmons 1991, Campbell and Borden 2006a, b), and land on host trees at random coupled with gustatory sampling (Hynum and Berryman 1980, Raffa and Berryman 1982, Pureswaran and Borden 2003) to select their host tree. Upon selecting a suitable host, aggregation pheromones are produced attracting both mates and others of the same sex. These pheromones may also attract individuals of other species of bark beetles, predators and parasitoids (Poland and Borden 1994, 1997, Savoie et al. 1998, Aukema et al. 2004, Miller et al. 2005).

Bark beetles create ovipositional galleries in the phloem tissue, with some scoring of the sapwood. Galleries vary in length and orientation to the grain of the wood, and may be curved or linear. Eggs are laid along the sides of the gallery or in carved niches, singly or in groups. Larvae feed either gregariously or solitarily away from the ovipositional gallery and pupation occurs in the phloem or in the outer bark (Wood 1982). Many species feed as newly eclosed adults prior to emergence to build up resources required for dispersal and host selection (Wood 1982, McNee et al. 2000).

A pair of species was selected to examine how interspecific interactions affect bark beetles. *D. ponderosae* is found in lodgepole pine forests throughout the southern half of the province of British Columbia and the north-western United States, but is a major pest of pine trees across its range. A great deal of research has examined the epidemic population phase of this species (Safranyik et al. 1974, Berryman 1976, Klein et al. 1978, Thomson and Shrimpton 1984, Safranyik and Linton 1985, Safranyik 1988, Safranyik et al. 1999), but the endemic phase has had very little work conducted on it (Carroll et al. 2006). Because of this, there is a knowledge gap in *D. ponderosae* population dynamics, specifically, the effect that the presence of other bark beetle species has on endemic *D. ponderosae* and how this influences population growth. Endemic *D. ponderosae* populations tend to be too small to allow for successful attack on healthy hosts, leaving them confined to weakened trees often previously infested by other bark beetles (Carroll et al. 2006). Carroll et al. (2006) found that endemic *D. ponderosae* were directly associated with nine species of secondary bark beetles on the lower bole, the most common being *Pseudips mexicanus* (Hopkins). This beetle is native to British Columbia and is found sympatrically with *D. ponderosae* (Bright 1976), but little is known about it. The studies that do exist describe *P. mexicanus* in Monterey pine in California (Trimble 1924) and Mexican white pine in Central America (Schwerdtfeger 1956).

A study of the effects of the presence of *P. mexicanus* in weakened lodgepole pine trees on endemic *D. ponderosae* was conducted to determine whether *P. mexicanus* played a competitive or facilitative role in its interactions with *D. ponderosae*. To successfully scrutinize this interaction, in Chapter 2, I examine *P. mexicanus* life history characteristics (e.g. development rate, voltinism, attack density) in lodgepole pine. Chapter 3 investigates the effect of *P. mexicanus* on endemic *D. ponderosae* in weakened hosts using naturally attacked trees. Chapter 4 explores the mechanism used by *D. ponderosae* to locate hosts previously infested by *P. mexicanus* with trapping experiments using a variety of potentially attractive baits. The examination of the relationship between these two species of beetle will improve our understanding of how an eruptive herbivore maintains populations while in the endemic state and will close some knowledge gaps in the field of bark beetle ecology.

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#### CHAPTER 2

# THE LIFE HISTORY OF A SECONDARY BARK BEETLE, *PSEUDIPS MEXICANUS*, IN LODGEPOLE PINE

## ABSTRACT

*Pseudips mexicanus* (Hopkins) is a secondary bark beetle native to western North and Central America that attacks most species of *Pinus* within its range. A pair of life history studies examined *P. mexicanus* in other host species, but until now, no work has been conducted in lodgepole pine (*Pinus contorta* var. *latifolia*). *P. mexicanus* in lodgepole pine was found to be polygynous. Gallery lengths were shorter, offspring smaller, and the number of eggs laid per niche and the number of potential progeny fewer than in populations from California and Guatemala. The size of each larval instar was corrected from previous work. The development rate from the time of female attack to emerged adult offspring was less than 50 days at 26.5 °C and the heat accumulation required to complete the lifecycle was determined to be 889.2 degree days above 8.5 °C, indicating that *P. mexicanus* in the northern portion of its range is univoltine. Determination of these life history traits will allow discussion of interactions between *P. mexicanus* and other bark beetles with which it often cohabitates in weakened lodgepole pine.

Key words: sex ratio; development rate; voltinism; fecundity; Dyar's rule

#### INTRODUCTION

Numerous species of bark beetles (Coleoptera: Curculionidae: Scolytinae) are phloeophagus, feeding subcortically on a wide range of tree species. Some specialize on particular hosts, while others are generalists, often attacking all of the species within a genus or across a number of genera (Wood 1982). The majority of bark beetles attack weakened hosts, while some species successfully attack healthy trees and may erupt into large, often economically devastating epidemics (Wood 1982). Host defences can be altered by a number of intrinsic and extrinsic factors such as age, injury, pathogen infection and drought, all of which influence a tree's suitability as a bark beetle host (Shrimpton 1973, Raffa and Berryman 1983, Safranyik and Carroll 2006).

In Canada, different bark beetle species go through life cycles that range from bivoltine to semivoltine, depending upon latitude and elevation (Bright 1976). They may overwinter in the adult stage and emerge early the following summer or overwinter as juveniles and emerge later in the summer (Wood 1982). Upon emergence, bark beetles locate hosts through a combination of olfactory cues, visual recognition and gustatory sampling of host material (Hynum and Berryman 1980, Moeck and Simmons 1991, Campbell and Borden 2006). Depending on the species, either sex may be responsible for host location and initial attack. Upon selection of a suitable host, the pioneering sex releases aggregation pheromones, which usually attract both sexes (Wood 1982). In systems where the female initiates attack, she usually mates with a single male that may stay the duration of the egg laying period or may leave soon after copulation to mate with other females (monogyny) (Kirkendall 1983). Scolytinae species in which the male attacks first are usually polygynous, where multiple females join a single male (Kirkendall 1983).

Ovipositional gallery construction is conducted mostly in the phloem tissue, often with some scoring of the sapwood. Galleries vary in length and orientation to the grain of the wood, and may be curved or linear. Eggs are laid along the sides of the gallery or in egg niches, singly or in groups (Wood 1982). An egg niche is a small notch carved into the side of the ovipositional chamber in which eggs are laid and often packed in with frass (a mixture of wood fragments and feces). Larvae feed either gregariously or solitarily away from the ovipositional gallery, and pupation occurs in the phloem or in the outer bark (Wood 1982). Many species feed as newly eclosed adults prior to emergence to reach sexual maturity, and to build up resources required for dispersal and host selection (Wood 1982, McNee et al. 2000).

One of the approximately 50 species of bark beetles that use lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) as a host (Bright 1976) in western Canada is *Pseudips mexicanus* (Hopkins). *P. mexicanus* is native to the lodgepole pine ecosystem of central and southern British Columbia, but its range extends northwest into Alaska and south along the west coast of North and Central America into Guatemala (Bright 1976, Wood 1982). Typical of most bark beetles (Wood 1982), it attacks unthrifty trees (Trimble 1924) at low densities, and it has not been noted as an economic pest in Canada. *P. mexicanus* is often found cohabiting trees with endemic or low populations of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Chapter 3), an eruptive species currently in outbreak across British Columbia and parts of Alberta. Potential interaction effects between *P. mexicanus* and endemic *D. ponderosae* have not been investigated; however; to assess how two species interact with each other, life history knowledge must be available for both.

*D. ponderosae* has been studied extensively, especially in the epidemic phase (Amman and Cole 1983, Raffa and Berryman 1983, Safranyik et al. 1999, Safranyik and Carroll 2006). In contrast, the life history of *P. mexicanus* has been studied by only a few researchers. Trimble (1924) examined some aspects of the basic biology of *P. mexicanus* in Monterey pine (*Pinus radiata* D. Don), e.g., attacking sex ratio, gallery construction, instar head capsule sizes, the duration of development for each life stage, and the number of eggs laid per egg niche. Schwerdtfeger (1956) examined Guatemalan specimens in Mexican white pine (*Pinus ayacahuite* Ehrenb. ex Schltdl.), and determined the length of time required to develop from egg to adult. A Forest Pest Leaflet by Struble (1961) marginally expanded on Trimble's (1924) paper by commenting on predators, parasitoids and potential control measures. Fox et al. (1991) examined the potential for *P. mexicanus* to vector pitch canker fungus (*Fusarium subglutenans* (Wollenw. and Reink.) Nelson, Toussoun and Marasas) in Monterey pine.

The objective of this study was to elucidate the life history characteristics of *P*. *mexicanus* in lodgepole pine trees in British Columbia. A field survey was conducted to examine attack and ovipositional characteristics, and a complementary laboratory study examined the developmental characteristics of *P*. *mexicanus*, voltinism and the number of larval instars. The determination of these life history traits will provide the necessary knowledge to allow an examination of how *P*. *mexicanus* interacts with other species, such as endemic *D*. *ponderosae*. Additionally, the study will ascertain whether the biology of *P*. *mexicanus* in lodgepole pine trees differs from populations inhabiting other pine species further south.

#### METHODS

#### Field study

To study the attack and ovipositional characteristics of *P. mexicanus*, infested trees were located in 2005 by conducting a detailed survey of seven lodgepole pine stands totalling 28 ha. The stands were either pine dominant or co-dominant, located at Angstad Creek ( $49^{\circ}$  51' N, 120° 46' W, mean elevation = 1300 m), 28 km south of Merritt, British Columbia. Pine dominant stands are characterized by a pine component of > 50 % of tree species present, and individual pine trees extending above the crowns of other species, while in pine co-dominant stands the largest pines are of similar height as surrounding trees species (Avery and Burkhart 2002). Diameter at breast height (DBH), phloem thickness and tree age were collected by randomly placing one variable radius plot in each stand. A basal area factor 5 prism was used in all plots. For all pine trees, phloem thickness at DBH was recorded as the mean of two measurements to 0.1 mm made on the bole at 90° from each other. Phloem was removed using a 1.5 cm diameter punch.

*P. mexicanus*-infested trees were identified by the fine grained mixture of reddish boring dust on the trunk at the base of the tree. Seventeen *P. mexicanus*-infested trees were located, and their DBH, phloem thickness and age were determined along with an assessment of damage (physical injury or infection) (Carroll et al. 2006). The infested trees were harvested in September 2005, and the bark was peeled between 1.0 m and 1.5 m from the base to check for the presence of *P. mexicanus*. As beetles were not noted above 1.0m, the bottom 1.0 m of the bole of each tree was taken to the laboratory in Victoria BC. The ends of each log were sealed with hot paraffin wax to reduce desiccation and then stored at 4  $^{\circ}$ C until they were examined in detail. In March 2006 the logs were placed in individual rearing cages enclosed

in mesh in a greenhouse chamber kept at 20 °C, so the resident beetles could complete their lifecycle. Emerging beetles were collected daily and placed in 70 % ethyl alcohol for storage. *P. mexicanus* were sorted by sex using the large median tubercle present on the male frons and its absence or reduction on the female frons (Wood 1982) as the identifying characteristic. The pronotal width of each insect was measured to the nearest 0.1 mm using a dissecting microscope equipped with an ocular micrometer. Other beetle species (*D. ponderosae, Orthotomicus latidens* (LeConte), *Hylurgops porosus* LeConte and *H. rugipennis* (Mannerheim)) were also collected as they emerged and identified (Bright 1976).

After the emergence of beetles was complete (no emergence in any cage for > 5 consecutive days) the logs were peeled and the galleries of all species were inspected, but only those of *P. mexicanus* were examined in detail. During peeling, the sites of attack were examined for pitch exudation and evidence of necrotic lesions, the presence of which would indicate a defensive response by the tree upon attack (Reid et al. 1967, Nebeker et al. 1993). The galleries of other species were identified using descriptions in Bright (1976) and Wood (1982). A total of 81 attacks on the 17 logs (mean =  $7.0 \pm 1.3$  (SE) attacks/log) were assessed, and the shape of the gallery system assessed. Gallery systems were categorized as follows: 'amorphous', numerous gallery arms with no evidence of egg laying (Fig. 2.1A); or 'ovipositional', at least one gallery arm present with egg niches (Fig. 2.1B). The presence of a nuptial chamber, the number of ovipositional gallery arms extending from the nuptial chamber, ovipositional gallery length, and density of egg niches were recorded for ovipositional galleries only. The number of eggs laid per niche was determined by counting the number of larval traces moving away from each of three randomly selected egg niches



Figure 2.1. (A) Amorphous gallery of *Pseudips mexicanus* typical of overwintering beetles. Note lack of egg niches and apparent randomness in gallery length and direction. (B) Ovipositional gallery of *Pseudips mexicanus*. Note distinctive shape of the gallery and the presence of egg niches on the outside of the curve. NC = nuptial chamber, EN = egg niche.

from 12 randomly selected galleries. Potential progeny was calculated by multiplying the mean number of eggs laid per niche by the mean number of niches per gallery. The sex ratio of attacking adults was not quantified exactly, as not all gallery systems had adults present when the logs were dissected. However, the sex ratio of attacking adults was estimated by assuming one female created each gallery arm as suggested by Trimble (1924). One male was assumed present if a nuptial chamber was evident. The sex ratio of offspring was determined by counting the number of each sex that had emerged.

Additional qualitative observations of gallery shape were made in early May 2005 on ten trees mass attacked by *D. ponderosae* the previous year and subsequently attacked by *P. mexicanus* in late 2004 (post-*D. ponderosae*). Trees mass attacked by *D. ponderosae* were identified by the red crown and pitch tubes on the bole (Safranyik and Carroll 2006). Fresh boring dust was used to locate *P. mexicanus* attacks and the lower 1.0 m of the bole was peeled *in situ* to reveal gallery systems. Four amorphous galleries with beetles present were observed on three of these trees. A number of ovipositional galleries were also present but details were not noted.

#### Laboratory study

Life history characteristics of *P. mexicanus* were determined by rearing insects in lodgepole pine logs at different temperatures. The study was conducted in 2004 and repeated in 2005. Source (parent) beetles used in the study were reared out of logs cut from infested trees at Angstad Creek as described above or collected from Lindgren funnel traps (Lindgren 1983) baited with racemic ipsenol and ipsdienol (Phero Tech, Inc., Delta, BC) hung at Angstad Creek and Aberdeen Plateau (50° 5' N, 119° 11'W, elevation approximately 1300

m). Additional beetles were manually collected from the base of *D. ponderosae*-infested trees at Angstad Creek. Beetles were maintained alive until needed in 120 ml capped vials containing moist paper towel and were used within eight days of emergence or collection.

Two healthy lodgepole pine trees were felled early in the summer of 2004, and three trees were felled in 2005 at Angstad Creek. Each tree had a diameter at breast height of approximately 20 cm. The lower three metres of the bole was removed and cut into 20 cm long logs. The bark was scored vertically to the sapwood in a one centimetre wide strip on opposite sides of the log to create two separate resource patches. Hot paraffin wax was applied to all cuts to reduce desiccation. Each side of the scored log was manually infested with *P. mexicanus* using the gelatine capsule technique (Lanier and Wood 1968). One male was introduced into an entrance hole prepared in the centre of each side and left overnight (Safranyik and Linton 1983). Beetles which did not initiate attack after 24 hours were replaced. Once the male beetle had successfully entered the log, two females (Trimble 1924) were placed in the gelatine capsule. Females that did not enter logs after 24 hours were replaced. After successful beetle introduction, logs were placed in individual 20 L plastic buckets, each ventilated by a 5 cm diameter hole covered with 0.25 mm polyethylene mesh, and fitted with a 20 ml scintillation collecting vial.

