

DISSERTATION

MOUNTAIN PINE BEETLE-CAUSED MORTALITY, HOST SELECTION, AND
PERFORMED TREE DEFENSES IN LODGEPOLE AND PONDEROSA PINE OF THE
FRONT RANGE, COLORADO

Submitted by

Daniel R. West

Department of Bioagricultural Sciences and Pest Management

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2013

Doctoral Committee:

Advisor: William Jacobi

Jennifer Briggs

Jason Sibold

Louis Bjostad

Copyright by Daniel R West 2013

All Rights Reserved

ABSTRACT

MOUNTAIN PINE BEETLE-CAUSED MORTALITY, HOST SELECTION, AND PERFORMED TREE DEFENSES IN LODGEPOLE AND PONDEROSA PINE OF THE FRONT RANGE, COLORADO

Eruptive mountain pine beetle (*Dendroctonus ponderosae*) populations that developed in the late 1990's and early 2000's over the southern Rocky Mountains have resulted in widespread pine-mortality throughout the region. Concerns escalated in 2007 as to whether northern Colorado ponderosa pine- (*P. ponderosa*) dominated stands were susceptible to attack from beetles moving from adjacent stands of mountain pine beetle killed lodgepole pine (*P. contorta* var. *latifolia*). Early observations by A. D. Hopkins suggested alternate host species are unlikely to sustain attack from beetles that developed in a different host species. This dissertation addressed the research question of whether mountain pine beetles were likely to infest ponderosa pine trees from populations that developed within lodgepole pine. I used three approaches to answer the question: 1) a monitoring survey of Front Range, CO., USA, lodgepole-ponderosa pine ecotone stands for five time periods (2004-7, 8, 9, 10, 11) to quantify mortality levels in both host species (Chapter 1); 2) a controlled-field choice experiment where beetles that developed in either lodgepole or ponderosa pine were offered cut-logs of both lodgepole and ponderosa pine (Chapter 2); and 3) a bioassay where individual female mountain pine beetles that developed in either host species were offered bark/phloem units of both host species (Chapter 2). Results from monitoring mountain pine beetle-caused mortality indicated both species were attacked in similar proportions over time. Both the controlled-field studies and laboratory bioassays of mountain pine beetle host selection found beetles preferred ponderosa pine over lodgepole pine 2:1. Results from the choice experiments were consistent with the findings of primary host selection

in laboratory bioassays, indicating beetle behavior in the presence of aggregation pheromones within the field arenas was no different than host selection behavior in the absence of aggregation pheromones in the bark/phloem laboratory bioassay arenas.

The initial conifer defenses against bark beetle attack are preformed quantitative and qualitative defenses produced prior to being wounded or attacked. The objective of Chapter 3 was to assess the preformed (24-hr) oleoresin defenses of lodgepole pine and ponderosa pine in mixed pine ecotone stands. The amounts of exuded preformed oleoresins – the quantitative defenses – were almost four times greater in ponderosa pines than lodgepole pines. Differences were found in all but three of the constitutive monoterpenes – the qualitative defenses – between the two tree species. Greater concentrations of limonene, β -phellandrene, and cymene were identified in lodgepole pine than ponderosa pine oleoresins. The monoterpenes of β -pinene, 3-carene, myrcene, and terpinolene were all greater in ponderosa pine than lodgepole pine oleoresins. Although we found differences both in quantitative and qualitative preformed oleoresin defenses, hypotheses linking the ecological relevance of these differences to bark beetle susceptibility have not been tested. The results from the field monitoring surveys where both species were attacked in similar proportions differ from the findings of the choice tests where the beetles infested greater proportions of ponderosa pine logs than lodgepole pine logs. I infer that, under natural conditions, the greater amount of oleoresin flow and/or the amount of specific monoterpenes in ponderosa pine oleoresin compared to lodgepole pine may have limited the rate of mass attacks on ponderosa pine and served to equilibrate the amount of mortality we recorded in both species in the forest stands.

To my knowledge, this is the first documentation of mountain pine beetles infesting both lodgepole pine and an alternate host, ponderosa pine, at landscape scales during epidemic

conditions in Colorado. Managers should be aware there are no apparent major host characteristics the mountain pine beetle cannot overcome that might prevent the movement from lodgepole pines into ponderosa pines. Rather, my results indicate that the availability, condition, and defense capability of host trees influence their susceptibility to the mountain pine beetle.

ACKNOWLEDGEMENTS

This dissertation and associated manuscripts would not have been possible without the support and guidance of numerous family, friends, and colleagues. The contributions and efforts of the following individuals are greatly appreciated.

A special thanks to my wife, Barbara, for her unwavering support, patience, and love. She put up with 6 months a year of field work between 2009 and 2011, much of which I was gone for weeks at a time. She supported me while I constructed emergence cages, rearing boxes, and choice arenas in our garage into the wee hours of the night. She tolerated turning our home into a working laboratory during trials of bioassays and the mounting of hundreds of insects for measurement. She nursed me back to health and tolerated me after I herniated and ruptured two discs in my back. Overall, she shared a wealth of laughter and provided support throughout. Hansi inquisitively listened to concerns, provided an excuse for daily exercise, listened while I practiced presentations, and offered continuous comic relief. Thanks to my parents Bob and Cheryl, and in-laws Trudy and Don for continued support and encouragement.

I would like to thank my academic committee for their assistance and guidance throughout my studies. Bill Jacobi offered continued support and guidance throughout the program and offered advice in a multitude of conundrums. As my major professor/advisor, Bill was invaluable to the completion of this dissertation. Bill offered invaluable expertise and shared his knowledge in forest health, which I will always be grateful for. I would especially like to thank Jenny Briggs, who hired me as a field crew technician with the U.S. Geological Survey while I finished my Master's thesis. Jenny provided funding, assisted with all aspects of the survey experimental design, field surveyed sites with me, assisted with interpreting results and tirelessly offered

support in writing manuscripts. Both Bill and Jenny supervised cooperative agreements and ensured financial stability throughout the program. The majority of this dissertation would not have been possible without the support of both Jenny and Bill. Funding from the USGS was awarded through grants from the Central Region State Partnership Program and the Rocky Mountain Area in a Cooperative Ecosystem Studies Unit agreement between Colorado State University and the USGS Rocky Mountain Geographic Science Center. Lou Bjostad provided lab space and time for the chemical analyses of resins, insect lipids, and tree carbohydrates. Lou joined the committee in the last year of my program and offered expertise as well as moral support. Jason Sibold provided valuable input interpreting the results and the presentation of the findings.

Sheryl Costello and Jose Negrón offered valuable discussion in designing and implementing the surveys of Front Range mountain pine beetle-caused mortality. Sheryl Costello helped with contacting personnel and access within the US Forest Service and with arranging permits in Rocky Mountain National Park. Sheryl was a tremendous help with logistics and an overall moral support. She is greatly missed. Jose Negrón provided me lab space and offered logistic support for field crews at the US Forest Service, Rocky Mountain Research Station. Dave Hattis and Dice Gayton provided logistic for, and permits for field work on US Forest Service managed lands. Pam Mauritsen, USGS, offered logistic support for field vehicles and always had a solution during maintenance and accident repair.

I would like to thank Sky Stephens and Joe Duda, Colorado State Forest Service, who provided funding for field crews and facilitated access to several site locations. Therese Glowacki, Scott Golden, and Chad Julian, Boulder County Parks and Open Space, provided funding for field crews, and offered logistic support for permits of field-survey work and access to sites within

their managed lands. Dan Weber, Colorado State Parks, and Jeff Connor, Rocky Mountain National Park, provided logistic support and permits for site access.

The following individuals worked tirelessly as field crew members and/or lab technicians: Brison Bishop, Eric Eden, Justin Pomeranz, Tim Mapoles, Patrick Flynn, Paul Cigan, Rebecca Howell, Kevin Miller, Zach Wehr, Jake Thomsen, Peter Pavlowich, and Lea Pace. This dissertation would not have been possible without their hard work, understanding, comedic refuge, and moral support.

The support staff of the Bioagricultural Sciences and Pest Management department provided logistic and overall help with teaching, paperwork, and account management. Special thanks to Janet Dill, Paula White, Maggie Hirko, and Department Head Tom Holtzer.

I would like to thank the students, past and present, in Bill Jacobi's Forest and Shade Tree Lab. In particular, I would like to thank Meg Dudley, Javier Mercado, and Christy Cleaver for their support, putting up with me in the office, engaging in conversations over conundrums, offering comic relief, and providing a pleasurable working environment.

PREFACE

The mountain pine beetle (*Dendroctonus ponderosae* Hopk.) is one of only a few bark beetles out of over 600 Scolytinae species that periodically erupt to populations causing widespread-pine mortality. Much of western North America experienced beetle-caused mortality in the late 1990's and early 2000's. In Colorado, the vast majority of beetle-caused mortality occurred in lodgepole pine dominated stands from the 1990's to 2007. As mortality expanded through the mixed conifer lodgepole-ponderosa pine ecotone, concerns from land managers mounted regarding the susceptibility of ponderosa pine dominated forests. Previous observational and manipulative studies provided mixed evidence whether the beetle would transition between hosts. This dissertation significantly advances our understanding of mountain pine beetle host selection between two alternate host species in mixed stands and is the first to combine field monitoring surveys of mortality in two host species over multiple years with manipulative experiments to address mountain pine beetle host selection behavior. Chapter 1 focuses on monitoring of mortality in the ecotone of lodgepole and ponderosa pine conducted along northern Colorado's Front Range. There were 19 sites in the ecotone selected to address the research question of whether mountain pine beetle-caused mortality levels in ponderosa pine stands would be similar to lodgepole pine stands. Sites spanned 140 km located just south of the Wyoming state border to Golden Gate Canyon State Park, just south and west of Denver. Surveys of mountain pine beetle-caused mortality were conducted to compare mortality levels of ponderosa pines and lodgepole pines for the years of attack from 2008 through 2011. We estimated mortality for the years of 2004 through 2007, which we considered background or endemic levels.

Chapter 2 focuses on manipulative experiments of host selection and emergence phenology of the mountain pine beetle in mixed lodgepole-ponderosa pine ecotone stands. Choice experiments were conducted in the field in 2010 and 2011 to determine host selection behavior of beetles that developed in each host species and were offered both alternate host species for future infestation. The mountain pine beetle uses in part, tree volatiles, visual cues, and random landing to find suitable host trees. Once a tree has been selected, gustatory (feeding) acceptance allows bark and phloem mining to occur. These stepwise processes are known as primary host selection. During the mining process, host volatiles are oxidized and emitted from the beetles as monoterpenes within the subcortical tissues. These serve as aggregation pheromones that attract con-specifics, or other mountain pine beetles, to the tree. The aggregation of con-specifics in response to the pheromone blend is known as secondary host selection. To test whether host selection behavior of groups of beetles in the field choice experiments differed from primary host selection, bioassays were conducted using individual insects and offering them sections of both host species for infestation in small closed containers.

Annual emergence phenology of mountain pine beetle that developed in either lodgepole or ponderosa pine was previously unresolved. Weekly beetle emergence frequencies from the two hosts were conducted in the field using emergence cages built from bronze screen. Peak emergence periods and sex ratios were quantified for beetles that developed in both lodgepole and ponderosa pine. This is the first study comparing *Dendroctonus* emergence times from two host species under attack in the same forest stands.

Chapter 3 describes host defense studies comparing lodgepole pine and ponderosa pine in the Front Range, CO. Trees have two types of preformed host defenses which may serve as deterrents or toxins to attacking insects: The quantitative (the amount of oleoresin released in

response to the attack) and qualitative (the chemical constituents of that oleoresin). The choice experiments described in Chapter 2 offered beetles cut-logs or bark/phloem units where oleoresin flow had been debilitated by cutting otherwise healthy trees. To compare preformed defenses between the two hosts, oleoresin flow was measured in live trees at three locations in northern Colorado. Trees from both species were paired in the field based on growing conditions, diameter, and dominance status. Twenty-four hour amounts of oleoresin flow (quantitative defenses) were collected to test for differences in amount between the two host species. Oleoresin constituents (qualitative defenses) were extracted from the oleoresin from each host using hexane as a solvent. The chemical constituents were identified and quantified using gas chromatography-flame ionization detection. The quantification of differences in host defenses in these two pine species provides the groundwork for future study of the complexity in mountain pine beetle host selection-host defense relationships.

TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	v
PREFACE.....	viii
TABLE OF CONTENTS.....	xi
LIST OF TABLES.....	xiii
LIST OF FIGURES.....	xiv
CHAPTER 1.....	1
MOUNTAIN PINE BEETLE-CAUSED MORTALITY CHRONOLOGY IN LODGEPOLE AND PONDEROSA PINES IN THE SOUTHERN ROCKY MOUNTAINS.....	1
Summary.....	1
Introduction.....	2
Methods.....	6
Data analyses:.....	7
Results.....	9
Discussion.....	11
References.....	28
CHAPTER 2.....	29
MOUNTAIN PINE BEETLE HOST SELECTION BETWEEN LODGEPOLE AND PONDEROSA PINES IN THE SOUTHERN ROCKY MOUNTAINS.....	29
Summary.....	29
Introduction.....	30
Materials and Methods.....	37
1.1 Emergence phenology.....	37
1.2. Sex ratio of emerging beetles.....	38
1.3. Host selection: controlled field experiment.....	39
1.4. Host selection: bioassays with individual beetles.....	41
1.5. Reproductive success.....	43
Results.....	43
2.1. Emergence phenology.....	43
2.2. Sex ratios.....	44
2.3. Host selection: controlled field experiment.....	45
2.4. Host selection: bioassays with individual beetles.....	46

2.5. Reproductive success	46
Discussion	47
Sympatric host races and sex ratios	48
Hopkins' Host Selection Principle.....	48
Host defenses and future research needs	51
References	66
CHAPTER 3	70
HOST DEFENSE MECHANISMS AGAINST BARK BEETLE ATTACK DIFFER BETWEEN PONDEROSA AND LODGEPOLE PINES	70
Summary	70
Introduction.....	71
Methods and Materials.....	77
Study Sites.	77
Quantitative host defense mechanisms.	78
Qualitative defense mechanisms.....	79
Chemicals.....	80
Results.....	80
Discussion.....	81
References.....	91

LIST OF TABLES

Table 1.1. Live trees per hectare and basal areas (m ² /ha greater than 2.5cm diameter at 1.4 m) in 2008 at 19 Front Range, CO. sites with 3 plots per site (N=57).....	18
Table 1.2. Number and percentage of plots on which trees were killed by the mountain pine beetle, and the mean and maximum basal area infested across all plots. Plots were located in the lodgepole-ponderosa pine ecotone in the Front Range, CO. (N=57 plots). Sites comprised 3-0.20 ha plots (N=19 sites).	18
Table 1.3. Uninfested lodgepole pine and ponderosa pine density and basal area in 2008 along Front Range, CO. sites (N=19). Sites comprised 3-0.20 ha plots in the lodgepole-ponderosa pine ecotone (N=57).	19
Table 1.4. Average cumulative (2004-2011) mountain pine beetle-caused mortality in <i>Pinus contorta</i> and <i>Pinus ponderosa</i> within Front Range, CO sites from north to south. Sites comprised 3-0.20 ha plots in the lodgepole-ponderosa pine ecotone (N=57).	20
Table 1.5. Average diameter (cm) by year of mountain pine beetle-caused lodgepole and ponderosa pine mortality along the Front Range, CO. Differing letters indicate significance at alpha=.05. N=57 plots. Mean (lower LSD, Upper LSD).....	21
Table 1.6. Mixed model regression effects for predicting current year mountain pine beetle-caused ponderosa pine mortality (basal area (BA); m ² /ha) in response to the lagged (previous year's) mountain pine beetle-caused mortality in ponderosa pine and lodgepole pine, with the available ponderosa pine basal area by year (2008-2011).	22
Table 2.1. Emerged mountain pine beetles (meter ² of each tree) and the number of trees with emergence per week from lodgepole pine (<i>P. contorta</i>) and ponderosa pine (<i>P. ponderosa</i>) between 2590 and 2650 m of elevation. Collections were conducted near Molly Lake area (2010) and Pennock Pass areas (2011) on the Arapaho-Roosevelt NF. CO. A total of 60 trees were randomly selected in mid-June each year; 30 traps were attached to lodgepole pine and 30 to ponderosa pine.	54
Table 2. 2. Effect significance from four models (y=response) describing mpb host selection, offspring production, offspring per female, and gallery length per female between lodgepole and ponderosa pine in controlled-field-choice experiments (2010-11; α=0.05).	55
Table 3. 1. Qualitative constitutive oleoresin composition (micrograms/gram oleoresin) from lodgepole pines (<i>P. contorta</i> var. <i>latifolia</i> ; n=10) and ponderosa pines (<i>P. ponderosa</i> ; n=10) sampled from Front Range, Colorado. Collections were conducted in July and August of 2011. Monoterpene compositions were identified using Gas Chromatography-Flame Ionization Detection and quantified using Clarity software.	86

LIST OF FIGURES

Figure 1.1. Study sites (2009-11) in the lodgepole-ponderosa pine ecotone along the Front Range, CO. USDA Forest Service and its partners, 2008 Aerial Detection Survey data depicting mountain pine beetle-caused mortality in lodgepole pines and ponderosa pines in 2007. 23

Figure 1.2. Study sites (2009-11) in the lodgepole-ponderosa pine ecotone along the Front Range, CO. USDA Forest Service and its partners, 2012 Aerial Detection Survey data depicting mountain pine beetle-caused mortality in lodgepole pines and ponderosa pines in 2011. 24

Figure 1.3. Plot design used in mountain pine beetle-caused mortality surveys in lodgepole-ponderosa pine ecotone sites along the Front Range, CO. (N=57 plots (0.2 hectares) at 19 sites; 3 circular sub-plots per plot (200 meters² each)). 25

Figure 1.4. Annual percent basal area (m²/ha; mean \pm ½ LSD) of mountain pine beetle-caused mortality of that year’s available lodgepole and ponderosa pine basal area along the Front Range, CO. in 2004-2011. Differing letters indicate significant differences between years ($\alpha=0.05$). N=19 sites with 57 plots; 3 plots per site. 26

Figure 1.5. Predicted current year’s basal area of ponderosa pine mountain pine beetle-caused mortality in response to the lagged (previous year) basal area of mountain pine beetle infested ponderosa pine, average previous years’ mountain pine beetle infested lodgepole pine basal area, and available uninfested median ponderosa pine by year (2008-11). 27

Figure 2. 1. Mountain pine beetle-caused mortality in lodgepole and ponderosa pines from 2010 Aerial Detection Survey along the Front Range, CO with lodgepole-ponderosa pine ecotone site locations for 2010 and 2011. 56

Figure 2. 2. Host Selection experiment with two ponderosa pine natal hosts providing freshly emerged mountain pine beetle to offered lodgepole pine and ponderosa pine cut-logs. Choice logs were replaced weekly (July and August) for five weeks in 2010 and 2011. 57

Figure 2. 3. Host Selection experiment with offered lodgepole pine and ponderosa pine cut-logs to freshly emerged mountain pine beetle from either lodgepole pine or ponderosa pine natal hosts. Choice logs were replaced weekly (July and August) for five weeks in 2010 and 2011. ... 58

Figure 2. 4. Bioassays for mountain pine beetle host selection between fresh bark/phloem units from lodgepole and ponderosa pines. Individual females from either lodgepole pine or ponderosa pine were offered a choice between the two alternate host bark/phloem units (32 cm²). N=64; half from each natal host. 59

Figure 2. 5. Percent of total emerged mountain pine beetle from naturally infested lodgepole pine and ponderosa pine in 2010 and 2011. Trees were randomly located in the lodgepole-ponderosa ecotone between 2590 to 2650 m of elevation on the Arapaho-Roosevelt NF, CO. N=60 emergence cages (0.18 m²) split between species per year. 60

Figure 2. 6. Female to male ratio of mountain pine beetles emerged from combined lodgepole pine (n=60 trees) and ponderosa pine (n=60 trees) in 2010 and 2011 (Mean \pm 1/2 LSD; N=120 trees split between species per year; emergence cages covered 0.18 m²/tree). Trees were randomly located in the lodgepole-ponderosa ecotone between 2590 to 2650 m of elevation on the Arapaho-Roosevelt NF, CO..... 61

Figure 2. 7. Mountain pine beetle oviposition galleries created on log sections. A. Beetle source species: Mean number of oviposition galleries constructed on log sections by mountain pine beetles that had parental lines of either lodgepole and ponderosa pine host trees (source species). N=240 log sections (120 sections from arenas with lodgepole as natal source of beetles, 120 from arenas with ponderosa as natal source of beetles). (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$). B. Beetle choice species: Mean number of mountain pine beetle constructed oviposition galleries in log sections of lodgepole or ponderosa pine chosen by beetles from both natal tree species. N=240 log sections (120 sections of ponderosa as choice species, 120 sections of lodgepole as choice species) (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$). 62

Figure 2. 8. Mountain pine beetle brood produced from log sections. A. Beetle source species: Mean number of mountain pine beetle brood produced in log sections that had parental lines of either lodgepole and ponderosa pine host trees (source species). N=240 log sections (120 log sections from arenas with lodgepole as natal source of beetles, 120 log sections from arenas with ponderosa as natal source of beetles). (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$). B. Beetle choice species: Mean number of mountain pine beetle brood produced in log sections of lodgepole or ponderosa pine chosen by beetles from both natal tree species. N=240 log sections (120 sections of ponderosa as choice species, 120 sections of lodgepole as choice species) (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$)..... 63

Figure 2. 9. Mountain pine beetle brood per female. A. Beetle source species: Mean number of mountain pine beetle brood per female produced in log sections that had parental lines of either lodgepole and ponderosa pine host trees (source species). N=240 log sections (120 log sections from arenas with lodgepole as natal source of beetles, 120 log sections from arenas with ponderosa as natal source of beetles). (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$). B. Beetle choice species: Mean number of mountain pine beetle brood produced in log sections of lodgepole or ponderosa pine chosen by beetles from both natal tree species. N=240 log sections (120 sections of ponderosa as choice species, 120 sections of lodgepole as choice species) (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$)..... 64

Figure 2. 10. Average mountain pine beetle oviposition gallery length. A. Beetle source species: Mean length of mountain pine beetle oviposition galleries in log sections that had parental lines of either lodgepole and ponderosa pine host trees (source species). N=240 log sections (120 log sections from arenas with lodgepole as natal source of beetles, 120 log sections from arenas with ponderosa as natal source of beetles). (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$). B. Beetle choice species: Mean length of mountain pine beetle oviposition galleries in log sections of lodgepole or ponderosa pine chosen by beetles from both natal tree species. N=240 log sections (120 sections of ponderosa as choice species, 120 sections of

lodgepole as choice species) (Mean \pm $\frac{1}{2}$ LSD). Differing letters indicate statistical separation ($\alpha=0.05$)..... 65

Figure 3. 1. Total preformed (24 hr) quantitative oleoresin (mean milligrams oleoresin \pm $\frac{1}{2}$ Least Significant Differences) from lodgepole pine (*P. contorta* var. *latifolia*) and ponderosa pines (*P. ponderosa*) in the Front Range, CO. Differing letters indicate significance $p < 0.05$ determined from Analysis of Variance (Proc GLM; SAS). Means \pm $\frac{1}{2}$ Least Significant Differences. 87

Figure 3. 2. Total cumulative constitutive terpenoid concentrations (mean ug/mg oleoresin \pm $\frac{1}{2}$ Least Significant Differences) from lodgepole pine (*P. contorta* var. *latifolia*; n=10) and ponderosa pines (*P. ponderosa*; n=10) in the Front Range, CO. Differing letters indicate significance $p < 0.05$ determined from a non-parametric ANOVA corrected using Exact Wilcoxon tests (Proc NPAR1WAY; SAS)..... 88

Figure 3. 3. Constitutive oleoresin profile (mean ug/mg) from Front Range, CO. lodgepole pines (*P. contorta* var. *latifolia*; n=10) and ponderosa pines (*P. ponderosa*; n=10) derived from GC-FID. Differences between pine species were determined through a MANOVA model (Proc GLM; SAS). Significance: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ns $P > 0.05$ 89

Figure 3. 4. Limonene concentrations (mean ug/mg oleoresin \pm $\frac{1}{2}$ Least Significant Differences) from constitutive oleoresin obtained from lodgepole pine (*P. contorta* var. *latifolia*; n=10) and ponderosa pines (*P. ponderosa*; n=10) in the Front Range, CO. Differing letters indicate significance $p < 0.05$ determined from a non-parametric ANOVA corrected using Exact Wilcoxon tests (Proc NPAR1WAY; SAS)..... 90

CHAPTER 1

MOUNTAIN PINE BEETLE-CAUSED MORTALITY CHRONOLOGY IN LODGEPOLE AND PONDEROSA PINES IN THE SOUTHERN ROCKY MOUNTAINS

Summary

Eruptive mountain pine beetle (*Dendroctonus ponderosae*, MPB) populations that developed in the late 1990's and early 2000's across the southern Rocky Mountains have resulted in widespread pine-mortality through western North America. Early observations by A. D. Hopkins suggested when alternate host species are available, MPB will prefer to breed in the host to which it has become adapted. In an attempt to understand the susceptibility of adjacent ponderosa pine-dominated stands from lodgepole pine-natal beetles, we surveyed MPB-caused mortality in mixed ponderosa and lodgepole pine stands over five beetle flight periods. We statistically modeled site and stand characteristics in stands with MPB-caused mortality in either species. We surveyed 19 sites in the mixed conifer lodgepole-ponderosa ecotone spanning 140 km of the northern Front Range Mountains, CO. At each site, we established sets of 3-0.20 ha monitoring plots (N=57) where we assessed each tree for beetle attacks during the annual MPB flights of 2008 through 2011. The average uninfested live basal area of lodgepole and ponderosa pines accounted for 88% of the total plot basal area prior to increased levels of MPB-caused mortality. Over the course of five time periods (2004-7, 2008, 09, 10, 11) we found differences in basal areas of attacked trees between years, though not between pine species. Annual percent basal areas of attacked trees were greater in the second year (2009) of infestation than endemic levels (2004-07). Basal areas of attacked trees in all other years were not different from background years. Modeling results infer host selection from lodgepole pine natal hosts into

ponderosa pine the following year, though do not infer host selection from ponderosa pine natal hosts into lodgepole pine the following year. Our statistical models found basal areas of uninfested ponderosa pine and the previous year's mortality levels in both ponderosa and lodgepole pines were significant predictors of the next year's ponderosa pine mortality. Conversely, a separate model indicated basal areas of lodgepole pine mortality in the next year were predicted by available basal areas of lodgepole and ponderosa pines along with the previous year's MPB-caused lodgepole pine mortality. We found diameters of attacked trees decreased over the years and were significantly smaller after four years of beetle-caused mortality. These results indicate managers and the scientific community should be aware of mountain pine beetle host selection of both lodgepole pines and ponderosa pines in the same flight period within mixed conifer stands.

Introduction

The mountain pine beetle (*Dendroctonus ponderosae*, MPB) is considered the most destructive of all native western North American forest insects (Furniss and Carolin, 1977). Eruptive mountain pine beetle populations that developed over the past two decades have resulted in widespread-pine mortality, affecting tens of millions of hectares since the 1990's (Raffa et al. 2008). The mountain pine beetle attacks all western North American native pines and exotic pine plantings (Safranyik and Carrol, 2006). In Colorado, lodgepole (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm. ex S. Watson) and ponderosa pines (*Pinus ponderosa* Lawson var. *scopulorum* Engelm.) are considered the primary hosts, with smaller proportions of mortality occurring in limber pine (*P. flexilis* James) and bristlecone pine (*P. aristata* Engelm.). The mountain pine beetle affected over 1.6 million hectares in Colorado and southern Wyoming from 1996 to 2011 (USFS, 2011). Concerns from land managers escalated over mountain pine beetle

movement from higher elevation lodgepole pine into lower elevation ponderosa pine as beetle populations progressively infested stands prior to 2007 to the east of the Continental Divide along Colorado's Front Range. Leading up to 2007, mountain pine beetle-caused mortality was considered to be within endemic levels along the Front Range ponderosa pine dominated forests where currently attacked trees were not located near brood trees or only a few trees were attacked in groups of three trees or less (Safranyik and Carroll, 2006). As both ponderosa pine and lodgepole pine were attacked in mixed-conifer stands, managers and the general public raised increasing concerns regarding potential large-scale mortality in ponderosa pine dominated stands.