Nine rearing buckets were placed into each of three walk-in growth chambers (Conviron PGV36, Controlled Environments Ltd., Winnipeg, Manitoba, Canada) in 2004 and five growth chambers in 2005. In 2004 the chambers were set at constant temperatures of 20.0, 23.5 and 28.8 °C and in 2005 at 14.1, 20.5, 23.4, 28.9 and 33.6 °C. Subtle variation in chamber temperature was due to differences between individual chambers. Temperatures were determined by placing a data logger ((HOBO<sup>®</sup> H8 Pro Temp, Onset Computer

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Corporation, Pocasset, MA, USA) in each chamber for the period of the study and temperatures were maintained at  $\pm$  1.5 °C with ambient humidity. A light:dark regime of 16:8 h was used to encourage emerging beetles to enter the collecting vials. Buckets were checked every five days until the first beetle appeared in a collection vial, after which time they were checked daily. Collected beetles were placed in 70 % ethyl alcohol.

Development rate. The developmental characteristics of *P. mexicanus* were assessed by recording the amount of time it took *P. mexicanus* to develop from the time of introduction of female beetles to the logs until emerging adults appeared in the manually infested pine logs at different temperatures. From this information, the minimum threshold and optimum temperature of development were determined and the development rate at each temperature was calculated by taking the reciprocal of the mean length of time in days (Campbell et al. 1974). The mean length of time for development from infestation to adult was estimated by counting the number of days between the time of beetle introduction to the logs and the first day of emergence of greater than four beetles per temperature. The minimum temperature was estimated by finding the maximum of a quadratic curve (see Analysis below).

*Voltinism.* Voltinism of *P. mexicanus* was determined by calculating the number of degree days required by *P. mexicanus* to complete its lifecycle from infestation to adult and comparing with temperature data from Environment Canada weather data (Environment Canada 2007) and data loggers (see below). The minimum and the optimum temperature of

development were used in equation (1) to determine the number of degree days required for *P. mexicanus* to fully mature and emerge:

$$dd = D(OT - MT) \tag{1}$$

where dd = number of degree days; D = number of days required for development from infestation to adult; OT = optimum temperature of development (°C); MT = minimum temperature of development (°C) (Snyder et al. 1999). The calculated number of degree days was compared with the total number of available degree days over a period of one year at two different locations. Environment Canada weather data were used from the Merritt STP station (50° 6'N, 120° 48'W, elevation 588 m) between June 1, 2004 and May 31, 2005. A second set of data were collected at Angstad Creck with HOBO temperature data loggers from July 2003 to June 2004. The Merritt site was chosen as the closest permanent weather station with current data. If the calculated number of degree days was less than the total available number of degree days at a particular station then *P. mexicanus* can be described as univoltine. If the calculated number of degree days was greater than the available number of degree days, then *P. mexicanus* could not complete one generation per year (semivoltine). *P. mexicanus* developing in fewer degree days than half of the available degree days at a weather station would indicate a potential multivoltine population.

*Instars. P. mexicanus* larvae were collected from the manually infested logs to determine the head capsule width of each instar. During both 2004 and 2005 one log per growth chamber temperature was periodically chosen at random and dissected to assess developmental progress. Larvae were collected from all peeled logs from the 23.5 °C chamber in 2004 for head capsule measurement. This temperature was chosen to ensure that all larval stages were present at the time of log dissection. At other temperatures, eggs may not have hatched or larvae had progressed into a later life stage at the time of the dissections, resulting in one or more missing instars. Head capsule widths and additionally, the number of larval instars, were determined by examining 195 larvae (Prebble 1933).

### Data analysis

*Field study.* Data not meeting assumptions of normality and homoscedasticity were transformed by  $Y = log_{10} (X + 1)$ . The characteristics (DBH, phloem thickness and age) of infested host trees were compared to healthy trees sampled in the prism plots using an independent samples *t*-test. Gallery characteristics were measured in the naturally infested trees and the means ( $\pm$  SE) reported. An independent samples *t*-test was used to detect differences in pronotal widths between the males and females collected from the naturally infested trees in the field. A log-likelihood ratio test (G-test) was performed to assess divergence of observed sex ratios in wild populations of both attacking adults and emerged offspring from the ratios reported in the literature.

*Laboratory study.* A regression analysis of development rate as a function of temperature was done. The development rates calculated at six temperatures (not including temperatures where beetle survival was zero) were used to estimate the optimum temperature of development for *P. mexicanus* by determining the maximum point of the curvilinear model fit to the data. Simple linear regression was used with rate and temperature to estimate the lower threshold of development through extrapolation of the model to Y = 0. Only temperatures through which development rate increased approximately linearly, and

excluding temperatures higher than would be normally encountered by the insect in nature, were included in this model (Gilbert and Raworth 1996). Larval head capsule widths were grouped graphically by examining natural breaks in the data, and the rate of change between instars was compared with previous research. All tests followed Sokal and Rohlf (1995) and were conducted using SPSS 13.0 (SPSS 2004) with  $\alpha = 0.05$ .

#### **RESULTS AND DISCUSSION**

#### Field study

Typical of Ipini bark beetles (Scolytinae), P. mexicanus focus their attack on suppressed, weakened or recently killed trees (Wood 1982). Observations of the 17 trees harvested and brought to the laboratory in 2005 revealed that the diameter at breast height was significantly smaller (t = 3.70, df = 59, P < 0.001) and phloem thickness was significantly thinner (t =4.03, df = 59, P < 0.001) for trees infested with *P. mexicanus* than for the healthy trees in the prism plots. Age was not significantly different (t = -1.66, df = 59, P = 0.10) (Table 2.1) in attacked versus unattacked trees, however. All trees with P. mexicanus attack had sustained physical injury in the past (broken tops, bole scars) or were infected by lodgepole pine dwarf mistletoe, Arceuthobium americanum Nutt. ex Engelm. (see Chapter 3). Inspection of the pine logs revealed no evidence of host defence in the form of resinous pitch tubes or necrotic lesions under the bark associated with P. mexicanus attack sites, confirming that the selected hosts had weakened defences at the time of attack. Trees which are capable of resistance should react to the presence of *P. mexicanus* by exudation of resin caused by physical trauma to resin canals and the production of induced lesions in the phloem and xylem tissues, a response to the inoculation of *P. mexicanus* fungal associates as seen in other bark beetle systems (Berryman 1972, Shrimpton 1973, Paine et al. 1997). Dissection of the logs taken to

**Table 2.1.** Characteristics of lodgepole pine trees (*Pinus contorta* var. *latifolia*) assessed by variable radius plot from seven stands at Angstad Creek, BC., and individual trees attacked by *Pseudips mexicanus*.

Mean $\pm$ SE	
<i>P. mexicanus</i> -infested trees $(n = 17)$	Stand $(n = 42)$
15.7 ± 0.9 a	$21.3 \pm 1.0$ b
$1.0 \pm 0.1 a$	$1.4 \pm 0.1 \text{ b}$
118.1 ± 1.9 a	111.5 ± 2.3 a
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Note: Within each row, means followed by the same letter are not significantly different from one another (independent samples t-test,  $\alpha = 0.05$ ).

the laboratory, as well as observation in the field of the portion of the bole 1.0 m to 1.5 m on each tree, revealed that *P. mexicanus* prefer the lower 1.0 m of suppressed lodgepole pine trees, 1.0 m of suppressed lodgepole pine trees, as no attack was noted above that point. Thus, *P. mexicanus* attack behaviour on lodgepole pine in British Columbia differs from that of California beetles (Wood 1982), which in *P. radiata* were found thriving in slash (Trimble 1924) as well as in the boles, branches and cones of trees infected with pitch canker fungus (Fox et al. 1990). Schwerdtfeger (1956) also found *P. mexicanus* in the bole, branches and slash of Mexican white pine, *P. ayacahuite*. The variation in attack behaviour may be the result of different trophic interactions, such as competition and symbiotic relationships, or climatic factors within the different ecosystems. The propensity of *P. mexicanus* to attack low on the bole in lodgepole pine may be an overwintering adaptation of northern populations. Snow often covers the lower trunk, insulating it and possibly reducing risk of cold-induced mortality.

*P. mexicanus* was found cohabiting with a number of other bark beetle species such as endemic *D. ponderosae*, *O. latidens*, *H. porosus* and *H. rugipennis*. Most of these species, except for *O. latidens* and endemic *D. ponderosae*, attacked at very low densities (< 2 attacks per tree on average) and were only present in a few trees. The attack density of *P. mexicanus* (Table 2.2) was lower than that of endemic *D. ponderosae*, which was present on approximately 37 % of the trees at a mean density of 55 attacks/m<sup>2</sup>. *O. latidens* was present on approximately 50 % of the trees at densities slightly greater than *P. mexicanus*. *O. latidens* were normally found higher on the bole than *P. mexicanus* and appeared to prefer more deteriorated phloem tissue (Miller and Borden 1985), while the *Hylurgops* species were

**Table 2.2.** Mean attack and ovipositional gallery characteristics ( $\pm$  SE) for wild populations of *Pseudips mexicanus* reared from naturally attacked lodgepole pine trees.

	<u> </u>		
Characteristic	Mean $\pm$ SE	Range	N
Attack density (attacks/m <sup>2</sup> )	$17.0 \pm 3.6$	1.9 - 46.8	17
Number of galleries per attack	$1.5\pm0.06$	1.0 - 3.0	81
Gallery length (cm)	$5.4 \pm 0.2$	1.1 - 17.0	125
Number of egg niches per gallery	$7.2\pm0.4$	1.0 - 23.0	125
Number of egg niches per cm of gallery	$1.4 \pm 0.07$	0.2 - 5.6	125
Number of eggs per niche	$3.5\pm0.2$	1.0 - 4.0	36
Potential progeny	$25.2 \pm 0.1$		

found lower on the bole than *P. mexicanus*, at or below the root collar. Trees with endemic *D. ponderosae* did not have *O. latidens* present, although *O. latidens* attacks may have been present higher on the bole. *D. ponderosae* tended to attack slightly above, but never overlapping, *P. mexicanus* gallery systems. It was not possible to determine which of the secondary species arrived first at these trees, but it appeared that all were spring attacks of the same year, while endemic *D. ponderosae* attacks followed *P. mexicanus* infestation.

Observation of gallery systems during the spring field surveys found four amorphous P. mexicanus galleries in three trees mass attacked by D. ponderosae (Fig. 2.1A). These types of galleries have also been noted in weakened hosts, not attacked by D. ponderosae (A. Carroll, unpubl. data). Each of these four galleries had four or more beetles, and none had any evidence of egg laying at the time of observation. *P. mexicanus* has been found to fly in low numbers throughout the summer, with a peak in late May and early June, and a smaller peak in early to mid-August (A. Carroll, unpubl. data). Similar late summer flights occur in a number of secondary species (Ayres et al. 2001). The purpose of this late flight is unclear, but it may be used to establish a secondary gallery or to find suitable sites for overwintering in hosts with available resources. Some bark beetle species, e.g., D. ponderosae, overwinter under the bark, while others, e.g., *Ips pini* (Say), emerge and drop into the duff at the base of their natal tree (Thomas 1961, Safranyik et al. 1996). P. mexicanus has not been found to overwinter in the duff (Safranyik et al. 1999) and so likely overwinters under the bark. Overwintering in a host with resource still available could be advantageous, as re-attack rather than spring dispersal could occur, reducing mortality risk. Dispersal in search of new hosts is very costly (Amman 1984), as beetles must be able to locate a suitable host, avoid predation and attract mates. The opportunity to ameliorate these risks would be beneficial.

All of the 81 *P. mexicanus* gallery systems observed on the 17 naturally infested trees brought to the laboratory were ovipositional (Fig. 2.1B). Nuptial chambers were associated with 97.5 % of the ovipositional gallery systems, indicating that the majority of the time a male initiated attack (Miller and Borden 1985). The galleries lacking a nuptial chamber indicate the lack of a male and may have been started by a female which had been previously mated in an earlier, but unsuitable gallery that she abandoned (Wood 1982). Alternatively, mating may have occurred prior to leaving the natal tree. *P. mexicanus* has the opportunity to engage in sibling mating prior to emergence, as, like many other scolytids (McNee et al. 2000), *P. mexicanus* undergo a period of pre-emergence feeding as callow adults, often in groups. This phenomenon of sib-mating has been noted in a number of other bark beetle species, but not in any Ipini (Kirkendall 1983), suggesting that sib-mating is somehow avoided by *P. mexicanus*, as it is in *I. pini* (Domingue and Teale 2007). Therefore, solitary galleries without nuptial chambers strongly suggest the presence of females that had mated during the construction of previous galleries and re-emerged rather than sib-mated females creating ovipositional galleries for the first time.

The ovipositional gallery in systems where only one gallery was associated with the nuptial chamber were either straight or curved. Egg niches in straight galleries were on either side, while niches in the curved galleries were on the outside of the curve (Fig. 2.1B). In systems where two galleries were present, the galleries were curved away from each other often in an 'S' formation with the nuptial chamber at the centre and egg niches on the outside of the curves as reported by both Trimble (1924) and Schwerdtfeger (1956). Food is often a limiting factor for larvae (De Jong and Sabelis 1988). The shape of the gallery system when two ovipositional galleries are present is likely an adaptation to reduce competition among

offspring and improve access to host material (Trimble 1924). The 'S' shape permits larvae to fan out away from the ovipositional gallery without having to come into contact with larvae from the other gallery. Although the 'S' shape likely reduces intraspecific interactions, it does not eliminate them. Cannibalism was observed in two cases during log dissections where a larger larva altered the direction of its gallery. In both instances, these larvae turned into neighbouring galleries and partially consumed the inhabitants prior to moving on.

The mean number of individual ovipositional galleries associated with each nuptial chamber (Table 2.2) was slightly less than that reported by Trimble (1924) and Schwerdtfeger (1956), both of whom stated two galleries were the most common. The mean of 1.5 galleries per attack that I found was significantly less (G = 9.47, df = 1, P < 0.05) and suggests an attacking sex ratio of approximately 1.5 females per male, assuming one female was present to create each gallery. Although females in some species have been observed creating multiple galleries per nuptial chamber (e.g., *O. latidens*, Reid 1999, Miller and Borden 1985), my observations could not verify this phenomenon, as parent adults were not always present when logs were peeled.

*P. mexicanus* was categorized as a polygynous species by Trimble (1924) and Schwerdtfeger (1956), but as monogynous by Bright and Stark (1973). Monogynous scolytid species in which the male initiates gallery construction are rare, while polygyny is very common within the Ipini, which includes *P. mexicanus* (Wood 1982, Kirkendall 1983). I found that > 50% of the gallery systems examined on trees naturally attacked by *P. mexicanus* had at least two radial arms, suggesting that this species is polygynous, but does not appear to be obligate. This finding and results from a trapping experiment that found five
times more females than males being attracted to *P. mexicanus* attractant pheromone (unpubl. data), correspond strongly to Kirkendall's (1983) definition of polygyny which states that: 1) more than one female will join a male in a gallery system; and 2) males produce an aggregation pheromone attractive to both sexes, but usually more attractive to females. Additionally, males of polygynous species tend to be the larger sex (Kirkendall 1983), and *P. mexicanus* males were on average 9.4% wider across the pronotum than females (Fig. 2.2). Male *P. mexicanus* offspring in lodgepole pine were significantly larger than females ( $t_{361} = -10.477$ , *P* < 0.001), with mean pronotal widths ( $\pm$  SE) of 1.7  $\pm$  0.1 mm and 1.6  $\pm$  0.1 mm respectively (Fig. 2.2). Males ranged in pronotal width from 1.4 to 2.0 mm and females from 1.4 to 1.9 mm.

At a mean length of 5.4 cm, galleries in lodgepole pine were shorter (Table 2.2) than in other species reported by Trimble (1924) and Schwerdtfeger (1956), where they averaged 6.5 cm and 9.0 cm, respectively. The mean number of egg niches per gallery in this study (Table 2.2) was less than half that reported by Schwerdtfeger (1956), who found a range of 16 to 20 niches per gallery. Egg niche density (number of egg niches per cm of gallery) was also smaller than either Trimble (1924) or Schwerdtfeger (1956) reported with densities of 2.0 niches/cm and 3.5 niches/cm respectively. The mean number of eggs per egg niche was again less than that found in the previous studies, where 4.0 was the norm (Trimble 1924, Schwerdtfeger 1956). The difference between the number of eggs laid per egg



Figure 2.2. Mean pronotal widths (mm  $\pm$  SE) of *Pseudips mexicanus* by sex reared from naturally infested lodgepole pine trees. Different letters above bars indicate significant differences (P < 0.05).

niche in my study and that reported by others may be a result of counting larval traces instead of eggs. I may have introduced error and lowered the mean number of eggs per niche by not accounting for occasional egg mortality prior to hatch.

The potential progeny produced per female was based on the mean number of eggs per niche and the mean number of niches per gallery, giving approximately 25 eggs per female in this study. Trimble (1924) reported 90 eggs laid per female, while Schwerdtfeger (1956) found 64 to 80. The large differences may be the result of (i) warmer temperatures and (ii) larger beetles in the southern populations. Amman (1972) found that D. ponderosae creating ovipositional galleries at higher temperatures had greater rates of egg production. Beetles in the south likely experience warmer temperatures, suggested by the higher egg niche density found in Mexican white pine (Schwerdtfeger 1956) than in my study. Beetle size significantly affects the egg-laying capacity, with larger beetles able to lay more eggs (McGhehey 1971). Larger size also confers a greater ability to disperse and locate new hosts, and energetic requirements for the attacking sex are usually greater than for the sex responding to aggregation pheromones (Pureswaran et al. 2006). Beetles from populations in Mexican white pine were reported to be larger (Schwerdtfeger 1956) than the ones I found in lodgepole pine, possibly contributing to the greater reproductive potential. Wood (1982) reported that P. mexicanus tend to be 2.5 times longer than wide. Using this ratio and length measurements, Wood's (1982) pronotal width range (no sex distinction) is 1.4 mm to 2.0 mm, almost identical to that of the males in my study. Similarly, Schwerdtfeger (1956) reported a pronotal range, again with no sex distinction, of 1.6 mm to 2.2 mm, with a minimum that was 10.6 % greater than the minimum I found for females, and a maximum

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10.7 % greater than for males in lodgepole pine. The female size difference could account for the greater egg production by southern beetles.

Many of the *P. mexicanus* galleries observed in lodgepole pine had stained phloem, but only moderate staining of the underlying wood was noted. This indicates the presence of a potentially phytopathogenic fungus associated with P. mexicanus, as suggested by Struble (1961), who observed staining of the sapwood as a result of fungal inoculation by P. mexicanus attacking Monterey pine trees. Numerous bark beetles have fungi associated with them (reviewed in Six 2003). P. mexicanus pupal chambers were noted to contain white fungal spores and it appears that *P. mexicanus* teneral adults consume these spores, as they were not detected in chambers with teneral beetles present. Spore consumption is known to take place in other species (Whitney 1971). The spores I observed are likely of the fungal species associate, Leptographium terebrantis Barras & Perry (K. Bleiker, S. Massoumi-Alamoutim, G. Smith, A. Carroll and C. Breuil 2007, unpubl. data), which has recently been isolated from the exoskeleton of P. mexicanus freshly excised from naturally attacked lodgepole pine. This fungus is associated with a number of other bark beetles species (Six et al. 2003) and is highly phytopathogenic in pine trees (Eckhardt et al. 2004). In addition to being detrimental to the host, evidence has been presented that some fungi confer a nutritional benefit to the associated beetle species when individual teneral adults ingest the spores (Ayres et al. 2000, Bleiker and Six 2007). The presence of this fungus may be more beneficial to secondary bark beetles as a nutritional supplement than as an aid to reduce host defences.

The sex of a total of 296 freshly emerged beetles was determined. The common sex ratio of one female per male found in *Ips* species (Miller and Borden 1985) was used for comparison purposes, as *Ips* are closely related to the small genus *Pseudips* (Cognato 2000). The resulting sex ratio for emerging offspring of 1.6 females/male was significantly greater than the expected ratio (G = 13.36, df = 1, P < 0.001). Cole (1973) suggests that beetles breeding at low densities should produce offspring of a sex ratio close to 1:1. As crowding increases, the smaller sex is reduced in number. Crowding is likely not occurring with *P. mexicanus* as attack densities are quite low, but if crowding were a limiting factor, the sex ratio should be biased toward the males, the larger sex (Safranyik 1976). While cold is normally a factor in differential survival, the beetles in this study did not experience temperatures below 0 °C, as the logs were collected in the fall prior to frost. It is possible that the parent beetles are preferentially selecting female offspring when laying eggs, however this behaviour is only common is species which inbreed (Borsa and Kjellberg 1996, West et al. 2005), and I have suggested that sibling mating does not occur in *P. mexicanus*. The difference between observed and expected sex ratios may be the result of using species of a different genus as a standard for comparison.

## Laboratory study

Development rate. In the 28.9 °C and 33.6 °C chambers all parent beetles died in 2005 prior to oviposition and consequently no data were collected. It is not completely clear why the beetles in the 28.9 °C chamber died, when beetles in the 28.8 °C chamber from 2004 successfully produced numerous offspring. Temperature and humidity traces from the 28.9 °C chamber did not indicate any erratic behaviour of the chamber, but upon examination, the parent beetles were found to be covered in an unidentified white fungus. One possible cause of the rapid mortality of these beetles was the residual presence of an entomopathogenic

fungus from a previous study not eliminated during sterilization of the chamber. The 28.9  $^{\circ}$ C data were not used in the models, but the 33.6  $^{\circ}$ C data were assumed to be accurate.

The development rates of insects tend to follow similar patterns of nearly linear increase followed by a peak and then rapid decrease (Wagner et al. 1984, Logan and Powell 2001). The apparent decrease in the rate of development for *P. mexicanus* at 28.8 °C (Fig. 2.3) indicates that this species does not tolerate temperatures of this magnitude well in at least one of its life stages. Beetles in the 33.6 °C environmental chamber may have died due to heat stress or desiccation of the study logs, and it is assumed that this represents the rapid drop-off to zero development that would be expected. A quadratic curve was fitted to the temperature data that had beetles surviving from both 2004 and 2005. The peak of the curve is the optimum temperature at which development rate is highest (Trudgill et al. 2005). In my study, this point was approximately 26.5 °C at a rate of 0.0198 d<sup>-1</sup> (Fig. 2.3). By taking the inverse of this rate, the optimum number of days required for *P. mexicanus* to develop from infestation to adult was determined to be 50.5 days at a constant temperature of 26.5 °C. This model (Fig. 2.3) appears to be a fairly good predictor for the species. Schwerdtfeger (1956) found that P. mexicanus in Mexican white pine took a period of 12 to 14 weeks at 17 °C to complete their life cycle. By solving the equation in Figure 2.3 for X = 17 °C and taking the reciprocal, the model predicts it would take 11.4 weeks for complete development, which is fairly close to Schwerdtfeger's (1956) results. Trimble (1924) reported development being completed in nine weeks but did not state a temperature at which this occurred. Faster development in northern climates has been shown for other species as an adaptation to shorter developmental periods dictated by cooler summers (Bentz et al. 2001) The development rate for P. mexicanus within the range of temperatures normally encountered in



**Figure 2.3.** Development rate  $(d^{-1})$  of *Pseudips mexicanus* from infestation to fully mature adult versus temperature. Quadratic curve applied to determine optimum temperature of development (arrow). Open circles indicate data collected in 2004; solid circles indicate data collected in 2005. Error bars represent 1 SE of the mean.

the environment is shown in Figure 2.4. The minimum temperature of development, the lowest point at which metabolic activity contributes to growth and development (Trudgill et al. 2005), was estimated to be 8.5 °C. Assessment of beetles reared at 23.5 °C by periodic peeling of logs showed that teneral adults were present after 45 days, but no emergence occurred until midway through day 48. Based on this, it appears that *P. mexicanus* can complete maturation feeding, commonly conducted by many bark beetles (Wood 1982), in less than 4 days and be prepared to emerge.

*Voltinism.* Degree day calculation can be made for each separate immature life stage or for the whole life cycle from infestation to emerging adult (Bentz et al. 1991, Bonhomme 2000, Logan and Powell 2001). In my study, development from the time of infestation to adult was modelled. From equation (1), the number of degree days required for complete development at 26.5 °C was calculated to be 889.2 degree days above 8.5 °C. At the Merritt STP weather station, 1662 degree days were available between June 1, 2004 and May 31, 2005. At higher elevation within the Angstad Creek watershed a minimum of 982 degree days were available between July 2003 and June 2004 for *P. mexicanus* development. The number of degree days required for one generation of *P. mexicanus* corresponds closely with the number of degree days available at Angstad creek, indicating populations there are univoltine. In the arid valley near Merritt, *P. mexicanus* may have 1.5 generations per year, but not two; however, at that site, *Pinus ponderosa* P. Laws. Ex C. Laws. is the dominant cover type.

Univoltine and multivoltine lifecycles may benefit *P. mexicanus* in both northern and southern populations as they allow for a more rapid population growth compared to



Figure 2.4. Minimum temperature of development (arrow) extrapolated from regression of temperatures within normally encountered conditions of *Pseudips mexicanus*. Open circles indicate data collected in 2004; solid circles indicate data collected in 2005. Error bars represent 1 SE of the mean.

semivoltine populations, and the shorter development time may reduce the potential effect of mortality agents, e.g., cold and predation, which may manifest themselves more during a longer developmental period (Hansen and Bentz 2003). The rapidity with which *P. mexicanus* can progress from infestation to adult at high temperatures suggests that it is well adapted to produce more than one generation in regions which have longer summers, as shown by Struble (1961), where three generations are common in California. Although degree day estimations are often calculated for pest insect species to establish timing of control measures to coincide with susceptible life stages, *P. mexicanus* has not been the subject of any such practices. However, it is important to know the number of generations that *P. mexicanus* is capable of producing per year so evaluation of interactions with other species can be made (see Chapter 3).

#### Instars.

The head capsule widths of *P. mexicanus* larvae were reported by Trimble (1924), but it appears an error occurred either in his measurements or during the printing of his findings, where the same head capsule width is listed for both  $3^{rd}$  and  $4^{th}$  instars (Table 2.3). I confirmed that *P. mexicanus* does have four instars (Fig. 2.5) and report the correct head capsule width for each (Table 2.3). Another indication of the possible error in Trimble's (1924) data was found by comparing the rate of change from one instar to the next between our data and those of Trimble (1924). Out data revealed a linear progression as was expected (Dyar 1890), while Trimble's data were non-linear (Table 2.3), not following the regular geometric progression described by Dyar (1890). Dyar (1890) found that for Lepidoptera, the ratio of the head capsule width of any instar to that of the next instar should be a constant



**Figure 2.5.** Histogram of *Pseudips mexicanus* larval head capsule widths from logs incubated at 23.5  $^{\circ}$ C indicating four distinct instars, n = 195.

**Table 2.3.** Comparison of mean head capsule (HC) widths, range ( $mm \pm SE$ ) and rate of change (ROC) between instars of *Pseudips mexicanus* from manually infested lodgepole pine logs (Smith) with those reported by Trimble (1924). Trimble did not report standard error or standard deviation.

			Study		
	Trimble 1	924		Smith	
Instar	HC width	ROC	HC width	ROC	HC width range
1	0.40		$0.38\pm0.003$		0.37 - 0.40
2	0.90	0.44	$0.50\pm0.006$	0.75	0.43 - 0.57
3	1.20	0.75	$0.68\pm0.005$	0.75	0.60 - 0.78
4	1.20	1.00	$0.93 \pm 0.004$	0.73	0.83 - 1.04

and therefore the increments in size between instars should closely resemble a straight line (Ghent 1956). Prebble (1933) and Reid (1962) showed that this rule was applicable for the bark beetles *Dendroctonus simplex* LeConte and *D. ponderosae*, respectively. The rate of change in these two studies was approximately 0.75, similar to our findings for *P. mexicanus* (Table 2.3).

In lodgepole pine forests, P. mexicanus differ from southern populations in a number of ways. Northern beetles may produce between 1.0 and 1.5 generations per year, unlike populations in California which can have as many as three generations per year (Struble 1961). Attack behaviour differs as well, as northern populations overwinter in the lower portion of the bole, which may be an adaptation to use snow cover to gain protection from lethal cold temperatures. Southern beetles tend to make use of the whole tree plus downed material. Similarities across the populations in terms of polygyny, ovipositional characteristics (i.e. # of eggs per egg niche) and development rate are apparent. In Canada, *P. mexicanus* is a common forest insect that is not an economic threat to the forestry industry. Populations of this species do not increase to levels where live, healthy trees are attacked, unlike *I. pini* which can cause mortality when conditions are optimal (Thomas 1961). *P.* mexicanus has, however; been a concern in Monterey pine because of its ability to disseminate the pitch canker fungus in California (Fox et al. 1991). Moribund trees are typical hosts in Canada, and through removal of low-quality material, aided by other bark beetle species, *P. mexicanus* is likely beneficial by maintaining stand health. Determination of attack dynamics, development rate and voltinism will facilitate future discussions examining interactions between P. mexicanus and other species such as endemic D. ponderosae.

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### **CHAPTER 3**

# AN EXAMINATION OF THE INTERACTION BETWEEN A SECONDARY BARK BEETLE, *PSEUDIPS MEXICANUS* AND ENDEMIC *DENDROCTONUS PONDEROSAE* IN WEAKENED LODGEPOLE PINE TREES.