Inherent differences between lodgepole pine dominated stands and ponderosa pine dominated stands created uncertainty for managers and the general public as to how the mountain pine beetle might behave if populations moved from more dense, even-aged lodgepole to more open, multi-cohort ponderosa pine dominated stands (Witcosky, 2008). Colorado has approximately 800,000 hectares of ponderosa pine, commonly found from 1800 to 2750 meters of elevation, while lodgepole pine occupies about 600,000 hectares between elevations of 2450 to 3050 meters (CSFS, 2013). Ponderosa pine stands with higher tree density are likely to be more prone to increased beetle activity than lower density stands, while average stand diameter and age are also suggested to be positively correlated with higher beetle-caused lodgepole pine mortality (Bollenbacher and Gibson, 1986). Based on previous beetle outbreaks, ponderosa pine stands in north-central Colorado had a 50% greater probability of infestation when basal areas exceeded 17 m²/ha (Negron and Popp, 2004). Conversely, lodgepole pines stands in the central Rocky Mountains of Montana with ages 60-125 years and basal areas exceeding 29.8 m²/ha were

suggested to be susceptible to mountain pine beetle disturbance (Bollenbacher and Gibson, 1986).

Early observations by A. D. Hopkins suggested when alternate hosts are available in mixed stands, the mountain pine beetle will prefer to breed in the host to which it has become adapted (Hopkins 1916, 1917). If this hypothesis is supported, mountain pine beetles are unlikely to infest ponderosa pine if they developed within lodgepole pine. The hypothesis is commonly known as Hopkins' Host Selection Principle, however tests have generated mixed evidence leaving the principle mired in controversy in recent decades. Results from controlled-manipulative experiments of the mountain pine beetle and a related bark beetle (*Ips paraconfusus*) did not support the hypothesis (Richmond, 1933; Wood, 1963). Conversely, two Rocky Mountain observational studies in mixed conifer stands found higher levels of mortality in the natal host species, indicating support for Hopkins' Host Selection Principle (Baker *et al.*, 1971, Dean, 2007). As reviewed by Barron (2001), various insect groups have demonstrated host preference as Hopkins described, though the mechanisms behind the behavior have been questioned. Specifically, entomologists have debated whether the host adaptation of larvae ("learning") can be transferred to the adult stages of development, or whether the host selections made by adults are independent of their experiences as larva. In spite of its strong relevance to forest management decision-making challenges during epidemic bark beetle outbreaks where multiple potential host species are present, few empirical tests of Hopkins' Host Selection Principle have been conducted in bark beetles, and no study to date has thoroughly investigated mountain pine beetle host selection through both observational field studies and controlled experiments.

Annual aerial survey data of mountain pine beetle outbreaks throughout Colorado is available for most National Forests from the USDA Forest Service, Forest Health Management, Rocky Mountain Region, from the years of 1956-1970, 1980-87, and 1994-present. We assessed each of these map sets for evidence of mountain pine beetle-caused mortality from one host species to another over time. We found no such documentation of outbreak beetle populations transitioning from one alternate host into another over time from these aerial detection data. As an additional data source, we evaluated the USDA Annual Reports (USDA Forest Service, Region 2, Forest Health Management) in Colorado looking for written documentation of beetle infestation that initiated in either lodgepole or ponderosa pine host species. Again, we found no such evidence throughout the reports from 1975 through 2005.

To evaluate the susceptibility of lower elevation ponderosa pine forests in Colorado's Front Range to infestation by epidemic mountain pine beetle populations in nearby lodgepole pine, we asked the question "Will mountain pine beetle-caused mortality in ponderosa pine be similar to that in lodgepole pine in the mixed conifer ecotone?" Since site and stand characteristics have been positively correlated with mountain pine beetle-caused mortality in both host species, we asked "Will site factors or stand characteristics affect mountain pine beetle-caused mortality in either species?" As the mountain pine beetle progressed through stands, potentially exhausting one suitable host, we asked "Over time, is one pine host preferred over the other?" Finally, to further our understanding of which size classes are likely to sustain impacts between lodgepole and ponderosa pine hosts, we asked "What size trees are preferentially attacked over successive mortality years?" We addressed these questions at sites in the southern Rocky Mountains by measuring mountain pine beetle-caused mortality over five time periods within mixed lodgepole and ponderosa pine stands.

Methods

We identified 19 sites in the mixed conifer lodgepole-ponderosa ecotone spanning approximately 140 km of the northern Front Range mountains, CO, (2440-2740 m) where recent mountain pine beetle attack was detected in several lodgepole pine trees from the previous year (Figures 1.1, 1.2). Sites were located on lands managed by various agencies: US Forest Service, National Park Service, Colorado State Parks, Colorado State Forest Service, and Boulder County Parks and Open Space. At each site, we established sets of 3-0.20 ha (1/2 ac) monitoring plots (each 30m x 100m; spaced >0.8 km apart). Within ~5 m of each plot, at least one lodgepole pine had been mass-attacked by mountain pine beetles in the previous summer, which provided evidence of active mountain pine beetle populations in or near the plots. Within each 0.20 ha plot, we inspected all trees during 4 separate surveys: prior to the annual mountain pine beetle flight in 2009 (beetle emergence from 2008) and after the beetle flight in 2009, 2010, and 2011. Beetle emergence predominantly occurs annually in mid-July through mid-August throughout our sites (West *et al.*, in prep). We recorded characteristics of all trees affected by mountain pine beetle and/or other detectable agents of disease/mortality (species, diameter (at breast height – 1.4m), signs of insect activity, presence of disease).

Mountain pine beetle activity for each tree attacked in 2008-2011 was classified as a mass attack if mountain pine beetle had infested 360 degrees of the tree's circumference. If a tree had been killed by mountain pine beetle between 2004 and 2007 (i.e. prior to our initial survey), we estimated the year of death based on the degradation status of needles and fine twigs retained in the tree canopy (Klutsch *et al.*, 2009). Within each plot, we established three- 0.02 hectare (1/20-ac) subplots (radius 8.3 m; Figure 1.3) in which we recorded diameter (at 1.4m) and health status of all trees > 2.5cm dbh (live, dead, affected by mountain pine beetle and/or other agents of

disease/mortality). We used these subplot data to calculate stand attributes (basal area, trees per acre) and to quantify site characteristics (elevation (m), slope (%), aspect quadrants) that could affect mountain pine beetle activity.

In 2009, the first year of this study, 15 sites (with 45 plots) were established and surveyed in June-July (pre-MPB flight; 2009) and Sept-Nov (post-MPB flight; 2009-11). Prior to our 2010 fall surveys, some sites were affected by recent forest management activities such as removal of recently MPB-infested trees, or stand-level thinning. Because these activities had the potential to alter the mountain pine beetle populations in/near our monitoring plots, and therefore may have influenced the probability of mountain pine beetle attacks, we dropped the affected plots from the study (4 plots on Boulder County Parks and Open Space lands and 1 plot on National Park Service lands). In 2010, we established 4 additional sites (12 plots) throughout the study area and collected the same data on mountain pine beetle activity and stand characteristics at these sites, spanning the same time period (2004-2011). By 2011, we had surveyed a total of 19 sites (57 plots) over the five time periods.

Data analyses:

We calculated the average number of trees per hectare (TPH) and associated basal area (BA; m²/ha) for the 19 sites (57 plots) from the data collected at the 9 subplots per site. These data were used to determine the uninfested stand characteristics of each pine species (BA/ha and TPH; mean \pm SE) – available for infestation to the mountain pine beetle – as well as overall stocking levels for all species. We quantified mountain pine beetle activity at each site as the mean TPH and BA infested per species per hectare across the 3 plots per site.

To test for differences in mountain pine beetle infestation levels across years between lodgepole and ponderosa pine (2004-2011), we used a mixed model approach (Proc Glimmix, SAS) comparing annual mean mountain pine beetle-caused mortality in the two species (m^2 basal area/ha) treating species, year, and species*year as fixed effects and site as a random effect. We averaged the annual mortality from 2004-07 across our sites, and considered the mortality from this time period as endemic, or population maintaining background levels. We compared the mortality we measured in each year from 2008 through 2011 to the endemic mortality levels prior to 2008. Due to skewness in the distributions of mountain pine beetle-caused tree mortality, square root transformations were performed prior to analyses. Means were back-transformed for presentation of the results. Upper and lower confidence bounds (half Least Significant Differences ($1.4 \times \text{Standard Error of Mean}$)) were calculated from square root back-transformed ($\text{mean} \pm 1/2 \text{ LSD}$) values to compare mortality between years in both species.

To assess whether mountain pine beetle preferentially selected larger diameter trees in either pine species during the incipient-epidemic phase of infestation, we tested the mean diameter of infested trees each year using a mixed model, while treating host species and year as fixed effects (Proc Glimmix, SAS). We performed a linear contrast of diameter distributions over the last four years (2008-11) to test whether sizes of infested trees decreased as mountain pine beetle populations increased, compared to the size of trees infested under endemic population conditions prior to 2008 (2004-07). Diameters of infested trees were \log_{10} -transformed prior to analysis and back-transformed for presentation of results, as previously described.

To determine which factors predicted mountain pine beetle-caused mortality levels (basal area; m^2/ha) in either ponderosa or lodgepole pine in a given year ($t+1$), we used a mixed-model (Proc Glimmix in SAS) treating the available basal area of uninfested ponderosa and lodgepole pine,

overall basal areas of all species in plots (live and standing dead), basal areas of all species in plots other than pines, previous year-mountain pine beetle-caused mortality levels (basal area) in both lodgepole and ponderosa pine species (t), year of infestation, plot slope, plot aspect, and plot elevation as fixed effects. To reduce the influence of high leverage points due to skewed values within the distribution of available basal area and mountain pine beetle-caused mortality in ponderosa and lodgepole pines, we performed \log_{10} -transformations on these variables. Upper and lower confidence bounds were calculated from \log_{10} back-transformed (mean $\pm 1/2$ LSD) values. Mortality estimates were back-transformed from the \log_{10} values for presentation of the results.

Results

Sufficient basal area of both ponderosa pine and lodgepole pine was available for infestation by the mountain pine beetle in our plots at the start of our surveys. The average live stand composition in our plots along the Front Range was dominated by the alternate mountain pine beetle-hosts of lodgepole pine (50%; 396 ± 36 TPH; mean \pm SE) and ponderosa pine (27%; 212 ± 24 TPH; Table 1.1). The average live basal area of lodgepole pine and ponderosa pine accounted for 88% of the total plot basal area, 12.1 ± 0.8 m²/ha and 9.7 ± 0.8 m²/ha respectively (Table 1.1). The occurrence of mortality varied among the plots in both lodgepole and ponderosa pines and across years (Table 1.2). Although the amount of uninfested pine basal area and trees per hectare varied across the sites, all sites experienced mountain pine beetle-caused mortality in at least one of the three plots per site in both lodgepole and ponderosa pines over the surveyed periods (Tables 1.3, 1.4).

Our comparison of mountain pine beetle-caused mortality in lodgepole pine vs. ponderosa pine over the course of five time periods (2004-7, 2008, 09, 10, 11) found differences in annual

percent basal areas of trees attacked between time periods ($F_{1,4}=3.32$, $p=0.0014$), but not between species ($F_{1,4}=4.65$, $P=0.07$). There were no species*year interactions ($F_{1,4}=0.99$, $P=0.41$). Average cumulative percent mortality levels across sites in both lodgepole and ponderosa pine was 41% basal area infested/adjusted uninfested (mortality was subtracted from uninfested basal area over time) across the five measurement periods. Average cumulative ponderosa pine mortality adjusted for previous year's mortality of uninfested was 40.7% (100% max, 0.7% min), while lodgepole pine was 41.4% (98% max, 9% min). The percent of attacked per uninfested trees during the endemic-mountain pine beetle phase from 2004-07 for combined lodgepole and ponderosa pine was lower (2.4 % (1.0, 4.5); mean percent (lower ½ LSD, upper ½ LSD)) than in 2009 (11.4 % (7.73, 15.89)). However, average annual percent mortality in the time period of 2004-07 was not different from annual average percent mortality in 2008 (6.5 % (3.89, 9.70)), 2010 (5.2 % (2.73, 8.34)), and 2011(5.2 % (2.70, 8.55); Figure 1.4).

Overall, we found mountain pine beetle attacked larger diameter trees than the stand average, but comparing the two host species showed that beetles infested significantly smaller diameter lodgepole pine (24.8 cm (24.2, 25.4); mean (lower ½ LSD, upper ½ LSD) compared to ponderosa pine (27.5 cm (26.7, 28.3); $F_{1,275}=13.8$, $P=0.0002$; Table 1.5). At the onset of our surveys, the average uninfested lodgepole pine diameter was 18.0 cm \pm 0.2 (mean \pm SE), while the average uninfested ponderosa pine diameter was 21.2 cm \pm 0.4. We found overall differences between infested tree diameters across years ($F_{4,275}=3.3$, $P=0.01$), while no interactions between species and year were detected ($F_{4,275}=0.6$, $P=0.68$; Table 1.5). Our linear contrast analysis showed a significant decrease in diameters of infested trees in both species as the infestation progressed through time between the years of 2008 through 2011 ($P=0.0005$; Table 1.5).