# ABSTRACT

Endemic populations of the bark beetle *Dendroctonus ponderosae* attack weakened lodgepole pine trees that are often previously infested by other bark beetle species, such as *Pseudips mexicanus*. Interactions between these two species were assessed by examining attack dynamics and attributes associated with brood production of *D. ponderosae* in trees containing *P. mexicanus* and trees attacked solely by *D. ponderosae*. My findings suggest that *D. ponderosae* attacking ephemeral hosts previously occupied by *P. mexicanus* do so in greater densities and offspring emerge earlier when compared to hosts *D. ponderosae* attacked alone. Additionally, *D. ponderosae* larvae in *P. mexicanus*-infested trees were found to require significantly less resource to complete development. The presence of *P. mexicanus* may affect internal host chemistry, improving the subcortical environment for endemic *D. ponderosae*, and hosts in this condition should be preferentially attacked.

Key words: facilitation; interspecific competition; host selection; phenology

#### INTRODUCTION

Interactions between species sharing a common resource are often characterized as competitive (Bruno et al. 2003). All such interactions are not negative, however. A great deal of work has been conducted in the past fifteen years investigating positive interactions or facilitation, especially in plant (Callaway 1995, Callaway and Walker 1997) and inter-tidal communities (Stachowicz 2001, Bruno et al. 2003). Facilitation is defined as an interaction where at least one species benefits from the interaction while none are harmed (Bruno et al. 2003). In the bark beetles (Coleoptera: Curculionidae: Scolytinae), a number of studies have examined and characterized competition occurring between cohabiting beetles, (Rankin and Borden 1991, Schlyter and Anderbrant 1993, Poland and Borden 1994, Gara et al. 1995, Poland and Borden 1998, Safranyik et al. 1999, Miller and Borden 2000, Hedgren 2004). As yet, only a small number of papers have suggested facilitation occurring between bark beetle species (Amman and Schmitz 1988, Carroll et al. 2006a).

Most bark beetles attack woody plants, but less than half of the families described are phloeophagous, i.e., they consume and breed in phloem (Wood 1982). Phloeophagus bark beetles bore through the bark and create ovipositional galleries in the phloem region of host trees. Many of them carry fungi, which have been suggested to aid in the killing of the host and provide nutritional benefits (Paine et al. 1997, Bleiker and Six 2007). While feeding on the phloem, bark beetle larvae may ingest or come into contact with fungal spores prior to or after pupation; this facilitates transport of the fungi to new host trees.

Bark beetles are integral to healthy forest ecosystems, contributing to the degradation of senescent or recently dead woody plant material and are often categorized by their host preference. 'Primary' beetles are the first species to arrive at and successfully infest healthy

living hosts (Doliner and Borden 1984) but may have to subsist in weakened trees when populations are low. Primary beetles have the ability to increase rapidly in numbers during periods of host stress and to become severe pests (Wood 1982, Safranyik and Carroll 2006). The majority of bark beetles are termed 'secondary' (Wood 1982) and attack weakened or dying trees almost exclusively (Doliner and Borden 1984), with some species able to increase in numbers for short periods, allowing attacks on living trees (Thomas 1961).

Populations of primary beetles fluctuate through a number of stages (Carroll et al. 2006a, Safranyik and Carroll 2006). In the endemic stage beetle populations are very low. Eruptive beetle species are often present within a forest as innocuous endemic populations. During this period of low numbers, beetles are unable to aggregate and overcome the defences of vigorous hosts (Wallin and Raffa 2004, Safranyik and Carroll 2006). Aggregation is an important mechanism for gaining access to a healthy tree. For example, Raffa and Berryman (1983) found that the mountain pine beetle needed at least 62 attacks per m<sup>2</sup> to reduce host defensive chemicals enough to ensure beetle survival upon entry under the bark. Consequently, when beetle numbers are below this critical value, i.e., during the endemic population phase, mountain pine beetles are forced to subsist on weakened trees, where they have to interact with a number of secondary bark beetle species (Carroll et al. 2006a).

As forest conditions change (aging hosts, drought), the number of vigour-impaired trees increase and larger hosts become accessible (Safranyik and Carroll 2006). When this occurs, populations increase to the endemic-incipient stage (Carroll et al. 2006a). Small groups of large diameter trees are infested while interactions with other bark beetle species decrease as increasing numbers of the eruptive species engage in 'primary' attack behaviour (Wallin and Raffa 2004). As populations continue to increase the epidemic stage is reached, during

which large healthy trees can be colonized (Carroll et al. 2006a). Throughout an epidemic, eruptive species attacking living trees are the only ones able to utilize the well-defended hosts, and interactions with other species are relatively minimal. The epidemic stage lasts until most large host trees with thick phloem have been killed. Beyond this point, populations begin to decline into the post-epidemic or collapse stage, characterized by increased interspecific interactions with other bark beetle species and reliance upon weakened hosts again (Safranyik and Carroll 2006).

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins, is found in lodgepole pine forests throughout the southern half of the province of British Columbia and the north-western United States, but is a major pest of pine trees across its range. A great deal of research has examined the epidemic population phase of this species (Safranyik et al. 1974, Berryman 1976, Klein et al. 1978, Thomson and Shrimpton 1984, Safranyik and Linton 1985, Safranyik 1988, Safranyik et al. 1999) and many management strategies have been suggested (McMullen et al. 1986, Raffa and Berryman 1986, Amman et al. 1989, Lindgren et al. 1989, Lindgren and Borden 1993, Anonymous 2001, Carroll et al. 2006b). The endemic phase on the other hand, has been examined very little (Carroll et al. 2006a). Because of this, there is a knowledge gap in mountain pine beetle population dynamics, specifically, the effect that the presence of other bark beetle species has on endemic *D. ponderosae* and how this influences population growth. Previous work by Amman and Schmitz (1988) and more recently by Carroll et al. (2006a) suggests that the presence of secondary bark beetles in trees attacked by endemic *D. ponderosae* may assist in population maintenance and possibly aid in population increases.

Endemic *D. ponderosae* populations tend to be too small to allow for successful attack on healthy hosts, leaving them confined to weakened trees often previously infested by other bark beetles (Carroll et al. 2006a). In lodgepole pine ecosystems, over 50 bark beetle species make use of various parts of the tree, the majority of which attack vigour-impaired hosts (Bright 1976). Carroll et al. (2006a) found that endemic D. ponderosae were directly associated with nine species of secondary bark beetles on the lower bole. The most common of those beetles was *Pseudips mexicanus* (Hopkins). This beetle is native to British Columbia and is found sympatrically with D. ponderosae (Bright 1976). P. mexicanus has one generation per year in Canada and overwinters either under the bark in the same host in which it developed or in a new host (Chapter 2). They attack early in the summer, unlike D. ponderosae, which attack in late July to early August (Safranyik and Jahren 1970). Because P. mexicanus attacks earlier than D. ponderosae, their broods have time to mature to adulthood before D. ponderosae emerge for their annual dispersal. This provides a partial temporal separation between the two species, where larval-larval interaction does not take place, reducing the likelihood of interference competition.

The trees suitable for *P. mexicanus* and endemic *D. ponderosae* are an ephemeral resource and likely require the beetles to search for long periods (Hanski 1987). Carroll et al. (2006a) found that in the endemic phase less than two trees per hectare were infested by *D. ponderosae* and up to five or six per hectare in the endemic-incipient phase. *P. mexicanus*-infested trees were more common, with up to 15 trees attacked per hectare. In a hypothetical stand stocked with 1000 stems, less than two percent of the total number of trees would be infested by either species, making both relatively rare on the landscape during the endemic phase of *D. ponderosae*. In the study by Carroll et al. (2006a), an examination of the trees

attacked by endemic *D. ponderosae* showed that approximately 65 % of them had been previously infested by secondary bark beetles.

Some eruptive bark beetles, such as *Dendroctonus rufipennis* Kirby, survive in endemic phases by exploiting ephemeral habitats (Lewis and Lindgren 2002, Wallin and Raffa 2004), where populations are sustained in weakened material. Mountain pine beetles likely maintain populations in a comparable fashion, even though suppressed trees often have thin phloem, which provides a sub-optimal resource for the insects. Brood production in these types of hosts is low, which prevents build-up of a large enough population to facilitate aggregation and successful attacks on larger, healthier trees (Amman 1972a).

Interaction between two species of bark beetles can have a number of consequences for each. Although species often partition resources when they cohabitate, competition often takes place where niche overlap occurs (Ayres et al. 2001). Competition can be exploitative or interference, reducing the reproductive capacity of both species (Wood 1982, Light et al. 1983). Poland and Borden (1994) showed that *Ips pini* Say and *Pityogenes knechteli* Swaine both had reduced numbers of progeny in trees naturally attacked as a result of exploitation of the phloem resource.

As *P. mexicanus* was found to be the most common secondary beetle in the study by Carroll et al. (2006a), it was used to assess the potential effects of the presence of secondary bark beetles on endemic *D. ponderosae*. Facilitation by secondary beetles has already been suggested (Carroll et al. 2006a), however; it is unclear what role *P. mexicanus* may be playing. To determine what effect the presence of *P. mexicanus* in weakened lodgepole pine hosts has on endemic *D. ponderosae*, their interaction was examined in naturally infested trees. It was predicted that if *P. mexicanus* is competing with *D. ponderosae*, *D. ponderosae* 

would have lower attack densities in *P. mexicanus*-infested trees than in trees *D. ponderosae* attacked alone. Additionally, *D. ponderosae* females should produce fewer and smaller offspring as a result of *P. mexicanus* resource utilization forcing *D. ponderosae* into poorer quality sections of the host with reduced overall quantity of resource available when compared to broods from trees *D. ponderosae* attacked alone. However, if *P. mexicanus* is facilitating *D. ponderosae*, then *D. ponderosae* should attack *P. mexicanus*-infested trees in greater numbers and *D. ponderosae* offspring should be more numerous and larger than in trees attacked by *D. ponderosae* alone. Regardless of the direction of the interaction, the results of this study will improve understanding of *D. ponderosae* population dynamics in the endemic phase.

#### **METHODS**

#### Study area.

The field site was located near Angstad Creek, 28 km south of Merritt BC ( $49^{\circ}$  51' N,  $120^{\circ}$  46' W, mean elevation = 1300 (± 200) m). Trees were selected from 28 ha (seven 4 ha stands) of forest where lodgepole pine was either the dominant or co-dominant species. Pine dominant stands are characterized by the pine component being greater than 50 % of the species present and individual pine trees extending above the crowns of other species, while in pine co-dominant stands these are of similar height to surrounding tree species (Avery and Burkhart 2002). Tree characteristics were collected by randomly placing one variable radius plot in each stand. A BAF 5 prism was used in all plots. The level of beetle infestation was endemic to endemic/incipient where beetle numbers were too low to overcome large

diameter, healthy trees (Safranyik and Carroll 2006). The study ran from May 2005 – March 2006.

## Tree selection.

Study trees were a minimum of 50 metres from any other *D. ponderosae*-attacked tree and were attacked in 2005. Because of the rarity of such trees (Carroll et al. 2006a), only twelve trees were found, seven with both *D. ponderosae* and *P. mexicanus* present and five with *D. ponderosae* only. These will be referred to as the 'infestation types' throughout the chapter. Identification and determination of the presence of each attacking species was accomplished by assessing each pine tree in the 28 ha study area. The initial assessment in June 2005 identified trees with new *P. mexicanus* attacks by observing boring dust collected at the base of the tree. Stands were reassessed in August and *D. ponderosae* attacks were identified in a similar manner, along with any new *P. mexicanus* attacks occurring since the first assessment. Pitch tubes, indicators of *D. ponderosae* attack stimulating host resistance, were not noted on any of the trees, suggesting a diminished host defensive capacity (Amman 1984), which is typical in endemic *D. ponderosae* attacks.

Diameter at breast height (1.3 m) (DBH), phloem thickness, age, ten-year mean annual increment of growth and physical damage or pathogen infestation were recorded from each infested tree. Phloem thickness at DBH was recorded as the mean of two measurements, 90° from each other. Phloem was removed using a 1.5 cm diameter punch. Assessment of injuries from physical damage or pathogens was made following the methods of Carroll et al. (2006a). Trees were felled in late September and the bottom 1 m of each was transported to the Pacific Forestry Centre, Victoria, BC, where they were placed in individual rearing cages.

All cuts made to the logs were sealed with paraffin wax to prevent desiccation of the phloem and sapwood. Rearing of all beetles took place in a greenhouse room maintained at an ambient air temperature of 20 °C.

## Beetle rearing and gallery examination.

Emerging beetles were collected beginning in January 2006, placed in labelled vials, and frozen until later examination. After emergence was complete (determined as no emergence for a period of 7 days for all logs), the logs were placed in an autoclave at 120 °C and 2 atmospheres of pressure for an hour to soften the bark, which was then peeled off for examination of galleries (Carroll et al. 2006a). Remaining adult beetles under the bark were collected. Logs were assessed and attributes such as attack density (number of attacks per m<sup>2</sup>), interspecific gallery overlap (percent of total gallery systems overlapping systems of other species) and percent phloem-use were recorded for both species. Phloem-use was defined as any portion of the phloem consumed or discoloured as a result of proximate occupation by a beetle or its associated fungal growth. Surface area of the outer face of the sapwood plus the phloem thickness. Phloem volume was calculated by subtracting the surface area of the sapwood from the surface area of the outer phloem, multiplying by the phloem thickness and the percent use.

*D. ponderosae* were counted, and their sex and size determined. Sex of mountain pine beetles was established by looking for the male stridulatory apparatus on the 7<sup>th</sup> abdominal tergite (Lyon 1958). Since *P. mexicanus* had mostly emerged by the time the trees were cut, the sex and size of these beetles was not determined. The width of *D. ponderosae* female

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pronotums was measured as an indicator of size to the nearest 0.1 mm using an ocular micrometer (Haack et al. 1984). Only female *D. ponderosae* were examined, as they are the attacking and egg-laying sex. Where more than 100 mountain pine beetles had emerged, at least 40 females were sub-sampled using methods of Campbell and Borden (2006a). Unless otherwise indicated, all values are reported as mean  $\pm$  SE (one standard error).

## Analysis.

Transformation of data by Y = sqrt(X + 0.01) was done as needed to address assumptions of normality and homoscedasticity. Tree characteristics were compared between trees in both infestation types and healthy trees from the prism plots using analysis of variance (ANOVA) and a Ryan-Einot-Gabriel-Welsch F-test (REGW) for multiple comparisons. DBH and 10 year mean annual increment (MAI) data were transformed as described above. Regression analyses were used to test the effects of D. ponderosae attack density on (i) D. ponderosae ovipositional gallery length, (ii) phloem volume used per D. ponderosae attack, (iii) proportion of phloem used by D. ponderosae per log and (iv) D. ponderosae offspring mean pronotal widths. Regression was also used to test the effects of (i) the total number of D. ponderosae offspring per log on the phloem volume used per D. ponderosae offspring, (ii) P. mexicanus phloem volume use on the number of D. ponderosae female offspring produced per  $m^2$  and on D. ponderosae offspring mean pronotal widths, (iii) P. mexicanus attack density on *P. mexicanus* ovipositional gallery length and (iv) the mean number of Julian days after the emergence of the first D. ponderosae brood adult per infestation type on the cumulative percent D. ponderosae emergence. Curvilinear and non-linear regressions were used only when they explained significantly (P < 0.05) more of the variation than simple

linear regressions. Differences in sex ratios of *D. ponderosae* offspring were compared to an expected value using a log-likelihood ratio test (G-test). Independent samples t-tests were used to compare variables between infestation types. All tests were referenced from Sokal and Rohlf (1995) and statistical analyses were conducted with SPSS 13.0 (SPSS 2004) and SigmaPlot 9.01 (Systat 2004).