Our analysis of which factors influenced the current year's ($t+1$) mortality in ponderosa pine revealed that the basal area infested in the previous year (t) in both ponderosa pine ($P=0.007$, $F_{1, 221}=7.47$) and lodgepole pine ($P=0.009$, $F_{1, 221}=6.96$) were significant factors. In addition, the available basal area in ponderosa pine ($P=0.017$, $F_{1, 221}=5.8$) and the year ($P=0.0007$, $F_{3, 221}=5.9$) were also significant overall predictors of mountain pine beetle-caused mortality in a given year in ponderosa pine (Table 1.6; Figure 1.5). No other site or stand variables were significant predictors of the current year's ponderosa pine mortality. In comparison, the basal area of the current year's mountain pine beetle-caused mortality in lodgepole pine ($t+1$) was predicted by the previous year's (t) infested basal area of only lodgepole pine ($P<0.0001$, $F_{1, 221}=29.5$); the covariates of year ($P<0.0001$, $F_{3, 221}=18.6$) and the available lodgepole pine and ponderosa pine basal areas ($P=0.007$, $F_{1, 221}=7.5$; $P=0.03$, $F_{1, 221}=4.5$, respectively) were also significant. No other site or stand factors were significant predictors of the current year's lodgepole pine mortality.

Discussion

This study monitored mountain pine beetle-caused mortality in the lodgepole-ponderosa pine ecotone over four years of beetle emergence. Specifically, we addressed the research question (“Will mountain pine beetle-caused mortality in ponderosa pine be similar to that in lodgepole pine in the mixed conifer ecotone?”) by surveying mortality levels in both alternate hosts from the same stands. Sufficient basal area of both lodgepole pine and ponderosa pine was available for infestation as the beetle moved into the ecotone. We found the annual mountain pine beetle-caused mortality (percent of available basal area infested) were not different between species in each survey period (2004-7, 08, 09, 10, and 11). This finding differs from those of previous observational studies which reported a natal host species effect upon subsequent host selection (Baker *et al.*, 1971; Dean, 2007). However, our results support those of early manipulative

studies of host selection in the mountain pine beetle and *Ips* species (Richmond, 1933; Wood, 1963).

Tree mortality (% BA/ha) in the ecotone from mountain pine beetle activity was different between years, with beetle killed basal areas being higher than endemic mortality levels just two years into the survey. A curvilinear effect in percent mortality levels was evident over time as the beetle progressed through the stands. Percent mortality levels in both hosts transitioned back to no different than background levels three and four years into our survey. Total cumulative mortality percent (basal area infested/uninfested) across the five monitoring periods was 41 percent of available pine basal area. From this result, sufficient uninfested pine was available for further infestation, though it remained unattacked four years after mortality levels increased.

Whether additional mortality throughout our study areas and the greater Front Range will result from persistent beetle activity is uncertain and difficult to predict. Mountain pine beetle populations throughout the region are greater than recorded over the last hundred years. The pressure these populations have upon stands has likely contributed to alternate host selection and movements from lodgepole pine into ponderosa pine that we have not seen previously occur. Host condition and the ability to withstand attack is also likely a contributing factor to susceptibility of an alternate host species. Whether environmental conditions during the current beetle infestation of ponderosa pine and lodgepole pine in mixed conifer stands are different from past outbreaks has yet to be examined. Whether ponderosa pine dominated stands have only recently attained sufficient volume needed to sustain beetle populations of the current scale across the Front Range is also unknown. Taken together, all of these factors may have aligned to create an unprecedented movement from lodgepole pine dominated forests into mixed conifer and ponderosa pine-dominated forests documented in this study.

Mountain pine beetle risk ratings in mixed pine/conifer stands have not been reported for the area. Studies of single species stand susceptibility have suggested stocking levels for both lodgepole pine- and ponderosa pine-dominated stands should be well above the basal areas we surveyed in mixed conifer stands to be considered susceptible to attack. Negron and Popp (2004) suggest a 50% greater probability of infestation when ponderosa pine basal areas exceed 17 m²/ha. Bollenbacher and Gibson (1986) suggested basal areas exceeding 29.8 m²/ha are susceptible for increased mortality levels in lodgepole pine-dominated stands. Our average basal areas in combined lodgepole and ponderosa pine was 21.8 m²/ha, with 12.1 m²/ha in lodgepole pine and 9.7 m²/ha in ponderosa pine. Cumulative mortality levels (41% basal area infested/uninfested) across the five measurement periods indicate beetle pressure upon these stands was reasonably high. Whether stocking levels in mixed stands follow susceptibility ratings for single species models is unknown, though we surveyed substantial levels of mortality in both host species. These results provide groundwork for further investigations of stand level mortality from the mountain pine beetle in mixed pine/conifer stands where multiple hosts are available. The findings of this study led to controlled experiments both in the field and laboratory, investigating host selection behavior of the mountain pine beetle when the natal host species of either lodgepole or ponderosa pines was controlled and beetles were offered cut logs and bark/phloem of either lodgepole or ponderosa pines (West *et al.*, in prep; Chapter 2).

Relatively larger diameter trees have been suggested to be the preferred size class for the mountain pine beetle. The available average stand diameter for infestation was 18.0 cm ± 0.2 (mean±SE) for lodgepole pine and 21.2 cm ± 0.4 for ponderosa pine. Safranyik and Carroll (2006) suggest four phases for mountain pine beetle epidemics in lodgepole pine-dominated forest. A key factor in the transition from endemic levels to incipient-epidemic levels is the

diameter of infestation. When the infested diameter exceeds that of the average stand diameter, populations are considered to have transitioned from background to incipient-epidemic (Safranyik and Carroll, 2006). However, we found the average infested diameters prior to 2008 (MPB lodgepole: 25.8 cm; MPB ponderosa: 26.5 cm) were well above the average uninfested stand diameters (lodgepole: 19.7 cm; ponderosa: 23.5 cm). These results indicate levels of infestation may have been greater than endemic levels once we initiated the survey. However, we found the infested diameters were significantly lower in 2011 than the years prior in 2008 through 2010 and lower than endemic levels in 2004-07. We interpret this as a depletion of the available trees with larger diameters over time, though not exhausting the larger diameter trees from the stands.

Overall, our statistical models found basal areas of uninfested ponderosa pine and the previous year's mountain pine beetle-caused mortality levels in both ponderosa pine and lodgepole pine were significant predictors of the next year's ponderosa pine mortality. A separate model investigating current year's lodgepole pine mortality found that infested basal areas were predicted by the available basal areas of both lodgepole and ponderosa pines and the previous year's mountain pine beetle-caused lodgepole pine mortality. Other site- and stand-level variables (site elevations, slopes, aspects, and total stand tree basal areas) were not significant predictors of beetle-caused mortality levels. Taken together, these results indicate mountain pine beetle host selection movement from lodgepole pine natal hosts into ponderosa pine, though do not indicate a movement from ponderosa pine natal hosts into lodgepole pine.

The results from our statistical modeling suggest the rate of increase of predicted mountain pine beetle-caused mortality levels may be greater when the previous year's mortality levels are relatively low. For example, when average basal areas from the previous year's ponderosa pine

beetle-caused mortality were 0-0.9 m²/ha, the next year's predicted ponderosa pine mortality basal areas ranged from 0.08 to near 0.28 m²/ha. When the previous year's beetle-caused mortality levels were much greater (e.g. 7.6-9.6 m²/ha), the next year's mortality basal areas were predicted between 0.01 to near 0.05 m²/ha. These results suggest managers should be aware the rate of next year's infestation may be greatest when previous years' infested basal areas are relatively low. The variability of infestation from year to year and between plots was quite considerable in our study, which adds to the overall robustness of the model. Since the modeling input data was from locations with mortality ranging from relatively high to low, we chose not to further stratify locations into low or high beetle infestation levels which could constrain comparisons due to the reduction of sampled locations and may further constrain interpreting model results due to the narrow set of stand conditions required to make comparisons from such a stratification.

From our surveys, we infer the residual available basal areas and diameters were sufficient for further infestation from this outbreak. In order for landscape scale disturbance from the mountain pine beetle to occur and persist, relationships between beetle populations, suitable host abundance, and environmental conditions all need to align. The population of mountain pine beetle in the study area and the Rocky Mountain region is greater than ever recorded. Our surveys of overstory mortality showed that sufficient numbers of trees of susceptible sizes were present and still remain across the Front Range to sustain a large population of beetles capable of overcoming otherwise healthy trees. The environmental conditions over the course of beetle host transition from lodgepole pine stands into stands containing ponderosa pines may have aided the beetle's success by reducing preformed and induced tree defenses. Not only does climate affect the trees' defenses, a warmer climate reduces overwinter cold-induced mortality to the greater

population of mountain pine beetle in the region. Together, the components of beetle pressure, abundance of susceptible trees, and environmental factors may have aligned both temporally and spatially to allow for the large scale movement between lodgepole pines and ponderosa pines along the mountains of the Front Range during the study. It is unclear from our sampling whether we can expect host selection by the mountain pine beetle of ponderosa pine and lodgepole pine to persist as the epidemic continues in the near future, as each year the host is depleted and environmental conditions vary.

In conclusion, our surveys found mountain pine beetle attacked both ponderosa pine and lodgepole pine in similar proportions in mixed ecotone stands along the Front Range, CO. Statistical modeling showed that site factors and total stand density were not significant predictors of mountain pine beetle caused mortality in lodgepole or ponderosa pine. However, lodgepole pine and ponderosa pine mortality levels in the previous year from mountain pine beetle were good predictors of the next year's mortality in ponderosa pine, along with the amount of uninfested ponderosa pine. The next year's lodgepole pine mortality was predicted by the previous year's lodgepole pine mortality and uninfested levels of lodgepole and ponderosa pine. These results suggest mountain pine beetle movement from lodgepole pines into ponderosa pines, though not completely, as our surveys showed that lodgepole pine was still infested over the course of several years. Managers should be aware that mortality levels in mixed-pine stands may not be different between the two species. The findings of this study suggest the mountain pine beetle can readily overcome any physiological differences between the two host species. Host condition may be a greater predictor of beetle infestation. Further investigation is warranted in the confirmation of ponderosa pine host selection in the field by beetles that developed in lodgepole pine. This study confirmed mortality in both host species in mixed conifer stands,

however it did not provide evidence of the extent to which beetles that developed within lodgepole pine subsequently selected ponderosa pine hosts. Direct manipulative studies have shown beetles prefer ponderosa pine subsequent to lodgepole pine development when offered cut-logs in a choice test in the field and when offered bark/phloem units in bioassays (West *et al.*, Ch. 2).

Table 1.1. Live trees per hectare and basal areas (m²/ha greater than 2.5cm diameter at 1.4 m) in 2008 at 19 Front Range, CO. sites with 3 plots per site (N=57).

Species	TPH ^a	Std Error	BA m ² /ha ^b	Std Error	DBH ^c	Std Error
<i>Pinus contorta</i>	396	35.8	12.1	0.8	19.7	0.6
<i>Pinus ponderosa</i>	212	23.9	9.7	0.8	23.5	1.1
<i>Pinus flexilis</i>	80	18.8	1.2	0.3	13.4	1.5
<i>Pseudotsuga menziesii</i>	124	31.2	2.9	0.5	16.7	1.4
<i>Picea engelmannii</i>	54	21.7	2.2	1.2	20.1	1.9
<i>Abies lasiocarpa</i>	49	16.5	0.3	0.1	8.1	2.3
<i>Populus tremuloides</i>	128	25.8	1.1	0.3	11.4	1.1
<i>Juniperus scopulorum</i>	17	0.00	0.1	0.1	9.1	2.8

^aMean trees per hectare

^bBasal area; square meters per hectare

^cDiameter at 1.4 m (centimeters)

Table 1.2. Number and percentage of plots on which trees were killed by the mountain pine beetle, and the mean and maximum basal area infested across all plots. Plots were located in the lodgepole-ponderosa pine ecotone in the Front Range, CO. (N=57 plots). Sites comprised 3-0.20 ha plots (N=19 sites).

Ponderosa pine	2004-7	2008	2009	2010	2011
# plots with mortality	18	27	27	14	12
# plots no mortality	39	30	27	39	41
% plots w/mortality	32%	47%	47%	25%	21%
Mean infested m ² /ha	0.6	1.3	3.5	2.2	4.7
Max infested m ² /ha	2.2	4.0	11.0	5.0	10.4
Lodgepole pine	2004-7	2008	2009	2010	2011
# plots with mortality	35	48	44	22	18
# plots no mortality	22	9	10	31	35
% plots w/mortality	61%	84%	77%	39%	32%
Mean infested m ² /ha	1.1	1.4	2.0	2.0	1.7
Max infested m ² /ha	3.2	9.9	11.1	6.6	4.8

Table 1.3. Uninfested lodgepole pine and ponderosa pine density and basal area in 2008 along Front Range, CO. sites (N=19). Sites comprised 3-0.20 ha plots in the lodgepole-ponderosa pine ecotone (N=57).