## RESULTS

## Tree characteristics.

Descriptions of study-tree characteristics and stand tree measurements are shown in Table 3.1. All of these trees were alive and had green crowns at the time of attack, but were stressed in some manner. Damage assessment by visual examination (Carroll et al. 2006a) revealed that all trees had at least one putative vigour-impairing injury (forked tops, broken tops, scars) and all had evidence of lodgepole pine dwarf mistletoe, *Arceuthobium americanum* Nutt. ex Engelm., infestation in the branches. Additionally, all trees were suppressed or had crowns which did not extend into the main canopy.

The mean DBH of trees attacked by *D. ponderosae* either with *P. mexicanus* or alone was significantly smaller than the mean stand DBH (Table 3.1,  $F_{2,51} = 4.17$ , P = 0.02). Amman and Pace (1976) characterized different phloem thickness classes and defined 'thin phloem' as having a mean of  $1.8 \pm 0.3$  (SD) mm, which was greater than the mean phloem thickness of all trees examined in this study, including the healthy ones. A comparison of mean phloem thicknesses of trees infested by both *D. ponderosae* and *P. mexicanus*, trees infested by *D. ponderosae* alone and uninfested trees examined during prism plot data collection

**Table 3.1.** Characteristics of lodgepole pine trees attacked by *Dendroctonus ponderosae* (*D. ponderosae*) alone (*D. ponderosae* only) and trees attacked by *D. ponderosae* after *Pseudips mexicanus* infestation (*D. ponderosae* and *P. mexicanus*). Also included are tree characteristics of lodgepole pine from seven stands (Stand). These data were used to assess the interaction between endemic *D. ponderosae* and *P. mexicanus*.

Tree characteristic		Mean ± SE	
	D. ponderosae only $(n = 5)$	D. ponderosae and P. mexicanus $(n = 7)$	Stand $(n = 42)$
DBH (cm)	14.8 ± 1.2 a	$16.2 \pm 0.8$ a	$21.3 \pm 1.0$ b
Phloem thickness (mm)	$1.5 \pm 0.2$ a	$1.4 \pm 0.1$ a	$1.4 \pm 0.1$ a
Age (years)	105.6 ± 4.6 a	$118.0 \pm 3.3$ a	111.5 ± 2.3 a
10 year MAI (mm)	$0.20 \pm 0.05$ a	$0.20 \pm 0.04$ a	$0.45\pm0.04\ b$

Note: Within each row, means followed by the same letter are not significantly different from one another (REGW multiple range test,  $\alpha = 0.05$ ).

was not significant (Table 3.1,  $F_{2,51} = 0.16$ , P = 0.85). Tree age was not significantly different between *D. ponderosae*-only attacked trees, *D. ponderosae* with *P. mexicanus*attacked trees and stand data (Table 3.1,  $F_{2,51} = 1.24$ , P = 0.30). Ten-year MAI were small for all attacked trees, indicating senescence and prolonged stress and did not differ between *P. mexicanus* and *D. ponderosae*-attacked trees and *D. ponderosae*-only attacked trees. However, when compared to stand MAI, trees in both infestation types had growth reduced by over half (Table 3.1,  $F_{2,51} = 4.06$ , P = 0.02). All attacked trees had evidence of staining in the phloem and sapwood resulting from ophiostomatoid fungal infection.

## Attack density and gallery length.

The mean *D. ponderosae* attack density was significantly greater (approximately four times) in trees attacked previously by *P. mexicanus* than in trees attacked by *D. ponderosae* alone (Table 3.2, t = -3.88, df = 10, P < 0.05). *P. mexicanus* attack density tended to be lower than 17 attacks/m<sup>2</sup>, with a mean of  $5.9 \pm 2.1$  attacks/m<sup>2</sup>. Comparison of *D. ponderosae* ovipositional gallery lengths between infestation types revealed no significant difference (t = 0.32, df = 10, P = 0.76). Gallery lengths were expected to decrease as *D. ponderosae* attack density increased (Coulson et al. 1976), however no significant relationship was found with simple linear regression (N = 12,  $r_{adj.}^2 = 0.16$ , P = 0.18).

## Phloem use.

*P. mexicanus* attack density exhibited a strong linear relationship with proportion of *P. mexicanus* phloem use (Fig. 3.1, N = 7,  $r_{adj.}^2 = 0.62$ , P = 0.02) as expected. *P. mexicanus* 

<i>ponderosae</i> alone ± SE.	(D. p	onderosae only) an	id by D. ponderose	ae atter P. mexicam	us intestation (D.	ponderosae and P. 1	nexicanus). Mean
Attacking species	ц	D. ponderosae attacks/m <sup>2</sup>	<i>P. mexicanus</i> attacks/m <sup>2</sup>	D. ponderosae gallery length (cm)/attack	P. mexicanus phloem volume (%) used per log	D. ponderosae phloem volume (%) used per log	# D. ponderosae female offspring produced per attack
D. ponderosae only	S	14.0 ± 4.0 a	n/a	20.1 ± 2.2 a	n/a	37.6 ± 8.0 a	$4.8\pm1.6~\mathrm{a}$
D. ponderosae and P.	٢	$55.1 \pm 8.4 \text{ b}$	5.9 ± 2.1	19.4 ± 1.1 a	$5.0 \pm 1.4$	68.6 ± 2.4 b	7.0 ± 1.4 a
mexicanus							
Note: Within eacl test, $\alpha = 0.05$ ).	h colı	ımn, means followe	ed by the same lett	er are not significa	ntly different fron	1 one another (indep	endent samples t-



**Figure 3.1.** Proportion of phloem used per lodgepole pine log by *Pseudips mexicanus* in relation to *P. mexicanus* attack density (number of attacks per  $m^2$ ).

phloem use did not exceed 10 % of total phloem volume available on any log and the overall mean consumption was very low (Table 3.2). The proportion of phloem consumed by *D. ponderosae*, however; was nonlinear and increased rapidly to approximately 70 %, where it levelled off at a density of approximately 25 attacks/m<sup>2</sup> (Fig. 3.2, N = 12,  $R^2_{adj.} = 0.89$ , P < 0.0001). The maximum phloem use by both species combined was approximately 80 %, leaving at least 20 % of the total phloem resource per log unused by either species (Table 3.2). The greatest phloem use was by *D. ponderosae* in trees previously occupied by *P. mexicanus* (solid dots, Fig. 3.2; Table 3.2, t = -4.30, df = 10, P < 0.05).

Phloem use per *D. ponderosae* attack exhibited an inverse relationship with attack density (Fig. 3.3, N = 12,  $r_{adj.}^2 = 0.750$ , P < 0.001) and hosts attacked solely by *D. ponderosae* used the greatest amount of phloem per attack (Fig. 3.3, open dots, t = 3.251, df = 10, P < 0.05) with a mean of  $0.93 \pm 0.19$  cm<sup>3</sup>/attack compared to  $0.33 \pm 0.08$  cm<sup>3</sup>/attack in trees previously attacked by *P. mexicanus*. Unused areas of phloem were recorded between *D. ponderosae* and *P. mexicanus* gallery systems, and no interspecific egg or larval gallery overlap was observed.

The amount of phloem utilized per individual *D. ponderosae* offspring decreased dramatically with increasing number of offspring produced (Fig. 3.4, N = 12,  $R_{adj.}^2 = 0.70$ , P < 0.0001). *D. ponderosae* offspring in hosts attacked by *D. ponderosae* alone used 0.19 ± 0.08 cm<sup>3</sup> per attack and 0.03 ± 0.01 cm<sup>3</sup> in hosts attacked by both species. There was a significant difference in phloem use by *D. ponderosae* offspring between the two types of infested hosts (t = 2.40, df = 10, P = 0.04).


**Figure 3.2.** Proportion of phloem used per lodgepole pine log by *Dendroctonus ponderosae* in relation to *D. ponderosae* attack density (number of attacks per  $m^2$ ). Solid dots indicate trees attacked previously by *Pseudips mexicanus*, followed by *D. ponderosae* in the same year and open circles indicate trees attacked solely by *D. ponderosae*.



**Figure 3.3.** Lodgepole pine phloem volume  $(cm^3)$  utilized per attack by endemic *Dendroctonus ponderosae* in relation to *D. ponderosae* attack density. Solid dots indicate trees attacked previously by *Pseudips mexicanus*, followed by *D. ponderosae* in the same year and open circles indicate trees attacked solely by *D. ponderosae*.



**Figure 3.4.** Lodgepole pine phloem volume  $(cm^3)$  utilized per beetle by endemic *Dendroctonus ponderosae* in relation to the total number of emerged *D. ponderosae* offspring per tree. Solid dots indicate trees attacked previously by *Pseudips mexicanus*, followed by *D. ponderosae* in the same year and open circles indicate trees attacked solely by *D. ponderosae*.

## Number of offspring emerging.

The volume of phloem utilized per m<sup>2</sup> by *P. mexicanus* significantly influenced the total number of emerging *D. ponderosae* females per m<sup>2</sup>, where more offspring emerged as phloem use increased (Fig. 3.5, N = 12,  $r_{adj.}^2 = 0.30$ , P = 0.04). Trees infested by *P. mexicanus* (solid dots, Fig. 3.5) produced the most *D. ponderosae* offspring per m<sup>2</sup> (t = -2.89, df = 10, P = 0.02) with a mean of  $356.0 \pm 74.7$  females compared to a mean of  $76.9 \pm 42.8$  in trees *D. ponderosae* attacked alone. The number of *D. ponderosae* brood females produced per attack was not significantly related to *D. ponderosae* attack density (N = 12,  $r_{adj.}^2 = -0.09$ , P = 0.08). The number of female *D. ponderosae* produced per attack did not differ significantly between the two infestation types (t = -0.98, df = 10, P = 0.35), with *D. ponderosae* females (Table 3.2). The total phloem volume available to *D. ponderosae* did not significantly affect the number of *D. ponderosae* emerging offspring (N = 12,  $r_{adj.}^2 = 0.002$ , P = 0.89).

# Offspring size.

Pronotal widths of female *D. ponderosae* were measured as an indicator of beetle size. A multiple regression analysis indicated that *P. mexicanus* phloem volume consumption per m<sup>2</sup> did not contribute significantly to the model, but pronotal widths were negatively affected by *D. ponderosae* attack density (Table 3.3). However, the effect was small and the sizes of *D. ponderosae* from the two infestation types were not significantly different (t = 1.41, df = 10, P = 0.19), with *D. ponderosae* female offspring from hosts attacked solely by *D. ponderosae* 



**Figure 3.5**. Number of female endemic *Dendroctonus ponderosae* emerging per  $m^2$  in relation to phloem volume used (cm<sup>3</sup>) by *Pseudips mexicanus* per  $m^2$  in lodgepole pine. Solid dots indicate trees attacked previously by *P. mexicanus*, followed by *D. ponderosae* in the same year and open circles indicate trees attacked solely by *D. ponderosae*.

**Table 3.3.** Summary of a multiple linear regression analysis examining the effects of volume of phloem utilized (cm<sup>3</sup>) by *Pseudips mexicanus* per m<sup>2</sup> and *Dendroctonus ponderosae* attack density (attacks/m<sup>2</sup>) on female endemic *D. ponderosae* mean pronotal width (mm).

	Coefficients	SE	t	Р
D. ponderosae attack density	-0.003	0.001	-3.126	0.012
P. mexicanus phloem use per log	0.054	0.034	1.573	0.150
Intercept	2.204	0.034	64.888	< 0.001

Multiple  $R^{2}_{adj.} = 0.41$ ,  $F_{2,9} = 4.89$ , P = 0.04

having a mean pronotal width of  $2.17 \pm 0.03$  mm and female offspring from *P. mexicanus* infested trees having a mean width of  $2.10 \pm 0.03$  mm.

#### Sex ratio.

Mountain pine beetle offspring were expected to have a 1:2 male to female sex ratio (Reid 1958, McGhehey 1969). A comparison of the sex ratios of emerging adults for the two types of infestations to the expected ratio showed significant differences. Both offspring from trees attacked solely by *D. ponderosae* and offspring from trees attacked by both species had a sex ratio of 1:1.4 (G = 4.02, df = 1, P < 0.05 and G = 13.98, df = 1, P < 0.001 respectively).

#### Brood emergence phenology.

The number of days after first emergence and the cumulative mean percent D. ponderosae female emergence for both infestation types was described by a sigmoidal curve (Fig. 3.6, N = 12,  $R^2_{adj.} = 0.997$ , P < 0.0001). D. ponderosae offspring from trees infested with P. mexicanus emerged earlier than offspring from trees attacked by D. ponderosae alone, as determined by observing the length of time required to reach 50 % emergence. D. ponderosae offspring reached this point after approximately 25.5 days in hosts previously colonized by P. mexicanus and after 32.5 days in hosts attacked by D. ponderosae alone. Initial emergence was more rapid in trees previously attacked by P. mexicanus, but faster in the later half of the emergence period in trees occupied by D. ponderosae only. All logs had stopped producing brood after 48 days and were peeled on day 55 to collect any remaining adults.



Figure 3.6. Cumulative mean percent emergence of female endemic *Dendroctonus* ponderosae from lodgepole pine trees in relation to the mean number of days since emergence commenced per infestation type. Solid dots indicate trees attacked previously by *Pseudips mexicanus*, followed by *D. ponderosae* in the same year, open circles indicate trees attacked solely by *D. ponderosae* and grey dots represent overlapping data points. Error bars are 1 SE of the mean.

#### DISCUSSION

The findings of this study indicate that decadent trees previously colonized by *P. mexicanus* were better resources for endemic *D. ponderosae* than trees *D. ponderosae* attacked alone. Hosts attacked in both infestation types were similar both physically and likely chemically (Raffa and Berryman 1982). Even so, hosts occupied by *P. mexicanus* were more attractive to endemic *D. ponderosae*, resulting in larger numbers of *D. ponderosae* attacks and subsequently greater offspring production per tree. The number of female *D. ponderosae* offspring produced was positively affected by the volume of phloem consumed by *P. mexicanus* (Fig. 3.5), indicating that the presence of *P. mexicanus* contributed in some manner to the increased *D. ponderosae* attack density. Additionally, *D. ponderosae* offspring sizes were not different between infestation types (Table 3.3), but *D. ponderosae* in *P. mexicanus*-infested trees emerged carlier than in trees *D. ponderosae* attacked alone (Fig. 3.6). Thus, comparison of *D. ponderosae* fitness characteristics between infestation types is valid.

*P. mexicanus* is a secondary bark beetle that attacks weakened hosts and does not develop into outbreak populations, seldom aggregating within hosts in large numbers (Wood 1982, Chapter 2). Although there are advantages to aggregation, such as mate attraction (Borden 1974) and predator swamping (Aukema and Raffa 2004), secondary beetles tend not to benefit from this behaviour, as increases in density produce sharp declines in reproductive success (Robins and Reid 1997). Aggregation to individual trees by this species is therefore normally limited (Wood 1982). Additionally, *P. mexicanus* does not completely utilize the resource they occupy (Fig. 3.1), leaving opportunity for other species to attack.