Site	Lodgepole pine - <i>P. contorta</i>						Ponderosa pine - <i>P. ponderosa</i>					
	TPH ^a	SE	BA ^b	SE	DBH ^c	SE	TPH ^a	SE	BA ^b	SE	DBH ^c	SE
Sheep Creek	472	248	9.5	4.6	13.3	0.2	329	198	14.4	8.7	21.0	0.6
Creedmore Lake	362	110	10.5	3.8	16.7	3.5	390	120	12.8	2.0	20.2	3.6
Molly Lake	494	76	12.5	0.4	16.6	0.6	313	101	10.3	1.0	21.7	6.8
Manhattan Rd	280	126	9.2	3.9	17.9	3.3	209	20	9.6	0.9	20.4	0.2
Bennett Creek	264	41	12.1	2.2	22.5	1.1	165	29	9.4	2.3	25.0	1.8
Pennock Pass N	587	245	18.2	5.2	21.7	3.2	165	50	5.7	1.0	19.2	2.1
Pennock Pass S	642	167	17.7	6.2	17.3	1.1	121	20	6.3	2.3	22.1	3.3
Stringtown	247	49	8.8	0.7	19.5	2.1	104	43	4.1	0.7	20.6	7.7
Horseshoe Park	225	48	10.7	0.8	23.7	1.8	170	82	7.4	2.1	29.8	10.3
Upper Beaver Meadows	313	76	10.6	2.0	20.5	1.0	362	41	17.7	0.9	22.9	1.4
Hollowell Park	373	104	13.6	1.3	20.0	1.2	82	25	9.3	1.6	39.2	4.6
Wildbasin	104	40	5.0	1.5	24.4	3.4	412	222	16.8	4.0	22.9	2.9
Prescott	351	221	12.1	5.9	21.6	3.2	77	43	5.1	3.7	17.3	9.3
Delonde Meadows	357	197	11.7	5.0	23.8	5.0	357	143	14.4	2.4	23.9	4.5
Mud Lake	747	45	17.2	1.9	16.0	0.6	187	96	9.1	4.3	22.2	1.3
Reynolds Ranch [‡]	274	138	10.2	4.1	22.5	3.4	132	67	9.3	5.7	24.2	1.7
Kelly Dahl	406	15	12.4	2.7	17.7	2.1	137	45	8.6	2.2	26.0	2.0
Lump Gulch	681	311	15.8	6.2	16.5	1.2	274	124	11.9	4.8	23.6	3.3
Green Ranch	351	105	12.1	0.8	21.9	4.8	49	17	2.5	0.4	24.0	3.4

[‡]Site excluded beginning in 2010 due to management activities

^aMean uninfested trees per hectare > 2.5 cm diameter breast height from 3 plots per site

^bMean basal area, square meters per hectare of uninfested trees > 2.5 cm from 3 plots per site

^cAverage diameter at breast height (1.4 m) in centimeters of uninfested trees >2.5 cm from 3 plots per site

Table 1.4. Average cumulative (2004-2011) mountain pine beetle-caused mortality in *Pinus contorta* and *Pinus ponderosa* within Front Range, CO sites from north to south. Sites comprised 3-0.20 ha plots in the lodgepole-ponderosa pine ecotone (N=57).

Site	Lodgepole pine - <i>P. contorta</i>						Ponderosa pine - <i>P. ponderosa</i>					
	TPH ^a	SE	BA ^b	SE	DBH ^c	SE	TPH ^a	SE	BA ^b	SE	DBH ^c	SE
Sheep Creek	44	20	2.6	1.3	25.4	1.6	46	23	2.7	1.2	27.1	1.0
Creedmore Lake	60	17	3.3	1.3	25.0	1.5	60	19	3.3	1.0	25.6	2.2
Molly Lake	31	4	1.3	0.2	22.4	1.5	32	7	1.7	0.2	26.1	3.5
Manhattan Rd	16	6	1.0	0.3	30.5	3.9	27	13	1.9	0.7	29.8	2.7
Bennett Creek	52	6	2.7	0.2	25.6	1.4	25	10	2.1	0.9	31.1	1.2
Pennock Pass N	85	34	3.1	0.8	23.3	3.0	16	9	1.3	0.7	19.7	9.9
Pennock Pass S	54	16	2.4	0.7	23.4	0.7	12	4	1.0	0.5	30.0	2.6
Stringtown	23	3	1.2	0.1	24.7	0.9	2	2	0.1	0.1	9.0	9.0
Horseshoe Park	48	13	2.8	0.4	27.2	2.4	40	11	2.7	0.5	28.7	1.8
Upper Beaver Meadows	29	11	1.3	0.5	23.6	1.5	57	21	3.4	0.9	27.0	2.5
Hollowell Park	27	3	1.6	0.3	26.9	1.4	19	5	2.1	0.4	37.2	1.7
Wildbasin [†]	19	12	0.9	0.5	25.0	1.9	5	3	0.2	0.1	24.3	2.2
Prescott	9	4	0.7	0.1	32.9	3.4	2	1	0.1	0.1	18.2	9.3
Delonde Meadows	11	1	0.6	0.2	25.5	4.2	1	1	0.0	0.0	6.4	6.4
Mud Lake	18	10	0.7	0.4	21.4	2.0	6	5	0.3	0.3	16.8	8.5
Reynolds Ranch [‡]	5	1	0.3	0.0	26.0	1.7	2	1	0.1	0.1	20.2	11.8
Kelly Dahl	18	9	1.0	0.6	26.8	2.2	1	1	0.1	0.1	13.4	13.4
Lump Gulch	10	3	0.5	0.1	25.5	0.5	10	10	0.4	0.4	7.3	7.3
Green Ranch	11	4	0.6	0.1	27.5	3.9	2	2	0.1	0.1	9.4	9.4

[†] 1 plot excluded from means beginning in 2010 due to management activities

[‡] Site excluded beginning in 2010 due to management activities

^a Mean trees per hectare of mountain pine beetle-caused mortality from 3 plots per site

^b Mean basal area, square meters per hectare of mountain pine beetle-caused mortality from 3 plots per site

^c Average diameter at breast height (1.4 m) in centimeters of mountain pine beetle-caused mortality from 3 plots per site

Table 1.5. Average diameter (cm) by year of mountain pine beetle-caused lodgepole and ponderosa pine mortality along the Front Range, CO. Differing letters indicate significance at alpha=.05. N=57 plots. Mean (lower LSD, Upper LSD).

	2004-07	2008	2009	2010	2011	Overall Mean
Lodgepole pine	25.8 (24.5, 27.1)	26.5 (25.3, 27.7)	25.5 (24.4, 26.6)	24.8 (23.4, 26.3)	21.8 (20.4, 23.3)	24.8 (24.2, 25.4) ^c
Ponderosa pine	26.5 (24.6, 28.5)	29.4 (27.7, 31.2)	28.0 (26.5, 29.6)	28.0 (26.1, 30.0)	25.8 (23.9, 27.9)	27.5 (26.7, 28.3) ^d
Yearly Mean	26.1 (25.0, 27.3) ^a	27.9 (26.9,29.0) ^a	27.0 (25.8, 27.7) ^a	26.3 (25.1, 27.6) ^a	23.7 (22.5, 25.0) ^b	

Table 1.6. Mixed model regression effects for predicting current year mountain pine beetle-caused ponderosa pine mortality (basal area (BA); m²/ha) in response to the lagged (previous year's) mountain pine beetle-caused mortality in ponderosa pine and lodgepole pine, with the available ponderosa pine basal area by year (2008-2011).

Model:

Current MPB Ponderosa Basal Area Mortality = Year of MPB Mortality + Lagged Year MPB Ponderosa Basal Area Mortality + Lagged Year MPB Lodgepole Basal Area Mortality + Available Uninfested Ponderosa Basal Area

Effect	coeff	Num DF	Den DF	P value
intercept	-0.9598			
log ₁₀ Lagged Year Ponderosa BA	0.2191	1	221	0.007
log ₁₀ Lagged Year Lodgepole BA	0.2020	1	221	0.009
log ₁₀ Ponderosa Uninfested BA	0.2737	1	221	0.017
Year		3	221	0.0007
2008	0.1486	1		
2009	0.3358	1		
2010	-0.2055	1		
2011	0	1		

	LS Means
Year 2008	-1.3776
Year 2009	-1.1904
Year 2010	-1.7318
Year 2011	-1.5263

†The average lagged log₁₀lodgepole and median available log₁₀ ponderosa used in graphing were -1.2302 and 0.1955, respectively.

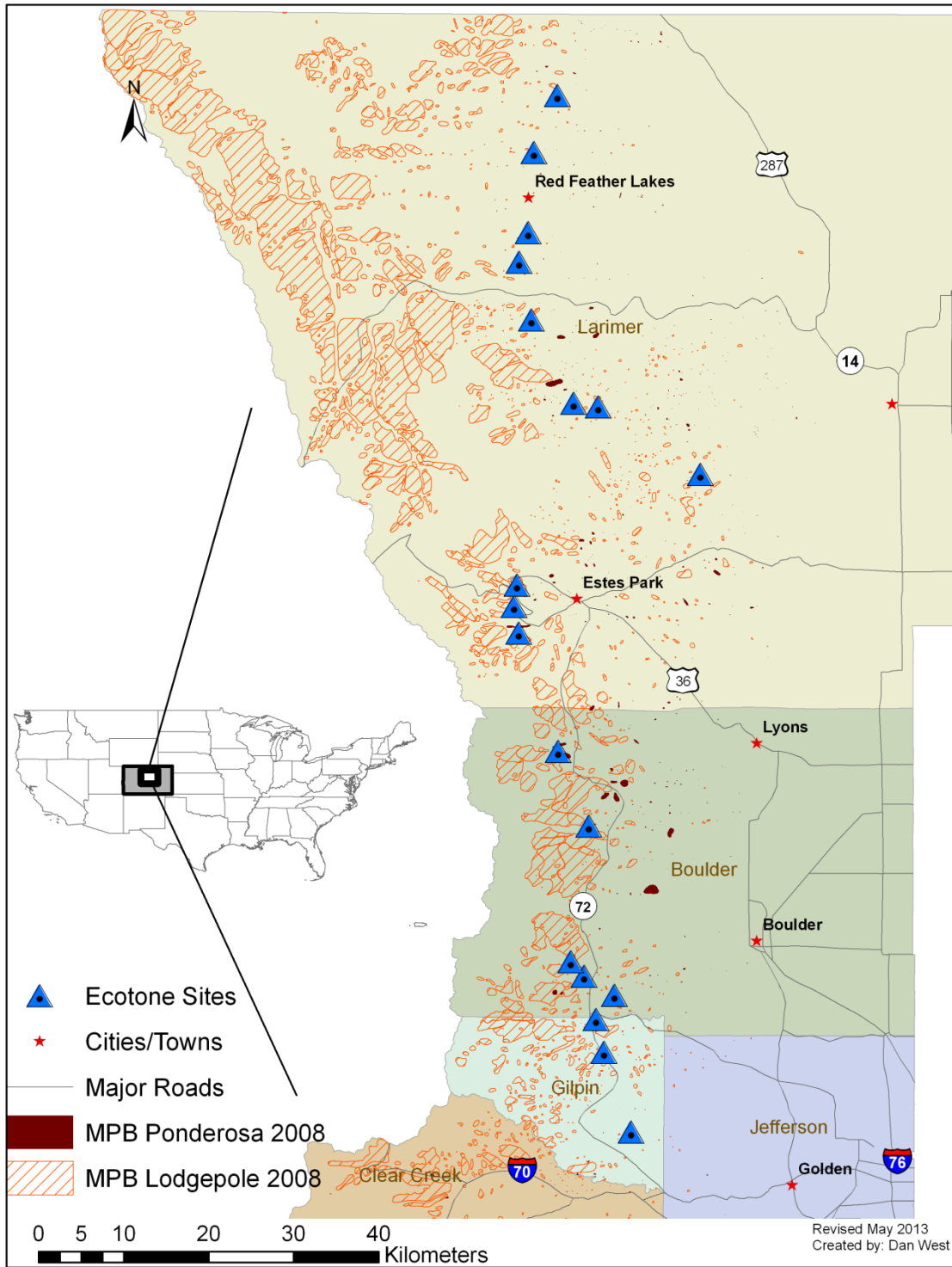


Figure 1.1. Study sites (2009-11) in the lodgepole-ponderosa pine ecotone along the Front Range, CO. USDA Forest Service and its partners, 2008 Aerial Detection Survey data depicting mountain pine beetle-caused mortality in lodgepole pines and ponderosa pines in 2007.

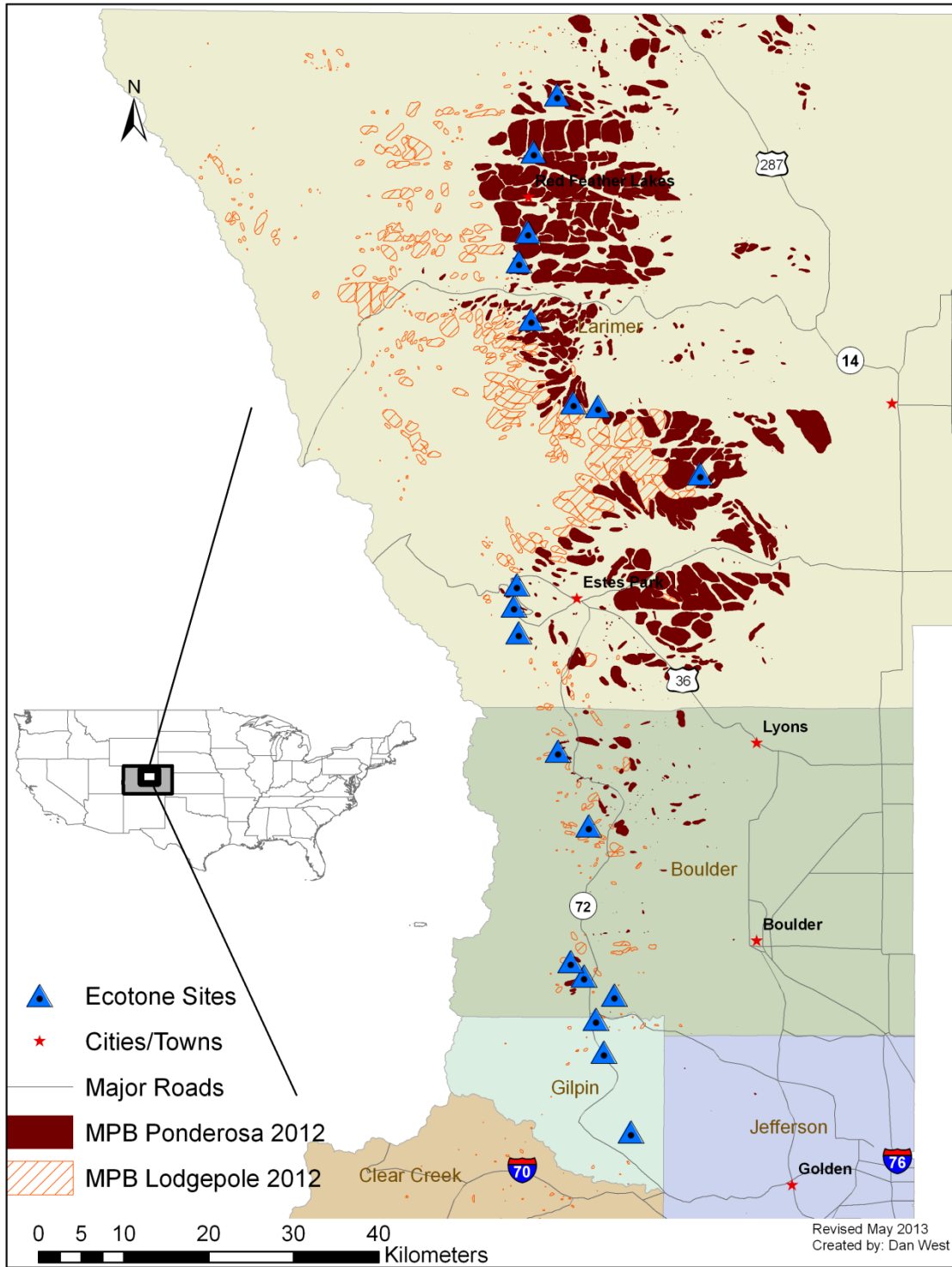


Figure 1.2. Study sites (2009-11) in the lodgepole-ponderosa pine ecotone along the Front Range, CO. USDA Forest Service and its partners, 2012 Aerial Detection Survey data depicting mountain pine beetle-caused mortality in lodgepole pines and ponderosa pines in 2011.