Endemic D. ponderosae typically attack injured or stressed, over-mature lodgepole pine trees, similar to the study trees (Carroll et al. 2006a). The lack of visible signs of defence (pitch tubes, necrotic lesions) on the study trees, in conjunction with the observed thin phloem, reduced growth and advanced age, suggest that these trees were highly susceptible to attack by endemic D. ponderosae, with greatly diminished constitutive and induced defences (Safranyik and Carroll 2006). In resistant hosts, attacking beetles initially encounter the preformed constitutive defence and the induced defence shortly thereafter (Raffa and Berryman 1987), both of which contain compounds toxic to attacking beetles (Cates and Alexander 1982). Constitutive defences are made up of large amounts of stored resin (secondary metabolites consisting of monoterpenes and resin acids) (Lombardero et al. 2000), but in a weak or non-resistant host, the constitutive defence is minimal or absent (Shrimpton 1973). The induced defence, a combination of necrotic lesion formation and secondary resin and associated chemical production, is not strongly linked to the magnitude of constitutive resin flow (Lombardero et al. 2000), as monoterpenes are either transported to the attack site or manufactured there de novo (Raffa and Berryman 1982). Trees encountered by endemic D. ponderosae do not have strong defences, but may still reduce beetle fitness. A reduction in defences by damage, pathogen infection or previous attack by other bark beetles could, therefore, benefit *D. ponderosae*, even in weakened hosts.

The induced defence is typically initiated in response to the presence of pathogenic fungi, inoculation of which by *P. mexicanus* may reduce host defences. Most bark beetle species carry fungal and yeast spores between hosts and many, such as *D. ponderosae*, have specialized invaginations of the exoskeleton called the mycangia, in which these spores are carried (Whitney and Farris 1970, Six and Paine 1998). These mycangial fungi are often mutualistic and are fundamental components of brood success in healthy hosts (Six and Paine 1998). Other fungal species are transported phoretically on the exoskeleton (Six 2003). *P. mexicanus* carries a diverse phoretic yeast and fungal complex, including *Leptographium terebrantis* Barras & Perry (Bleiker, Massoumi-Alamoutim, Smith, Carroll and Breuil 2007, unpub. data). This pathogenic fungus (Raffa and Smalley 1988) and others associated with *P. mexicanus*, may contribute to further weakening of attacked hosts. In a recent study, terpene production was compared in trees unattacked and previously attacked by *P. mexicanus* through inoculation with *Grosmannia clavigera* (Robinson-Jeffrey and Davidson) Zipfel, de Beer and Wingf. (previously *Ophiostoma clavigerum*), a *D. ponderosae* fungal associate. Prior to inoculation, trees had similar terpene concentrations, but trees previously attacked by *P. mexicanus* had a much weaker induced defensive response (less terpene production) than did the unattacked trees (C. Boone, unpubl. data).

Carroll et al. (2006a) suggested, based on evidence presented by Christiansen et al. (1987), that *D. ponderosae* may be relying upon secondary bark beetle species to reduce the defensive capacity of smaller, but still resistant trees in order to successfully reproduce. Christiansen et al. (1987) stated that small, suppressed trees do not use accumulated carbon stores for growth, but instead store the energy, making it available for inducible defence. The study trees had very low recent growth and lacked any sign of either a strong constitutive (pitch tubes) or induced (lesions) defensive response at *P. mexicanus* attack sites. If the trees were able to produce a strong induced response, the presence of *L. terebrantis* carried by *P. mexicanus* should have triggered one (Raffa and Smalley 1988). This lack of visible induced response, coupled with the work of Boone (unpubl. data), supports my hypothesis, and that of Carroll et al. (2006a), that *P. mexicanus*-infested trees are weaker after attack than similar

unattacked trees within the stand, and, therefore, are potentially better hosts for endemic *D*. *ponderosae*.

In hosts attacked solely by *D. ponderosae*, more phloem was used per attack (Fig. 3.3) and per individual offspring (Fig. 3.4) than in hosts previously attacked by *P. mexicanus*. It has been suggested that insects feeding on poor resources increase consumption to compensate for lower nutritional value (Mattson 1980, Six and Paine 1998, Safranyik and Carroll 2006). The greater resource use by individual *D. ponderosae* in hosts they attack alone suggests that offspring inhabit a less suitable host than *D. ponderosae* offspring from *P. mexicanus*-infested trees, and, therefore, may require larger amounts of resource per individual to obtain required nutrients. Phloem consumption was not complete on any study tree, and remained at fairly constant levels in *P. mexicanus*-infested hosts compared to consumption in hosts attacked by *D. ponderosae* alone, where the proportion of phloem used increased rapidly with attack density, but peaked at approximately 0.55 (Fig. 3.2). Overall consumption per log was higher in *P. mexicanus*-trees, due to the larger numbers of attacks and subsequent offspring production (Fig. 3.2), even though the mean total phloem volume available was not different between infestation types (P = 0.70).

Subcortical spacing between bark beetle species may be facilitated by rendering phloem unpalatable to other bark beetles by the presence of the mutualistic fungi of another species (Yearian et al. 1972). A number of authors have suggested that mutualistic fungi may increase the nutritional value of an otherwise poor host (Whitney et al. 1987, Six and Paine 1998, Ayres et al. 2000, Bleiker and Six 2007) and it has been proposed that the presence of fungi associated with secondary bark beetles may improve host conditions for *D. ponderosae* (Carroll et al. 2006a). Very little is known about the fungi or yeasts associated with *P*. *mexicanus* or their potential effects on adult and brood *D. ponderosae*. There was no visible evidence of fungal staining spreading throughout the logs prior to *D. ponderosae* attack; however; if *P. mexicanus*-associated fungi had colonized the logs, *D. ponderosae* may have benefited nutritionally from their presence, decreasing the amount of phloem required per beetle to obtain requisite nutritional requirements (Six and Paine 1998).

Consumption of greater volumes of phloem by beetles in trees attacked by *D. ponderosae* alone indicates that a longer period of time may have been required to reach maturity. The timing of egg laying was not determined, so exact length of development time could not be measured, but my results clearly show that *D. ponderosae* offspring took longer to emerge from trees they attacked alone. Since both groups were reared under the same conditions, the longer emergence time probably reflects slower development resulting from compensatory feeding due to poor nutritional value of the resource. Other insects have been shown to experience similar reductions in rate of growth and delays in emergence (Carisey and Bauce 1997).

The ability to develop quickly with minimal loss in body mass could confer a number of benefits to a beetle. Earlier emergence would give beetles the opportunity to locate and choose from among the best available hosts (Pureswaran et al. 2006). Once a host is selected, an optimum attack site can be located and oviposition commenced. The ability to begin egg laying prior to the arrival of conspecifics gives offspring time to develop into more robust life stages which can better survive intraspecific competition. Additionally, the ability to arrive earlier in relatively large numbers may also reduce losses due to predation while host searching, essentially beating predators to the host trees (Dixon and Payne 1979,

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Aukema and Raffa 2004). Beetles emerging later would have to contend with predators that have accumulated as a result of attraction to previous flights of beetles.

Offspring size is affected by growth rates as well as density of conspecifics. In this study, the size of female *D. ponderosae* offspring were found to be negatively affected by parental attack density, but the effect did not reduce offspring size significantly. D. ponderosae females from hosts attacked by this species alone were found to be 3.2 % wider across the pronotum than offspring from trees previously infested by P. mexicanus. Under laboratory conditions, McGhehey (1971) found that larger females tended to produce more eggs. Using the mean pronotal widths of female beetles from each infestation type and extrapolating from McGhehey's (1971) data, 140 eggs could potentially be laid by females emerging from D. ponderosae-only trees, compared to only 124 by the smaller females from P. mexicanus-infested hosts. This is a difference in potential fecundity for the larger females of approximately 11.4 % (Fig. 3 in McGhehey 1971). However, Amman (1972b) found that there was a great deal of variation in egg-laying capacity among individual beetles and Reid (1962) pointed out that only a fraction of the potential total number of eggs is ever laid in natural populations, suggesting that this potential improvement in fecundity for D. ponderosae in trees they attack alone may not be biologically significant. The number of D. ponderosae eggs laid could not be determined for the study trees, so actual fecundity was not calculated.

Larger body size has a number of associated costs and benefits. Bigger beetles tend to have greater fat stores (Atkins 1966), providing energy for longer periods spent searching for ephemeral hosts. Beetles emerging from a poor resource, regardless of their size, will have a diminished capability to secure hosts as a result of reduced lipid content (Elkin and Reid

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2005). Size also has been shown to affect pheromone production. More robust male *D. ponderosae* produce greater amounts of anti-aggregation pheromone, however; the production of aggregation pheromone by females is not affected by size (Pureswaran and Borden 2003). Therefore, the ability to attract mates is not diminished for smaller females. Additionally, smaller beetles may benefit in an endemic environment as smaller offspring would be more suited to colonize hosts with thin phloem (Pureswaran and Borden 2003). Larger beetles may accidentally break through the protective bark layer when the subcortical environment is not of suitable thickness, resulting in abandonment of the tree and exposure to the risks of finding a new host (Safranyik and Carroll 2006). Larger beetles attacking hosts with thin phloem may have to expend more energy engraving the sapwood (Amman 1972b), while smaller beetles would be able to mine more fully within the softer phloem tissue.

The sex ratio of emerging *D. ponderosae* was significantly different than that normally found in epidemic populations, where Reid (1958) and McGhehey (1969) found a ratio of 1:2 males to females, while I found ratios of 1:1.4 in hosts attacked by *D. ponderosae* only and in those attacked by *P. mexicanus* and *D. ponderosae*. During periods of stress, *D. ponderosae* has been shown to alter sex ratio in favour of females (Amman and Cole 1983); however; the number of females produced in this study was lower than that found in outbreak conditions, when numerous high quality hosts are available. I did not subject the beetles to cold, which could account for the large numbers of males present upon emergence, as they tend to be the most susceptible to freezing mortality (Safranyik 1976). However, it is possible that the ratio found during the endemic phase is the norm and that during an outbreak intraspecific competition reduces the number of male offspring, resulting in a female-biased ratio.

Alternatively, the risk of not obtaining a mate may be so great during the endemic phase that the number of males is kept high to ensure there are enough to find the attacking females.

Amman and Schmitz (1988) suggested that endemic *D. ponderosae* attacking hosts previously infested by secondary bark beetles should reduce risk of mortality through reduction of the time needed to locate a host by attraction to a tree infested with secondary beetles. They proposed that endemic *D. ponderosae* would be attracted to either the pheromones of the secondary beetles or the host volatiles emitted as a result of secondary attack. *P. mexicanus* attractant pheromone is not attractive to (Chapter 4) and, therefore, host location must be conducted through either detection of a volatile signature that *D. ponderosae* orients to (Moeck and Simmons 1991) or random landings (Hynum and Berryman 1980) or a combination of both (Campbell and Borden 2006).

I have shown that *D. ponderosae* attacking ephemeral hosts previously occupied by *P. mexicanus* do so in greater densities and offspring emerge earlier when compared to hosts *D. ponderosae* attacked alone. Additionally, *D. ponderosae* larvae in *P. mexicanus*-infested trees required significantly less resource to complete development. *P. mexicanus* likely has an effect on host chemistry, improving the subcortical environment in which endemic *D. ponderosae* can be more successful than in hosts they attack alone and possibly causing hosts to produce volatiles attractive to endemic *D. ponderosae*. Hosts with *P. mexicanus* present should be actively sought by endemic *D. ponderosae*, whereas *D. ponderosae* attacking trees with no secondary beetles present would likely do so as a last ditch effort to reproduce.

The form of the interaction between the two species appears to be facilitation, with *D. ponderosae* benefiting from the presence of *P. mexicanus* in weakened lodgepole pine trees. However, it is possible that during an emergence event *P. mexicanus* may re-infest the same

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tree in order to overwinter. This behaviour may help avoid dispersal losses, but has not been investigated. If this does occur, the presence of *D. ponderosae* may force *P. mexicanus* to find new host material, resulting in weak asymmetric exclusion competition, where *D. ponderosae* benefits greatly from the presence of *P. mexicanus* while *P. mexicanus* experiences mortality due to the presence of *D. ponderosae*. Regardless, the presence of *P. mexicanus* in these trees prior to *D. ponderosae* facilitates the maintenance of *D. ponderosae* populations during the endemic phase in lodgepole pine forests. Further work should examine how *P. mexicanus* alters the host chemistry upon attack and the chemical cues that endemic *D. ponderosae* respond to in order to find hosts initially. Additionally, the role of mutualistic fungi associated with *P. mexicanus* needs to be studied in terms of altering hosts chemistry and improving nutritional value of phloem for endemic *D. ponderosae*.

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#### CHAPTER 4

# THE RESPONSE OF ENDEMIC DENDROCTONUS PONDEROSAE TO VOLATILES ASSOCIATED WITH THE SECONDARY BARK BEETLE PSEUDIPS MEXICANUS

# ABSTRACT

A pair of experiments was conducted to assess the role of lodgepole pine host volatiles and the attractant pheromone of *Pseudips mexicanus* in host selection by endemic *Dendroctonus ponderosae* and other associated bark beetle species. Endemic *D. ponderosae* are often found in weakened hosts previously colonized by *P. mexicanus*, but did not respond to either pheromones or host volatiles produced from *P. mexicanus*-infested or uninfested cut logs, respectively. Both *P. mexicanus* and *Ips pini* were strongly attracted to *P. mexicanus* pheromone, a combination of racemic ipsdienol and ipsenol. Host volatiles presented alone or in combination with chemicals produced by feeding *P. mexicanus* were attractive to *P. mexicanus* and *Orthotomicus latidens*, while no *I. pini* were captured. These results suggest that *D. ponderosae* in the endemic stage locate hosts using cues not directly attributable to the presence of *P. mexicanus*, but rather identify volatiles associated with living host decline stemming from *P. mexicanus* attack.

Key words: pheromone; ipsenol; ipsdienol; population maintenance

## INTRODUCTION

The mountain pine beetle, Dendroctonus ponderosae Hopkins, is a major pest of lodgepole pine (Pinus contorta var. latifolia Engelmann) forests in western North America. During epidemics, large healthy trees are attacked and killed by aggregating beetles (Safranyik and Carroll 2006). Initial selection of these hosts is conducted by pioneering beetles through primary attraction (orientation to host volatiles) (Person 1931). Primary attraction has been studied extensively for epidemic populations of *D. ponderosae*, where it is generally accepted that orientation to host volatiles, recognition of silhouettes and random landing coupled with gustatory sampling are used by pioneering females to locate suitable hosts (reviewed in Safranyik and Carroll 2006). Like many tree-killing bark beetle species, D. ponderosae normally exists in healthy forests at low or endemic populations (Carroll et al. 2006), potentially for long periods (Raffa 1988). In the endemic stage, populations are so small that D. ponderosae cannot access healthy trees as there are not enough individuals available to overcome a well-defended host (Amman and Schmitz 1988). Strong resin flow and rapid induced responses in the form of monoterpene synthesis and necrotizing tissue prevent beetles and associated fungi from becoming established. Therefore, endemic beetles must subsist on smaller diameter, vigour-impaired trees (Wood 1982, Safranyik and Carroll 2006).

Vigour-impaired trees, in addition to being hosts for endemic *D. ponderosae*, are the principal resource for a large number of secondary bark beetles (Bright 1976, Safranyik and Carroll 2006). Over 50 species of bark beetle attack lodgepole pine in Canada, the vast majority of which only infest weak trees (Bright 1976). One of these beetles, *Pseudips mexicanus* (Hopkins) (previously *Ips mexicanus*, Cognato 2000), is often found in trees

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attacked in the same or subsequent season(s) by endemic *D. ponderosae* (Carroll et al. 2006). The presence of *P. mexicanus* has been found to facilitate *D. ponderosae* population maintenance where trees occupied by *P. mexicanus* experience greater *D. ponderosae* attack density, tend to produce more female offspring per attack that emerge earlier when compared to trees attacked by *D. ponderosae* alone (Chapter 3). If *D. ponderosae* is experiencing greater fitness in trees previously colonized by *P. mexicanus*, then there should be strong selection pressure for endemic *D. ponderosae* to orient to these beneficial hosts.

It is unclear how endemic *D. ponderosae* locate suitable hosts. Trees attacked by endemic *D. ponderosae* tend to be smaller than the stand average, relatively rare (Chapter 3) and produce lower quantities of phytochemicals compared to healthy trees (Raffa and Berryman 1982). Wallin and Raffa (2004) showed that in a closely related system, *Dendroctonus rufipennis* (Kirby) on spruce, there was a density-dependent host-selection behaviour governed by monoterpene concentrations. The study by Carroll et al (2006) suggested that a similar density-dependent behaviour occurs in *D. ponderosae* populations, but did not suggest a mechanism for host selection in the endemic stage.

Given that the majority of endemic *D. ponderosae* colonize trees that are either currently infested or were previously infested by other bark beetle species (Carroll et al. 2006), endemic *D. ponderosae* may employ secondary cues to locate hosts. Secondary attraction occurs when a beetle focuses on a cue other than that directly from the host, such as the aggregation pheromone of another species of beetle or a combination of aggregation pheromone and host volatiles. Depending upon the subsequent interaction between the sender and the receiver, the pheromone is classified as an allomone, kairomone or synomone. An allomone benefits the emitter upon response by the receiver, kairomonal attraction

benefits only the receiver of the chemical, e.g. many species of predators and parasitoids attracted to bark beetle emissions (Poland and Borden 1997, Aukema et al. 2004, Miller et al. 2005), while a synomone benefits both the receiver and sender (Nordlund 1981). Some bark beetles are cross-attracted to the semiochemicals of other species, for example *Pityogenes knechteli* Swaine to *Ips pini* (Say) (Poland and Borden 1994) and *P. mexicanus* to *Orthotomicus latidens* (LeConte) (Savoie et al. 1998) and may use these chemicals as either a pheromone or a synomone, contributing to resource partitioning (reviewed in Miller et al. 1991).

*P. mexicanus* utilize racemic ipsenol and ipsdienol as attractant pheromones (Carroll and Borden, unpubl.). Hunt and Borden (1988) examined attraction of epidemic level *D. ponderosae* to racemic ipsdienol and determined that beetles were attracted to high concentrations in the laboratory. In field trials no significant response to ipsdienol was detected, but upon the addition of host volatiles and *D. ponderosae* aggregation pheromone, trap catches improved significantly, however; catches were lower when ipsdienol was present. If a density-dependent host-selection strategy exists for *D. ponderosae*, endemic populations may respond to volatile cues differently than epidemic beetles. Since endemic *D. ponderosae* appears to preferentially colonize weakened hosts with *P. mexicanus* present (Chapter 3), it is possible that host selection is mediated by kairomonal or synomonal attraction to racemic ipsdienol with the addition of ipsenol or a combination of the two pheromones and host volatiles.

This study was designed to test the attractiveness of *P. mexicanus* attractant pheromone alone (Experiment 1) or in combination with volatiles generated by the presence of feeding *P. mexicanus* in host material (Experiment 2) to endemic level *D. ponderosae*. I predicted in

the first experiment that traps baited with *P. mexicanus* attractant pheromone would catch more *D. ponderosae* than control traps. In the second experiment, I expected that *P. mexicanus*-infested host material (i.e., bolts) would attract more *D. ponderosae* than either control traps or non-infested host material. If the number of beetles captured at the boltbaited traps was greater than at the pheromone-baited traps, more than just the pheromone is acting to make *P. mexicanus*-infested trees attractive to *D. ponderosae*. However, if the number of beetles captured in the pheromone-baited traps was equal or greater than in the *P. mexicanus*-infested bolt-baited traps, the pheromone can be said to be the main attractant.

## METHODS

## Study Site.

Two trapping experiments were conducted during the summer of 2005 in lodgepole pine stands near Angstad Creek, 28 km south of Merritt BC (49° 51' N, 120° 46' W, mean elevation = 1300 m). The stands used in Experiment 1 were pure pine, while the stands used in Experiment 2 were pine-dominant with a minor component of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). All stands within each experiment had similar species composition, age classes, height classes and stocking levels. During this study, *D. ponderosae* populations were shifting from endemic to incipient epidemic (Safranyik and Carroll 2006), where small groups of trees were being mass attacked. For both experiments, trapping took place between 20 July and 14 September 2005, during the main flight of *D. ponderosae* (Safranyik and Carroll 2006). Twelve-funnel Lindgren traps (Lindgren 1983) were suspended between two trees a minimum of 0.5 m away from the boles, with the collecting cup approximately 0.5 m above the ground. The collecting cup contained a piece

of Vapona No-pest<sup>®</sup> strip (Monsanto Canada Ltd, Mississauga, Ontario, Canada) to kill all captured insects (Campbell and Borden 2006).

## **Experiment 1 - Pheromone**

## Trap layout.

A randomized complete block design was laid out with 10 replicates, which consisted of a treatment trap and a control trap. The control was a blank trap, while the treatment consisted of a lure containing *P. mexicanus* attractant pheromone attached to the midpoint of the trap. The pheromone lure consisted of a pair of polyvinyl bubble caps, one with racemic ipsenol (2-methyl-6-methylene-7-octen-4-ol) and the other racemic ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) (Phero Tech, Inc., Delta, B.C., 2005). Both bubble caps contained 40 mg of their respective chemical with release rates of 0.4 mg/day for the ipsenol and 0.2 mg/day for the ipsdienol at 25 °C. The control and treatment traps were spaced > 40 m apart within the blocks and blocks were separated from each other by > 90m.

#### Experiment 2 - Pine bolt

#### Host material collection.

This experiment used 20 cm long lodgepole pine bolts as bait treatments either infested with *P. mexicanus* or left uninfested. Uninfested and infested pine bolts were used as baits because of the propensity of *Ips* and related species to produce pheromones when feeding (Vite and Gara 1962). Additionally, the bolts were employed as a source of host volatiles to test additive or synergistic properties when encountered with *P. mexicanus* attractant pheromone. Three 25 cm diameter lodgepole pine trees were felled at Angstad Creek on

May 28, 2005 and 3.0 m of the lower bole of each tree was removed and taken to the Pacific Forestry Centre in Victoria, B.C. Twenty-four 20 cm long bolts were cut from each of the logs and the bark was scored vertically on opposite sides to create two discrete bark sections. A three cm diameter eyehook was placed in the top of each. All cuts were sealed with paraffin wax to reduce desiccation and the bolts were placed in a cold room 4 °C until June 18, 2005.

#### Beetle collection.

For purposes of infesting the treatment bolts, *P. mexicanus* were collected alive at both Angstad Creek and on the Aberdeen Plateau, 30 km north-east of Kelowna BC ( $50^{\circ} 5^{\circ}$  N,  $119^{\circ} 11^{\circ}$ W, mean elevation = 1300 m) between June 13 and June 17, 2005, using pheromone-baited 12-funnel Lindgren traps. Collections were made daily to minimize escape, and damage and loss of beetles to predators. Additionally, beetles were collected manually on June 17, 2005 from active galleries at the base of lodgepole pine trees mass attacked in 2004 by *D. ponderosae* at Angstad Creek. All beetles were sorted by sex, placed in a jar with freshly peeled lodgepole pine phloem and stored at 4 °C until needed. *P. mexicanus* males were identified by the presence of a large median tubercle on the frons (Wood 1982), while females were separated by a reduced or absent tubercle..

## Bolt infestation.

On June 18, 2005, four bolts from each of the three harvested trees were randomly selected to be infested with *P. mexicanus*. The gelatine capsule method was used to introduce beetles to the logs (Lanier and Wood 1968). Holes were made in the middle of

each bark section and one male was placed in each hole, secured with a gelatine capsule and left overnight to initiate a nuptial chamber. Beetles that did not begin a nuptial chamber were replaced after 24 hours. When all beetles had successfully entered, two females per hole were introduced. Any female not entering under the bark after 24 hours was replaced (Safranyik and Linton 1983). All bolts were kept at room temperature for three days and subsequently stored at 4 °C until deployed on July 20, 2005.

## Trap layout.

Traps were laid out in a randomized complete block design with 12 replicates consisting of three traps each, two treatment traps and a blank control trap. All traps were 12-funnel Lindgren traps. The two treatments were laid out with either a randomly chosen infested bolt or a non-infested bolt as bait. The bolts were suspended by the eyehook on a steel pipe lashed to two trees and were placed close to, but not touching the traps. The bolts were hung at a height half way down the traps. All of the bolts were covered with sealed mesh sleeves (Poland and Borden 1994) to prevent the escape of resident *P. mexicanus* in the infested bolts and to inhibit attack by local bark beetles and other insects while the bolts were in the field. The control and treatment traps were spaced > 40 m apart within each replicate and the replicates were separated from each other by > 90 m.

## Beetle trapping and identification.

Traps for both experiments were checked on August 10, August 27, and September 14. Collected insects were stored in 70 % ethyl alcohol in 20 ml scintillation vials until sorted. Initial sorting separated bark beetles from other insects. Bark beetles were then identified to species (Linton 2005) and counted. *P. mexicanus*, *D. ponderosae*, *Ips pini* (Say) and *Pityogenes knechteli* Swaine were sorted by sex according to secondary sexual characteristics. *P. mexicanus* were identified as above. *D. ponderosae* males were distinguished by the presence of the stridulation apparatus on the 7<sup>th</sup> abdominal tergite (Lyon 1958). *I. pini* males were identified by the capitate 3<sup>rd</sup> dorsal spine on the elytral declivity (Bright 1976), while *P. knechteli* males were distinguished by a pair of hooked spines on the upper margin of the elytral declivity (Wood 1982). *Orthotomicus latidens* (LeConte) (previously *Ips latidens*; (Cognato and Vogler 2001)) were not sorted by sex.

#### Data analysis.

All trap catch data from both experiments were transformed by  $Y = log_{10}(X + 0.5)$  to address assumptions of normality and homoscedasticity (Tabachnick and Fidell 2001). Tests for outliers were conducted by plotting standardized residuals against dependent variables (beetle counts by species). Outliers were detected for all species in both experiments and all outlier traps were noted to have been located within 10 m of three or more trees recently mass attacked by *D. ponderosae*. A number of species of secondary bark beetles attack trees killed by *D. ponderosae* (Safranyik et al. 1999, Carroll et al. 2006) and, to eliminate any nontreatment effects, all outlier traps were removed from the analysis. In both experiments, an ANOVA was applied with transformed trap capture data as the dependent variable, treatment as a fixed factor and block as a random factor. A Ryan-Einot-Gabriel-Welsch (REGW) Ftest was used to assess differences between treatments in the bolt experiment. All means are reported as untransformed data  $\pm$  one standard error. Tests were reviewed in Sokal and Rohlf (1995) and analyses conducted with SPSS 13.0 (SPSS 2004). For all tests,  $\alpha = 0.05$ .

## RESULTS

## Experiment 1 - Pheromone

Total catches for D. ponderosae were small with four caught in pheromone traps and only one in a control trap. Catches for O. latidens were similar, with five beetles caught in pheromone traps and four in control traps. Both species subsequently were dropped from the The number of captured *P. mexicanus* males and females were significantly analysis. affected by treatment (Table 4.1). The number of males caught in the control traps was zero and a mean of 5.4  $\pm$  1.2 were captured in the pheromone-baited traps, while 0.4  $\pm$  0.4 and  $32.0 \pm 3.9$  females were caught in the control and baited traps, respectively (Fig. 4.1A). Females were captured in numbers approximately six times that of males in the pheromonebaited traps. Block effects were not significant for any species except for male *I. pini* (Table 4.1), where a pheromone trap in one block captured over 60 % of the total beetles (N = 26). This resulted in male *I. pini* captures in the pheromone traps not differing significantly from the control traps due to high variability in pheromone captures (Fig. 4.1B). However, significantly more females were captured in the pheromone-baited traps  $(11.8 \pm 4.5)$  than the control traps (0.2  $\pm$  0.2) (Table 4.1; Fig. 4.1B). Additionally, more than twice as many females as males were caught per pheromone-baited trap (Fig. 4.1B). Neither sex of P. knechteli was captured more in the pheromone-baited traps than in the control traps (Table 4.1), but more females were captured than males on average in the treatment traps  $(4.4 \pm 2.6)$ and  $1.4 \pm 1.2$  respectively).
**Table 4.1**. Analysis of variance test on log-transformed capture data for bark beetle species collected from a field trapping experiment with treatment as a fixed factor and block as a random. Treatments consisted of a control trap and a trap baited with *Pseudips mexicanus* attractant pheromone, racemic ipsenol and racemic ipsdienol.

			Male			Femal	e	
Species	Effect	d.f.	MS	F	Р	MS	F	Р
P. mexicanus	Treatment	1	2.658	108.596	<0.001	6.894	178.444	<0.001
	Block	4	0.024	1.000	0.500	0.073	1.879	0.278
	Intercept	1	0.460	18.802	0.012	4.477	61.659	0.001
I. pini	Treatment	1	0.153	1.000	0.500	3.239	14.431	0.019
-	Block	4	1.684	11.003	0.029	0.044	0.196	0.928
	Intercept	1	0.120	0.781	0.427	1.322	30.051	0.005
P. knechteli	Treatment	1	0.105	0.581	0.694	1.122	5.295	0.083
	Block	4	0.124	0.686	0.454	0.212	1.000	0.500
	Intercept	1	0.089	0.845	0.410	0.011	0.054	0.827



**Figure 4.1.** Number of bark beetles captured during a field trapping experiment using a combination of racemic ipsenol and ipsdienol as the pheromone bait; (A) *Pseudips mexicanus*; (B) *Ips pini*. Bars for each sex with the same letter are not significantly different (P < 0.05, N = 5). Con = control, Treat = pheromone treatment.