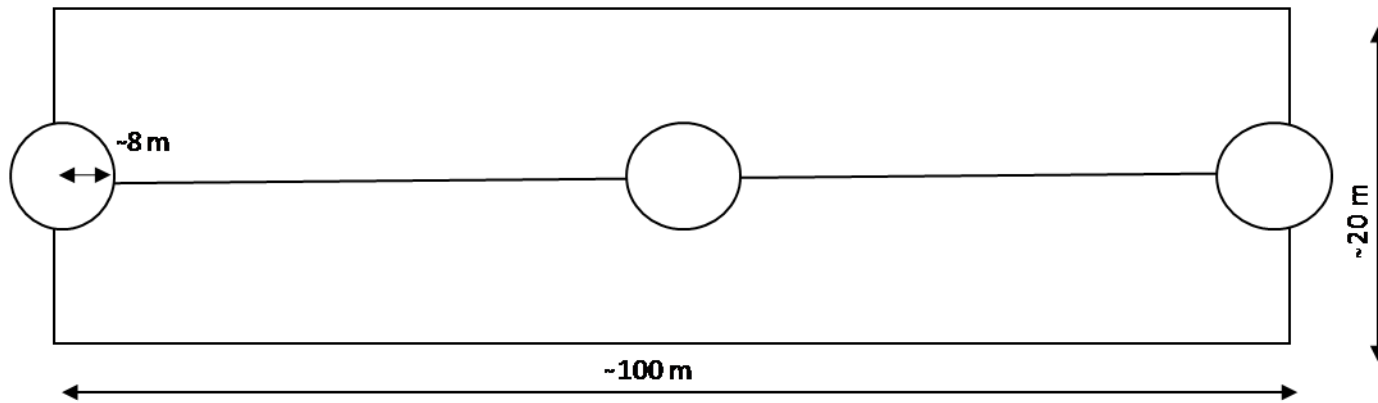


Figure 1.3. Plot design used in mountain pine beetle-caused mortality surveys in lodgepole-ponderosa pine ecotone sites along the Front Range, CO. (N=57 plots (0.2 hectares) at 19 sites; 3 circular sub-plots per plot (200 meters² each)).

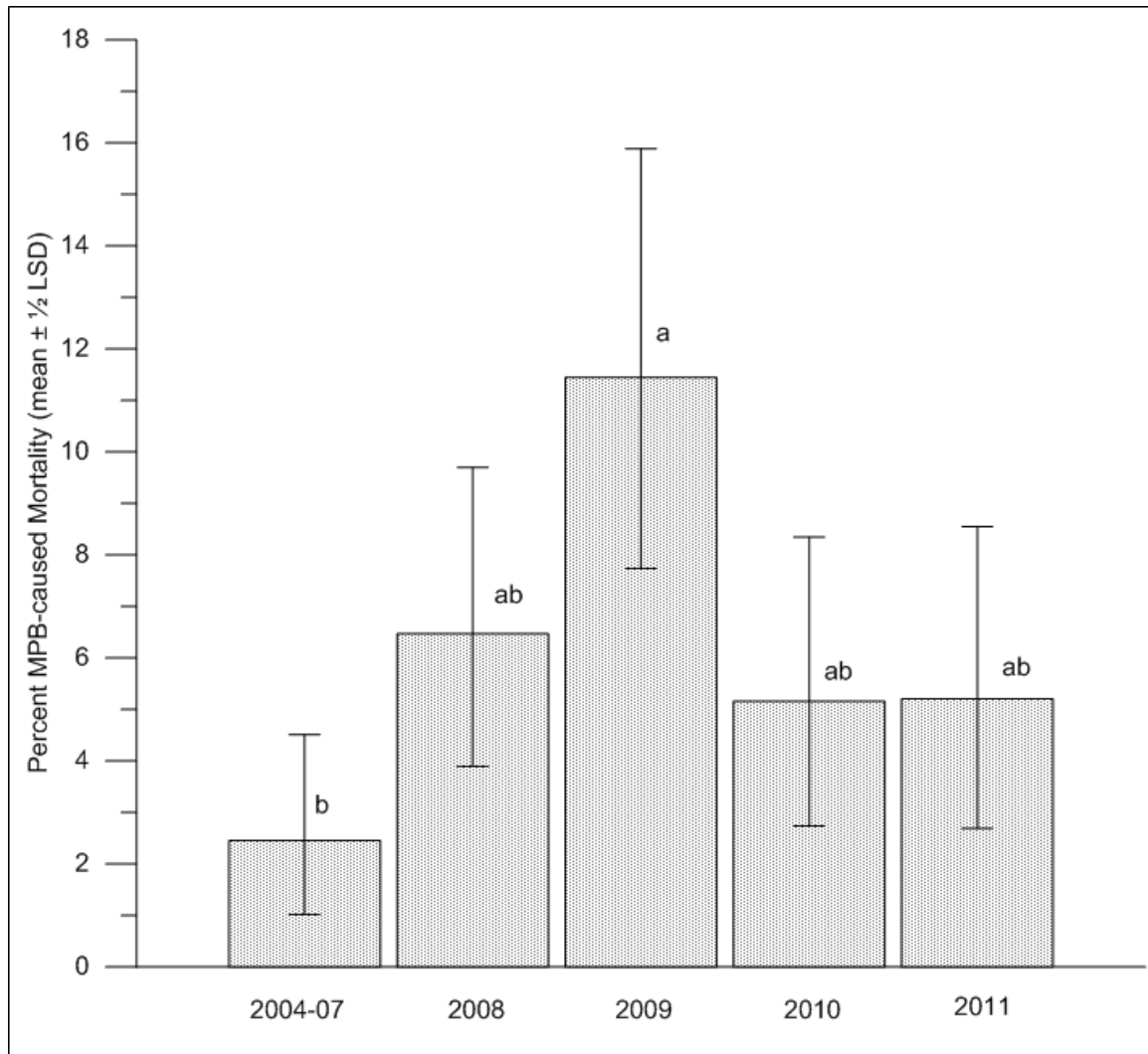


Figure 1.4. Annual percent basal area (m^2/ha ; mean \pm $\frac{1}{2}$ LSD) of mountain pine beetle-caused mortality of that year's available lodgepole and ponderosa pine basal area along the Front Range, CO. in 2004-2011. Differing letters indicate significant differences between years ($\alpha=0.05$). N=19 sites with 57 plots; 3 plots per site.

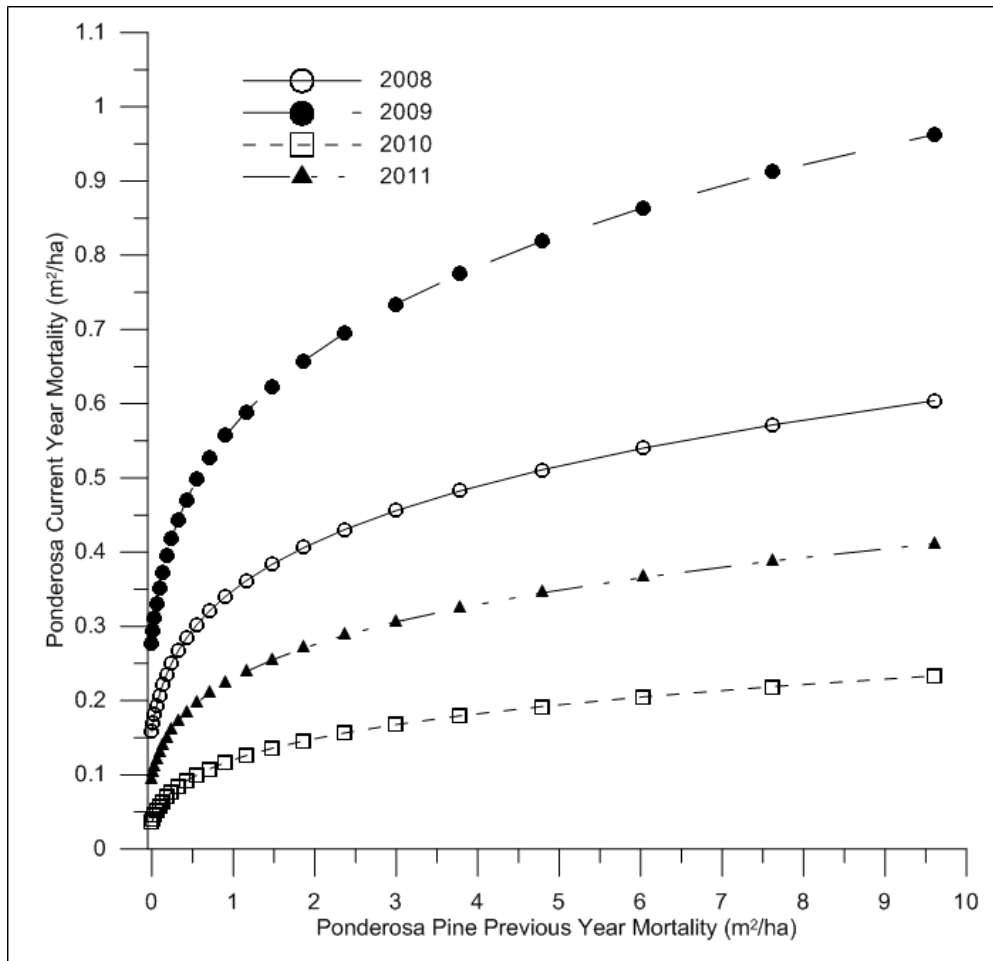


Figure 1.5. Predicted current year's basal area of ponderosa pine mountain pine beetle-caused mortality in response to the lagged (previous year) basal area of mountain pine beetle infested ponderosa pine, average previous years' mountain pine beetle infested lodgepole pine basal area, and available uninfested median ponderosa pine by year (2008-11).

See Table 1.5 for graph coefficients, average infested lodgepole basal area, and median available ponderosa pine values.

REFERENCES

- Barron, A.B. 2001. The life and death of Hopkins' Host-Selection Principle. *Insect Behavior* 14: 725-737.
- Bollenbacher, B., Gibson, K.E. 1986. Mountain Pine Beetle: A Land Manager's Perspective. R1-86-15. U.S. Department of Agriculture, Forest Service, Northern Region, Missoula, MT, 4 pp.
- CSFS. 2013. Colorado's Forest Types. *Colorado State Forest Service, Colorado State University*. Dec 12, 2012. <<http://csfs.colostate.edu/pages/forest-types-lpp.html>>
- Furniss, R.L., and Carolin, V.M. 1977. *Western forest insects*. US For. Serv. Misc. Publ. No. 1339. Washington, DC. 654 p.
- Hopkins, A.D. 1916. Economic investigations of the scolytid bark and timber beetles of North America. U.S. Department of Agriculture Program of Work for 1917. p. 353.
- Hopkins, A.D. 1917. A discussion of C. G. Hewitt's paper on "Insect Behavior." *Journal Economical Entomology* 10: 92-93.
- Klutsch, J.G., Negron, J.F., Costello, S.L., Rhoades, C.C., West, D.R., Popp, J., Caissie, R. 2009. Stand characteristics and downed woody debris accumulations with a mountain pine beetle (*Dendroctonus ponderosae* Hopkins) outbreak in Colorado. *Forest Ecology and Management* 258, 641-649
- Negron, J. F., and J. B. Popp. 2004. Probability of ponderosa pine infestation by mountain pine beetle in the Colorado Front Range. *Forest Ecology and Management* 191:17-27.
- Raffa, K. F., B. H. Aukema, J. Bentz, A. L. Carroll, J. A. Hicke, M. G. Turner, and W. H. Romme. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. *BioScience* 58:501-517.
- Richmond, H.A., 1933. Host selection studies of *Dendroctonus monticolae* Hopk. in southern British Columbia. *The Forestry Chronicle* 9:60-61.
- Safranyik, L., and Carroll, A. L. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. Pages 3-66 in L. Safranyik and B. Wilson, editors. *The mountain pine beetle: a synthesis of its biology, management, and impacts on lodgepole pine*. Canadian Forest Service, Pacific Forestry Centre, Natural Resources Canada, Victoria, British Columbia, Canada.
- USFS, 2011. Western Bark Beetle Strategy: Human Safety, Recovery and Resiliency. *US Dept. Agriculture, US Forest Service*. July 11, 2011. <http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5338089.pdf >
- Wood, D.L. 1963. Studies on host selection by *Ips confusus*. *University of California Publications in Entomology* 27: 241-282.

CHAPTER 2

MOUNTAIN PINE BEETLE HOST SELECTION BETWEEN LODGEPOLE AND PONDEROSA PINES IN THE SOUTHERN ROCKY MOUNTAINS

Summary

Along the Front Range in Colorado, concerns of forest managers, researchers, and entomologists escalated in 2007 as to whether ponderosa pine-dominated stands were susceptible to attack from mountain pine beetles (*Dendroctonus ponderosae*; MPB) that had caused mortality in nearby lodgepole pine stands. We tested Hopkins' Host Selection Principle which states "a species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted." We used a controlled field choice experiment, in which naturally emerged MPB adults from each host species in the lodgepole-ponderosa pine ecotone were offered uninfested cut-logs from both species as a choice for infestation. Ponderosa pine cut-logs were preferred over lodgepole pine cut-logs in both years at a ratio of almost 2:1, regardless of natal host species. More MPB entered the arenas from natal lodgepole pine, though more beetles chose ponderosa pine cut logs, suggesting a strong affinity for ponderosa pine over lodgepole pine under these conditions. We analyzed the reproductive success of MPB in the alternate host species by rearing insects in the infested choice logs from both natal hosts. On average, more than twice as many brood emerged from ponderosa pine logs than lodgepole pine logs. We found no differences in brood per female or gallery length per female between lodgepole and ponderosa pines, indicating similar reproductive success in both hosts. As a supplemental investigation of primary host selection, we assayed individual female beetles from either lodgepole or ponderosa pine and offered them bark/phloem sections from both alternate species in a closed container. In

two-thirds of the trials, the beetles preferred ponderosa pine over lodgepole pine bark/phloem sections. These results suggest a consistent preference for ponderosa pine, and indicate that host selection behavior of larger groups of beetles in the controlled-field-choice experiments was not different from primary host selection behavior of individuals. To investigate the question “Do MPB reproductively isolate in sympatric populations infesting lodgepole pine vs. ponderosa pine?” we examined differences in emergence phenology and sex ratios of emerging MPB collected from both alternate host species in mixed ecotone sites. The results revealed no phenological differences in temporal emergence synchronies or sex ratios between lodgepole and ponderosa pine-natal hosts. These results do not support the hypothesis of reproductively isolated populations based on emergence phenology. Overall, our findings suggest that Front Range ponderosa pine forests may incur elevated levels of MPB-caused mortality where adjacent MPB populations develop in lodgepole pines.

Introduction

Eruptive mountain pine beetle (*Dendroctonus ponderosae* Hopkins; MPB) populations have caused widespread mortality of pines in western North America, with tens of millions of hectares affected since the 1990's (Raffa *et al.*, 2008). In Colorado, major hosts of the native mountain pine beetle are lodgepole pine (*Pinus contorta* Dougl.) and ponderosa pine (*Pinus ponderosa* Lawson), while the less abundant limber pine (*Pinus flexilis* James) and bristlecone pine (*Pinus aristata* Englm.) are also host species. Throughout Colorado, ponderosa pine occupies approximately 800,000 hectares (ha) from 1800 to 2750 meters (m), while lodgepole pine occupies about 600,000 ha between 2450 to 3050 m (Colorado State Forest Service, Dec, 2012). In 2008, questions arose from the scientific community and land managers regarding whether lower elevation, ponderosa pine-dominated stands were at risk to high mortality levels

comparable to those measured in lodgepole pine stands, as annual aerial surveys identified an expansion of beetle-caused mortality from the western side of the Continental Divide along the Front Range to the eastern side where forests comprise ponderosa pine-dominated stands (USFS, Aerial Detection Survey (ADS) 2000-07). In order for both ponderosa and lodgepole pine hosts to sustain epidemic mountain pine beetle levels, host selection preference from lodgepole pine to ponderosa pine would have to occur as populations encountered increasing proportions of ponderosa vs. lodgepole pine. Alternatively, it was unknown if two mountain pine beetle biotypes or host races – reproductively isolated conspecific populations breeding within the two alternate hosts of ponderosa and lodgepole pine – would exist in sympatry, producing the illusion of alternate host preference.

Through field observations of the mountain pine beetle in lodgepole and ponderosa pines, A.D. Hopkins, a prominent early forest entomologist, developed hypotheses regarding host species selection by the mountain pine beetle when alternate pine hosts are available. Hopkins (1916, 1917) reported “a species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted.” Though few empirical tests of the hypothesis have been conducted in bark beetles (Coleoptera: Curculionidae: Scolytidae), controversy exists around the hypothesis on the basis of whether adult host preference behavior is derived from larval adaptation or other mechanisms. Richmond (1933) investigated mountain pine beetle host selection in British Columbia, Canada through controlled-choice experiments over three consecutive years, 1930-32 (2 replicates in 1930, 2 in 1931, and 1 in 1932). Sections of logs of either lodgepole pine or ponderosa pine infested with mountain pine beetle were placed in cages containing uninfested logs of similar size of lodgepole and ponderosa pines. From these five replicates of each natal host species, 66% of the beetles that emerged from ponderosa pine cut-

logs subsequently chose ponderosa cut-logs, whereas 73% of beetles emerging from lodgepole pine cut-logs subsequently chose ponderosa pine hosts over lodgepole cut-logs, contrary to Hopkins' Host Selection Principle.

In perhaps the most extensive test of Hopkins' Host Selection Principle of scolytid beetles, Wood (1963) investigated host selection behavior of serial populations of *Ips paraconfusus* (reported as *I. confuses*) in ponderosa pine, sugar pine (*P. lambertiana* Dougl.), and Jeffrey pine (*P. jeffreyi* Grev.; 22, 19, 31 generations, respectively). Choice experiments were conducted offering ten homogenized phloem discs prepared from the three alternate hosts. The choice experiments failed to support Hopkins' Host Selection Principle (Wood, 1963). Insects that had developed over multiple generations within one natal host did not subsequently select homogenized phloem discs from that host species. These two studies suggest Hopkins' Host Selection Principle may not prevail under experimental laboratory study.

Conversely, Aerial Detection Surveys conducted by the USDA Forest Service and partners across western pine forests over many decades indicate that outbreak populations of mountain pine beetle tend to remain primarily in the host species in which they developed (USDA, ADS, <http://www.fs.usda.gov/detail/r2/forest-grasslandhealth/>; Annual Biological Reports, USDA Forest Service, Rocky Mountain Region, Forest Health Management) The results of two observational studies under natural conditions in the Rocky Mountains are consistent with these reports and provide some support for Hopkins' Host Selection Principle. In Wyoming, US, Baker *et al.* (1971) surveyed three mixed lodgepole pine-whitebark pine-subalpine fir stands (*P. contorta* – *P. albicaulus* – *A. lasiocarpa*; each was approx. 10 km²) where mountain pine beetle-caused mortality initially occurred in either lodgepole pine or whitebark pine. Adjacent stands containing uninfested lodgepole pine or whitebark pine were available for subsequent attack by

the mountain pine beetle, but mortality predominated in the host species in which the outbreak initiated (Baker *et al.*, 1971). The pattern of mortality was suggested to have resulted from host preference for the same host in which the larvae had developed – the natal host. In another study conducted in the Medicine Bow Mountains, Wyoming, ten plots (0.13 ha) were established in mixed limber-lodgepole pine stands containing three MPB-infested limber pines per plot (Dean, 2007). After three annual surveys (2003-05), the proportion of available limber pine attacked was greater than that of available lodgepole pines, which the author attributed to beetles that had developed in limber pine natal hosts subsequently attacking limber pines, though no direct measurement of host selection behavior was conducted.

Though empirical studies addressing bark beetle larval host adaptation are few Barron (2001), some insect groups other than bark beetles display behaviors akin to host preference as described by Hopkins (1916,1917), particularly parasitoids (van Emden, 1996), flies (Barron and Corbet, 1999; Tulley *et al.* 1994; Ray, 1999;), ants (Jaisson, 1980), and bees (Breed *et al.*, 1998.) The principle is not resolved within all insect groups, and Barron (2001) suggests the apparent mechanisms behind host selection behavior may be of the utmost importance. The chemical legacy (Corbet, 1985) and proposed adult learning from these groups may elicit behavior consistent with Hopkins' Host Selection Principle (van Emden, 1996), although this notion departs from the foundation of the principle that Hopkins promoted. Fundamentally, the notion of adult host recognition being transferred from larval conditioning to adult stadia demonstrates a departure from Hopkins' Host Selection Principle, which was described from continuous attack over many generations of mountain pine beetle that preferred the host type for which they had previously developed. Hopkins (1917) speculated it may take several generations of mountain pine beetle breeding in an alternate host to develop future host selection preference for the

alternate species. Barron (2001) advocates the adult stage may have a greater influence upon host preference than preimaginal conditioning; in other words, the transfer of host preference from larvae to adult.

In light of the limited and often mixed nature of research results on natal host races or populations, host preference in general, and specifically natal host influence on future host preference by mountain pine beetle, we designed a series of experiments to address these questions. We first investigated whether natal host populations of beetles were separated by emergence phenology between lodgepole pine and ponderosa pine. We asked the question “Do populations of mountain pine beetle infesting lodgepole pine and ponderosa pine in sympatry isolate reproductively through emergence phenology?” We examined differences in emergence phenology and sex ratios of emerging mountain pine beetles collected from the two alternate host species at sites where they occur in mixed stands. Along the Front Range, CO., ponderosa pine commonly occurs at lower elevations, providing more degree days for beetle development and possible intraspecific host cues signaling the insects to emerge earlier than those that developed within higher elevation lodgepole pine. Populations of mountain pine beetle that developed within ponderosa pine may be adapted to earlier emergence synchronies based on host environment adaptations. Amman (1982) found adult mountain pine beetles emerged significantly earlier (8 days earlier; 50% of brood) in ponderosa pine than those from lodgepole pine natal host species. This finding suggests a resource advantage for selecting ponderosa pines over lodgepole pines.

The emergence periods of the mountain pine beetle in either host species along the eastern slopes of the Front Range Mountains in Colorado have not been fully documented. Lindgren funnel traps baited with insect attractants were used to collect mountain pine beetles in mixed conifer

lodgepole-limber pine stands in the Front Range, though peak emergence data were not reported (Mitton and Ferrenburg, 2012). Emergence periods have been described from cages attached to individual trees for lodgepole pine west of the Continental Divide in Colorado and in South Dakota for ponderosa pine developed beetles. In the Black Hills, SD., peak mountain pine beetle emergence from ponderosa pine occurred within the second and third weeks of August for 1966-67 between 1737m and 1767 m (Schmid, 1972), though most Colorado ponderosa occurs at greater elevations than those described from the Black Hills, SD. Conversely, lodgepole pine natal host populations may have developed adaptations for later seasonal synchronized emergence due to fewer degree days sufficient for suitable emergence, in part, and cues taken from the host. Insects that developed in lodgepole pine natal hosts at 2670m of elevation in Grand County, CO exhibited peak emergence during the first week of August in 2004 (Tishmack *et al.*, 2005). The discrepancies in peak emergence timing between these two study areas and both alternate host species identifies a gap in understanding whether differences exist in mountain pine beetle emergence phenology that could influence host selection between lodgepole and ponderosa pines in the same stands.

Our second assessment investigated whether sex ratios of emerging beetles differ over the duration of the emergence period and whether sex ratios differ between the two hosts, which would offer support for further investigations into potential biotype populations or host races in the mountain pine beetle. On average, sex ratios of emerging and attacking mountain pine beetles in northern Utah ranged from 60.1 to 70.1 percent female in 1974-5 at 2248m of elevation (Rasmussen, 1980). Initial female emergence increased throughout the first week of emergence and subsequently declined thereafter (Rasmussen, 1980). For this study, we asked “Do emerging

mountain pine beetle sex ratios differ throughout the emergence period between lodgepole and ponderosa pines, when both hosts are available?”

Our third question “Does the mountain pine beetle prefer ponderosa pine over lodgepole pine when natal host species is controlled?” was tested through two different experiments. First, we used a controlled-field-choice experiment, in which naturally emerged mountain pine beetle adults from each host species in the lodgepole-ponderosa pine ecotone were offered uninfested cut-logs from both species as a choice for infestation. Under these conditions, both primary and secondary host selection processes were assumed to be used in host selection by the beetles. The mountain pine beetle uses in part, tree volatiles, visual cues, and random landing to find suitable host trees. Once a tree has been selected, gustatory (feeding) acceptance allows bark and phloem mining to occur. These stepwise processes are known as primary host selection. During the mining process, host volatiles are oxidized and emitted from the beetles as monoterpenes within the subcortical tissues. These serve as aggregation pheromones that attract con-specifics, or other mountain pine beetles, to the tree. The aggregation of con-specifics in response to the pheromone blend is known as secondary host selection. Second, we conducted a laboratory assay in which individual female beetles from a known natal host (either lodgepole or ponderosa pine) were offered material from both alternate pine species for infestation in a closed arena. This method was intended to test only primary host selection behavior by the individual insects; there were no opportunities for aggregation pheromones to assist (secondary host selection) in host selection behavior.

Our fourth question, “Does reproductive success differ between mountain pine beetles infesting ponderosa pine vs. lodgepole pine?” was addressed by using the infested logs from our controlled field choice experiments and rearing the brood through to adult emergence. We

subsequently compared the number of offspring, the brood size per female, and the length of oviposition galleries between lodgepole and ponderosa pine host species. Higher reproductive rates in ponderosa pine host could potentially lead to increased severity in outbreak-caused mortality in lower elevation Front Range ponderosa pine forests compared to higher elevation lodgepole pine forest.

Materials and Methods

1.1 Emergence phenology

To address our question addressing the existence of sympatric mountain pine beetle biotypes or host races, we recorded the number of beetles emerging weekly from trees of both host species in two years (May-October of 2010 and 2011) at two sites in the Front Range, CO. lodgepole-ponderosa pine ecotone, Arapaho-Roosevelt NF, Colorado, USA (Molly Lake area in 2010, UTM NAD-83 Easting Northing: 13N 449861 4512902; Pennock Pass Area in 2011, UTM 13N 458090 4492379; Figure 2.1). We attached emergence cages in May, prior to adult emergence, on the north side of trees at 1.4 m from the forest floor. Cages were constructed of bronze screen (0.18 meters²) with glass jars affixed at the bottom. Thirty trees of each species were arbitrarily selected within an area of approx. 200 ha at the Molly Lake area and 33 ha at the Pennock Pass area. North aspects were chosen because greater densities of brood have been shown to be located on this aspect in lodgepole pine (Reid, 1963; McCambridge, 1964; Shepherd, 1965) though no aspect differences were detected in ponderosa pine (Schmid, 1972). Each week from mid-June to mid-Oct, we counted the number of adults that emerged from each tree and determined their sex. To investigate differences in emergence timing, we averaged the weekly adult cage collections for each host species and calculated half Least Significant Difference (LSD) values for confidence bounds (Proc Means; SAS 9.2). Log₁₀-transformations were

performed on weekly trap counts to reduce the influence of high leverage points. Mean estimates were back-transformed from \log_{10} values and the weekly collections were divided by the total collections to improve interpretation of the results. Upper and lower confidence bounds were calculated through \log_{10} back-transformed values (mean $\pm 1/2$ LSD).