# Experiment 2 - Pine bolt

D. ponderosae and I. pini were not attracted to either treatment or control traps, resulting in the capture of only three D. ponderosae and zero I. pini. Neither species was included in the analysis. Block did not significantly affect trap catches for any species (Table 4.2). A total of 13 female and five male P. knechteli were caught, but traps baited with either bolt treatment were not significantly more attractive than the control traps (Table 4.2). P. *mexicanus* males were more attracted to the traps baited with the uninfested bolts  $(4.4 \pm 1.3)$ than to the control traps (0.2  $\pm$  0.1), while attraction to the infested bolt-baited traps (2.8  $\pm$ 1.0) did not differ from either of the other two treatments (Table 4.2; Fig. 4.2). Female P. *mexicanus* were not captured at either treatment trap  $(8.0 \pm 2.4 \text{ and } 7.4 \pm 2.2 \text{ uninfested and})$ infested, respectively) in numbers significantly different from the control traps  $(1.9 \pm 1.0)$ (Table 4.2). Although captures at the traps baited with the bolts tended to be relatively high, the variance associated with the number of beetles caught in the control traps was large compared to control trap catches for *P. mexicanus* males and *I. latidens* (Figs. 4.2 & 4.3), resulting in the lack of difference between treatments. The number of females caught in traps baited with the uninfested bolts was approximately twice as high as the number of males, and the number of females caught in traps baited with infested bolts was over 2.5 times greater than the trap catches for males. Comparing the number of *P. mexicanus* caught between the two experiments, the pheromone treatment in Experiment 1 captured approximately four times as many females per trap as those caught in each of the bolt treatments in Experiment 2 (Figs. 4.1A & 4.2). Males were caught in similar numbers between treatments in each experiment.

**Table 4.2**. Analysis of variance test on log-transformed capture data for bark beetle species collected from a field trapping experiment with sex and treatment as factors. Treatments were comprised of a control trap, a trap baited with a lodgepole pine bolt infested with *Pseudips mexicanus* and a trap baited with an uninfested pine bolt.

			Male			Female	Female		
Species	Effect	d.f.	MS	F	P	MS	F	Р	
P. mexicanus	Treatment	2	1.141	7.080	0.006	0.810	2.424	0.120	
	Block	8	0.242	1.505	0.231	0.471	1.408	0.266	
	Intercept	1	1.092	4.504	0.067	5.395	11.462	0.010	
P. knechteli	Treatment	2	0.034	1.000	0.390	0.114	1.224	0.320	
	Block	8	0.040	1.188	0.365	0.074	0.798	0.613	
	Intercept	1	1.221	30.493	0.001	0.513	6.927	0.030	
O. latidens*	Treatment	2	1.740	4.897	0.022				
	Block	8	0.350	0.986	0.482				
	Intercept	1	5.136	14.668	0.005				

\* O. latidens were not segregated by sex; therefore, mean square, F and P values are reported for total number of beetles captured.



Treatment by sex

Figure 4.2. Number of *Pseudips mexicanus* captured during a field trapping experiment with lodgepole pine bolts as attractive baits either alone or infested by *P. mexicanus*. Bars for each sex with the same letter are not significantly different; REGW multiple comparisons test (P < 0.05, N = 9).



Figure 4.3. Number of *Orthotomicus latidens* captured during a field trapping experiment with lodgepole pine bolts either uninfested or infested by *P. mexicanus* as attractive baits. Bars with the same letter are not significantly different; REGW multiple comparisons test (P < 0.05, N = 9).

#### DISCUSSION

Contrary to predictions, my findings showed that endemic *D. ponderosae* was not attracted to either P. mexicanus attractant pheromone alone or in combination with volatiles associated with feeding P. mexicanus. Additionally, D. ponderosae did not perceive the uninfested bolts in Experiment 2 as attractive. Improved fitness correlates associated with endemic D. ponderosae in trees attacked by P. mexicanus (Chapter 3) suggest that these hosts should be actively sought by dispersing *D. ponderosae*. However, it appears that the presence of ipsenol does not mitigate the repellent effect of ipsdienol to flying D. ponderosae (Hunt and Borden 1988), explaining the lack of attraction to P. mexicanus pheromone-baited traps. Lack of attraction to the infested bolt treatment in Experiment 2 by D. ponderosae may have been a reaction to the production of ipsdienol by feeding male *P. mexicanus*, but since D. ponderosae was not captured at either of the bolt treatments, it is likely that the bolts were not producing the appropriate host volatile cues and may not have been sufficiently fresh. Interestingly, both *P. mexicanus* and *O. latidens* were strongly attracted to these traps, and both species are found in weak living hosts subsequently attacked by endemic D. ponderosae (Chapter 3) suggesting D. ponderosae seeks the same sort of host material. The lack of response by D. ponderosae to either P. mexicanus attractant pheromone or the two bolt treatments (infested or not) indicates endemic D. ponderosae use cues other than those employed in this study. Alternate cues may be host chemicals produced by living trees during defensive responses to P. mexicanus attack or inoculation with fungi associated with *P. mexicanus* or a combination thereof.

As was expected, attraction to the pheromone source was very strong for *P. mexicanus* in Experiment 1, especially for females. The sex ratio of attacking *P. mexicanus* is

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approximately two females/male (Chapter 2) while the observed response to the pheromone trap was six females/male. A number of factors conceivably contributed to the observed female bias. First, the two sexes are likely differentially attracted to attractant pheromones (Miller et al. 1991, Savoie et al. 1998, Miller et al. 2005). Lower numbers of males attracted to a pheromone source would be adaptive, helping to limit over-utilization of a scarce resource through moderation of attack densities, while attracting multiple mates to maximize fitness (Raffa et al. 1993). Second, the pheromone baits may have been releasing chemicals at a higher rate than would be experienced in the field, drawing more females to the traps than seen on attacked trees, as shown for *I. pini* (Miller et al. 2005). Third, the synthetic blend of the attractant pheromone did not include any host volatiles, which may have contributed to lower catches of males (Borden et al. 1983, Hunt and Borden 1988, Pureswaran and Borden 2005). The combination of these three factors may have artificially skewed the trap catches to favour female *P. mexicanus*, but findings for other species such as *D. pseudotsugae* Hopkins suggest that bias toward responders well beyond the sex ratio seen during attack and mating is not unusual (Pureswaran and Borden 2005).

Experiment 2 revealed a positive response by *P. mexicanus* to the host material, irrespective of the presence of feeding beetles. Based on the high catches at the pheromonebaited traps in Experiment 1, it was expected that the bolts with *P. mexicanus* feeding in them would attract more conspecific beetles, as *Ips* and related species produce attractant pheromone while the male is feeding and clearing frass (Vite and Pitman 1968), however; others suggest that as males acquire a complete harem, pheromone production decreases (reviewed in Miller and Borden 1990). The lack of difference between bolt treatments suggests a complete harem was present in the infested bolts and that the amount of pheromone being produced was not as compelling as the bolt volatile cues for either sex. The strong, but not significant (Fig. 4.2), kairomonal attraction of the females indicates that both sexes are capable of detecting suitable host material. This supports the findings in Chapter 2 of previously mated females having the ability to locate hosts in the absence of males. Other species, such as *O. latidens*, have not been observed to exhibit sex-specific responses to monoterpenes (Miller and Borden 1990). Thus, it is unclear why more female *P. mexicanus* than males would be attracted, since the males are the pioneering sex, and of the 81 galleries inspected in Chapter 2, 97.5 % had males associated with them.

Additional secondary bark beetle species commonly associated with *D. ponderosae* and *P. mexicanus* were captured. In the first experiment, *Ips pini* (Say) was attracted to the pheromone-baited traps (Fig. 4.1B), however; catches may have been limited by the presence of the ipsenol component (Furniss and Livingston 1979, Borden et al. 1992) which has been shown to reduce, but not completely preclude, *I. pini* attraction to ipsdienol. The second experiment captured no *I. pini*, clearly indicating a lack of attraction to the host material and to the presence of *P. mexicanus* within the infested bolts. *I. pini* colonizes recently dead, often horizontal trees and slash, as well as the tops of standing trees killed by *D. ponderosae* (Wood 1982). Because of this, it was suspected that *I. pini* would find both bolt treatments attractive, especially the uninfested ones; however, the lack of attraction suggests that the appropriate host volatile cues were not present or that populations of *I. pini* in those areas were particularly small.

O. latidens was caught in very small numbers in Experiment 1, indicating potential inhibition by P. mexicanus attractant pheromone. This was expected since Miller and Borden (1992) found trap catches of O. latidens significantly reduced when racemic

ipsdienol was added to racemic ipsenol. In Experiment 2, *O. latidens* was found to be attracted to both bolt treatments. The slightly lower numbers caught in traps baited with *P. mexicanus*-infested bolts compared to uninfested bolts may be due to inhibition by ipsdienol produced by feeding *P. mexicanus* (Vite and Gara 1962). However, bark beetles are able to distinguish quantitative differences in monoterpene plumes (Wallin and Raffa 2000, Pureswaran et al. 2004), so the presence of *P. mexicanus* degrading the host material by opening the phloem to oxidization and desiccation, inoculating the subcortical environment with fungi and yeasts (Whitney 1982), and physically destroying portions of the phloem and sapwood through gallery construction may have acted as an additional deterrent.

The final species to be caught in these experiments, *P. knechteli*, was caught in higher numbers in Experiment 1 than in Experiment 2. Savoie et al. (1998) found ipsdienol to be the aggregation pheromone of this species, but noted that the addition of (*S*)-(-)-ipsenol significantly decreased the number of beetles caught, however; the inclusion of host volatiles with racemic ipsdienol alone elicited significant attraction responses (Miller and Borden 2003). The number of beetles caught in Experiment 1 are very similar to the trap catches of Savoie et al. (1998), suggesting racemic ipsenol is also inhibitory to *P. knechteli*. The low numbers of beetles caught at the bolt treatments in Experiment 2 may be the result of missing host volatiles required to stimulate attack, and since *P. knechteli* are normally found attacking high in the canopy (Safranyik et al. 2000), they may not be present in large numbers within 1.5 m of the ground.

The results of this study demonstrate that the members of the community of bark beetles that exist in lodgepole pine forests in south-central British Columbia are differentially attracted to either *P. mexicanus* attractant pheromone or infested host material, likely as a mechanism to reduce competitive interactions between them (Safranyik et al. 2000). The focal species of this investigation, *D. ponderosae*, in the endemic stage attacks trees previously infested by *P. mexicanus*, but does not use secondary attraction to *P. mexicanus* attractant pheromones to locate such hosts. Additionally, dead hosts, regardless of the presence or absence of *P. mexicanus*, are not attractive to *D. ponderosae*. However, a living but severely weakened host previously occupied by *P. mexicanus* and subsequently attacked by *D. ponderosae* benefits *D. ponderosae* by facilitating population maintenance (Chapter 3). To take advantage of this, *D. ponderosae* must rely upon other stimuli such as host volatile signatures and silhouette recognition to locate suitable material while in the endemic stage.

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# CHAPTER 5

### GENERAL CONCLUSIONS

This group of studies was undertaken to quantify the potential effects of the presence of a secondary bark beetle, P. mexicanus, on endemic D. ponderosae cohabiting weakened lodgepole pine trees. To properly evaluate this interaction, the life history of P. mexicanus required elucidation (Chapter 2). Prior work had examined a number of life history traits of P. mexicanus, but these studies involved different host-tree species in different climatic regions (Trimble 1924, Schwerdtfeger 1956, Struble 1961, Fox et al. 1990). Three of these studies were conducted on populations breeding in Monterey pine in California and one in Mexican white pine in Guatemala. The populations of P. mexicanus that I studied in lodgepole pine forests of south-central British Columbia were found to be univoltine, but developed more rapidly than Californian or Central American beetles (Schwerdtfeger 1956). Beetles in lodgepole pine laid substantially fewer eggs than in either of the other tree species (Trimble 1924, Schwerdtfeger 1956), a likely result of different climatic and host conditions, since beetles in BC may have stopped egg laying early in the summer to allow offspring to prepare for overwintering. The attack behaviour of northern populations also differed significantly. In BC, the beetles attacked and overwintered exclusively in the lower portion of the bole whereas southern beetles tend to colonize the whole tree (Trimble 1924, Schwerdtfeger 1956). This may be an adaptation to diminish the effect of cold-induced mortality by confining attacks to the portion of the bole that is covered by snow during the

coldest periods of the winter. Southern US and Central American beetles also tended to make use of downed trees and slash, behaviours not noted during these investigations.

Northern populations were similar to southern ones in terms of polygyny, ovipositional characteristics (i.e., # of eggs per egg niche) and development rate. In Canada, *P. mexicanus* is a common forest insect that is not an economic threat to the forestry industry. It has, however; been a concern in California because of its ability to disseminate the pitch canker fungus in Monterey pine (Fox et al. 1991). Moribund trees are typical hosts for *P. mexicanus* in Canada and, through removal of suppressed and/or damaged trees, *P. mexicanus* likely functions to maintain lodgepole pine stand health.

The second study examined interactions between *D. ponderosae* and *P. mexicanus* (Chapter 3). Evidence from Carroll et al. (2006a) suggested that *P. mexicanus* may somehow improve host conditions for endemic *D. ponderosae*. Amman and Schmitz (1988) also proposed that endemic *D. ponderosae* may benefit from cohabitation with secondary bark beetles. My study quantified this phenomenon, by showing that *D. ponderosae* attacking hosts previously occupied by *P. mexicanus* did so in greater densities than in trees where *P. mexicanus* was absent, with no loss in offspring size from crowding. *D. ponderosae* offspring emerging from *P. mexicanus*-infested trees developed faster than in uninfested trees, suggesting that endemic *D. ponderosae* attacking trees previously infested by *P. mexicanus* have access to better resources than they do when attacking trees alone.

The final study was conducted based on predictions that *D. ponderosae* searching for hosts would actively seek trees infested with *P. mexicanus*. However, results of trapping experiments (Chapter 4) showed that *D. ponderosae* was not attracted to *P. mexicanus* attractant pheromone or to bolts of lodgepole pine containing feeding *P. mexicanus*. Since

secondary attraction to *P. mexicanus* attractant pheromones did not occur, it appears that other volatile phytochemicals from living trees play a dominant role in host selection during endemic *D. ponderosae* dispersal.

The principal results of my research provide evidence for population maintenance by an eruptive herbivore when in a non-eruptive population state. Furthermore, the interaction between *D. ponderosae* and *P. mexicanus* suggests that management of *P. mexicanus* populations may contribute to a reduction of endemic *D. ponderosae* on the landscape. Both species are a natural component of a healthy lodgepole pine ecosystem and when *D. ponderosae* is in endemic populations, both species play a role in reducing the number of standing unthrifty trees, keeping stands sanitized. However, the development of over-mature forests (Taylor and Carroll 2004), coupled with climate change trends (Carroll et al. 2004), have resulted in the most devastating *D. ponderosae* outbreak in recorded history (e.g. Kurz et al. 2008). Management in the mid-1990's for *P. mexicanus* may not have prevented this *D. ponderosae* outbreak, but the potential of future epidemics may be reduced by observing secondary bark beetle populations (Carroll et al. 2006a) and reacting accordingly through either direct (reduction of *D. ponderosae* populations) or indirect (reduction of susceptible trees) control methods (Carroll et al. 2006b).

Another important aspect of my work is the determination of positive interactions occurring between insect species. This example of facilitation in the insect world supports the work of Kaplan and Denno (2007), who advocate for the adoption of a new paradigm to account for positive interactions between insect species within a competition framework. Understanding that *P. mexicanus* improves host conditions for endemic *D. ponderosae* leads to the question of what role the other secondary species commonly associated with *P.* 

*mexicanus* (*Orthotomicus latidens*, *Hylurgops* spp.) play in *D. ponderosae* population dynamics. Further work to examine this complex trophic interaction would be needed to fully understand the bark beetle community ecology in weakened lodgepole pine trees.

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