1.2. Sex ratio of emerging beetles

To investigate whether sex ratios varied between beetles that developed within the two pine hosts, we counted the numbers of males vs. females present in weekly cage collections from each tree if less than 30 beetles had emerged during a given week. If more than 30 insects emerged from a tree in a week, we randomly sampled 30 individuals from the collection. Sex was determined by identifying the presence of the stridulating organ present in males on the seventh abdominal tergum (Hopkins, 1909; Lyon, 1958). Using a mixed model, we tested for differences in sex ratios from the two host species using the mean ratio of emerged females to total emerged beetles per tree per week in response to the fixed effects of host species, year, species*year interaction term, week within year, and species*week within year (Proc Glimmix; SAS 9.2). To reduce the effect of high leverage points resulting from some trees producing no or low emergence during the early and late weeks of the emergence period, we eliminated from the analysis the weeks that had emergence from less than nine of the thirty trees and weeks for which the standard errors of the mean emergence values were greater than 0.5. Thus, sex ratios were compared across five weeks for both years, 2010-11 (July 23 through August 21). Upper and lower confidence bounds were calculated for the mean number of collected females (mean $\pm 1/2$ LSD).

1.3. Host selection: controlled field experiment

To address the question of whether mountain pine beetle preferentially selected ponderosa or lodgepole pine hosts, controlled field experiments were conducted at the same two sites as the aforementioned phenology study. These sites had incipient-epidemic (Safranyik and Carroll, 2006) *D. ponderosae* populations that were infesting both lodgepole and ponderosa pine trees. Within 160 ha at the Molly Lake area, and 30 ha at the Pennock Pass area, we selected twelve pairs of trees that had been mass-attacked the previous year and attached emergence cages to them. Each pair of trees was of the same species (6 pairs of ponderosa pine, 6 of lodgepole pine) and served as sources of beetles with the same natal host. From the emergence cages attached to each pair of trees, we placed 5 cm-diameter-PVC pipes leading into a single choice arena on the forest floor which contained vertically oriented cut-log sections of both ponderosa pine and lodgepole pine (Figure 2.2). This design allowed beetles to naturally emerge from their natal host trees, travel down the pipes, enter the arenas, and select a host species to infest, either lodgepole or ponderosa. Arenas (122 x 61 x 61 cm) were constructed of oriented strand board (OSB) bases, 6 vertical frame supports (5 x 8 x 61cm), and wood framed tops, covered with aluminum screening which allowed ambient temperature fluctuations and air circulation.

Trees supplying the beetles had been mass attacked the previous year. When identifying these trees, we defined a mass attack as one in which (i) beetle entrance wounds (often with pitch tubes) surrounded the entire diameter of the tree; (ii) frass was present around the entire tree base; and (iii) fading of the foliage had begun. Additionally, attack status was verified through bark sampling on the south aspect of each tree. The densities of attacks on natal host trees were not standardized, as trees were selected for the experiment based on having high number of attacks and being adjacent to another mass attacked tree of the same species, so that beetles from

two different trees could access each choice arena. Emergence cages were placed high enough on the stem to allow the insects to travel downward in the PVC pipes into the choice arenas. The beetles entered the midpoint of the arenas via the PVC piping. In all replicates, each tube supplied freshly emerged adults from different emergence cages affixed to different trees, except in one case in 2011 when two arenas were separately supplied from one large *P. ponderosa* affixed with two emergence cages, one above another, due to a lack of two adjacent infested hosts in the vicinity of the arenas.

To provide breeding tree material for these choice experiments, one tree of each host species from the same stand was felled each week, limbed, and cut into 60 cm sections which we placed in the choice arenas. Trees were selected using the following criteria: no visible insect damage or disease; growing under similar conditions in the same stand; similar diameter (< 2.5 cm difference; > 20 cm dbh); and similar taper for the twelve log sections. Cut ends of sections were sealed with a water-based wax emulsion product to reduce desiccation (Waxlor End Sealant, Forestry Suppliers). Log sections of each host were matched by diameter and vertically positioned at each end of the arenas (Figure 2.3). The order in which the logs of the two tree species were placed at each end of the arenas was randomized each week. Log sections were replaced every seven days for five weeks from July 21 to September 1, 2010 and July 14 to August 22, 2011. New log sections were placed in the arenas the day they were felled and cut.

After seven days, each log section was removed from the field-choice arena and placed in a rearing box located in a temperature controlled laboratory ($20.4\text{ C} \pm 0.02$; mean \pm SE). Rearing boxes were constructed of OSB (61 cm x 61 cm x 61 cm) with stapled aluminum screened fronts covered with black landscape fabric to reduce light and allow moisture movement. Emerged

insects were collected weekly in glass jars affixed to holes in the bottom of the rearing boxes. Weekly collections of emerged beetles were tallied.

Each log section was removed from the rearing chamber after beetle emergence ceased. Cut-log diameters (center and ends) were recorded along with the length of the log section, phloem layer thickness on each cut end, and the total number of mountain pine beetle emergence holes. To test for differences in mean diameters and phloem thickness in response to the fixed effects of choice species, we used a mixed model where natal trees (n=48) and natal trees by species (lodgepole pine, ponderosa pine) were treated as random effects in the model (Proc Mixed; SAS 9.2). The bark from each log was peeled in approx 13 cm wide strips and oviposition galleries were measured using a map distance wheel. Oviposition gallery counts and lengths were used as an index of host preference.

We tested the host selection behavior of mountain pine beetle by comparing the number of oviposition galleries constructed in lodgepole pine vs. ponderosa pine. Females initiate oviposition gallery construction in *D. ponderosae* (Reid, 1962), therefore, the number of galleries is synonymous with the number of female selections of each host. We used a mixed model where year (2010, 2011), natal-host species (lodgepole pine, ponderosa pine), and choice log species (lodgepole pine, ponderosa pine) were fixed effects while natal trees (n=48) within natal species (lodgepole pine, ponderosa pine) by year (2010, 2011) and choice log species by natal trees within natal species by year were random effects. (Proc Mixed; SAS 9.2).

1.4. Host selection: bioassays with individual beetles

Bioassays were developed to test primary host selection of individual beetles, without any influence of secondary pheromone presence from previous beetle attack. In each replicate, one

adult beetle was placed in a plastic container (2450 cm³) with sections of bark/phloem from both ponderosa and lodgepole pines (Figure 2.4). To obtain the bark/phloem sections, six uninfested trees of each species were selected from the study site in 2010 and 2011, felled, limbed, and cut into 60 cm lengths. Log sections were marked in sequence as to match the taper between each tree species. In the laboratory, we removed the outer bark and phloem intact in 32 cm² units from log sections from each species. In plastic storage containers, one bark unit from lodgepole pine and one unit from ponderosa pine were suspended with the bark side up in paraffin wax. The paraffin reduced desiccation of the phloem while rendering the cut ends of the phloem unavailable for direct feeding. To retain leverage points for possible entry by beetles under bark flakes and furrows, the bark surface was not covered with wax or was otherwise altered. We obtained mountain pine beetles for this experiment from naturally infested trees that had been felled the previous fall and stored in unheated storage sheds. Female mountain pine beetles were chosen at random as they emerged from these lodgepole pine and ponderosa pine logs. Beetles were accepted for use in the bioassays provided their vigor was such that they could climb the sides of a Petri dish and right themselves when displaced to their dorsal side.

During each bioassay, we placed one female mountain pine beetle on the middle of either the lodgepole or ponderosa pine bark unit. The order of placement was randomized for each replicate. The beetle behavior was observed four times daily, for approx two minutes per replicate, until the beetle selected a host. At each observation period, the insect's path of travel or observed behavior was recorded via drawings for each beetle replicate. The criteria we used to identify host selection were penetration of the bark/phloem unit coupled with sustained feeding of the phloem. Sixty-four replicate tests were conducted in 2011-12, half with female beetles that had developed in lodgepole pine and half with female beetles from ponderosa pine. Replicates

experiencing insect mortality without penetration of the bark/phloem unit or sustained feeding were reported as such without a retrieval. We used chi square tests of independence to determine whether the proportion of host species chosen was affected by the beetles' natal host species. To determine whether the proportions of ponderosa vs. lodgepole pines selected were equal, we performed binomial proportion exact tests.

1.5. Reproductive success

We used a mixed model to analyze the variation in brood production from each natal-tree and choice-log treatment in the field experiment, treating the number of emerging adults as the dependent variable. To quantify female fecundity, the number of emerged adult brood were divided by the number of oviposition galleries constructed per female and tested as the dependent variable under a similar model in response to natal-tree and choice-log species. Lastly, we used the continuous length of each oviposition gallery as a metric for host suitability and tested for differences between treatments. To reduce the influence of high leverage points due to skewed values of gallery counts, emerged adults, adults per female, and gallery length per female, \log_{10} -transformations were performed on these variables. Mean estimates were back-transformed from \log_{10} values to improve interpretation of the results. Upper and lower confidence bounds were calculated through \log_{10} back-transformed values (mean $\pm 1/2$ LSD).

Results

2.1. Emergence phenology

Mountain pine beetle emergence from ponderosa and lodgepole pines occurred over 65 and 72 days in 2010 and 2011, respectively, with peak emergence in late July and early August (Table 2.1; Figure 2.5). Total beetle emergence for both pines ranged from 657 to 958 per meter² of

bark surface over the four month collection period. In 2010, beetles first emerged from our caged sample trees (n=60) on June 25; two ponderosa pine natal trees produced one adult each. One week later on July 2, the first mountain pine beetle emerged from one of the 30 lodgepole pines. The same pattern was seen in 2011, though the first emergence occurred two weeks later than in 2010. Single mountain pine beetles were collected from four individual ponderosa pine trees on July 9, 2011, while one lodgepole pine produced the first mountain pine beetle on July 15 (Table 2.1). In 2010, the last day on which beetles emerged from both hosts was Sept 24. On the final day of emergence, three of 30 lodgepole trees had adult MPB emergence (2, 2, 1 adults) while ponderosa had four trees produce one adult beetle each. In 2011, beetles stopped emerging after Sept 27 for lodgepole pine, when one adult was collected from each of two of the 30 trees. The last day of emergence from ponderosa pine was Oct 18, when four beetles were collected from each of two trees. The “peak emergence” for both species occurred over a two week period, the last week of July and the first week of August, for both years.

Our analyses identified differences in emergence frequencies between species, years, weeks within year, and species*weeks within each year. Across all weeks for both years, more insects emerged from lodgepole pine (3.4 MPB/m² (3.1, 3.6); mean (lower half LSD, upper half LSD) than ponderosa pine (2.3 MPB/m² (2.1, 2.6); P <0.0001, F_{1, 1447}=18.2), while more emerged overall from both hosts in 2010 (3.3 MPB/m² (3.0, 3.5)) than did in 2011(2.4 MPB/m² (2.2, 2.6); P=0.0003, F_{1, 1447}=13.1; Figure 2.5).

2.2. Sex ratios

Differences in mountain pine beetle sex ratios were detected between years, over the five week emergence period selected in 2010 and 2011, (P=0.046, F_{1, 417}=3.99), and weeks within years

($P < 0.0001$, $F_{8, 417} = 5.50$), though no differences were detected between the two pine species ($P = 0.61$, $F_{1, 417} = 0.26$). Female emergence outnumbered male emergence earlier in the flight period by approximately 2:1. As the emergence period progressed, female emergence declined resulting in female ratios of approximately 1:1 two weeks after the peak emergence period (Figure 2.6).

2.3. Host selection: controlled field experiment

Field choice experiments indicated mountain pine beetle preferred ponderosa pine log sections over lodgepole pine approximately 2:1, with 102.1 vs. 49.2 oviposition galleries/m² of log, respectively; $F_{1, 20} = 10.99$, $P = 0.003$; Figure 2.7). Of the 240 paired-choice logs offered over the ten weeks of controlled-field experiments during 2010 and 2011, 68.3% or 164 logs were selected overall (45 uninfested lodgepole pine; 31 uninfested ponderosa pine. The choice preference was likely not influenced by cut-log diameter or maximum thickness of the phloem, since no differences were detected in these variables between the host species ($F_{1, 215} = 0.01$, $P = 0.97$; $F_{1, 215} = 3.84$, $P = 0.051$, respectively). More insects entered the choice arenas from lodgepole pine natal hosts than from ponderosa pine ($F_{1, 20} = 12.16$, $P = 0.002$; Table 2.2). More insects chose a host within the arenas in 2010 than in 2011 most likely because more insects entered the arenas that year ($F_{1, 20} = 6.84$, $P = 0.017$). However we did not control for attack density on our randomly selected natal trees that supplied the parental lines in either given year. Given more insects entered the arenas in 2010, the year in which the trials were conducted had an overall effect ($F_{1, 20} = 4.76$, $P = 0.041$), though this finding may be ecologically less relevant, as we did not control for attack density on natal hosts.

More mountain pine beetles entered the arenas from lodgepole pines than ponderosa, in turn the parental source species (natal species) supplying the beetles was significantly greater from lodgepole pine ($F_{1,20}=7.98$, $P=0.010$; Table 2.2). However, about twice as many brood on average, 673.7 MPB/m² emerged from ponderosa pine (52 MPB per log (37, 71); mean $\pm 1/2$ LSD) than from lodgepole pine at 247.2 MPB/m² (19 MPB per log (14, 28); $F_{1,20}=8.41$, $P=0.009$; Figure 2.8).

2.4. Host selection: bioassays with individual beetles

In our bioassays, adult-female-mountain pine beetles from lodgepole pine natal hosts chose ponderosa pine bark units over lodgepole pine bark units 16:10 (50:31%). Across both years, six of 32 beetles from lodgepole pine developed host died prior to confirmed host selection (19%). Females from ponderosa pine natal host chose ponderosa pine bark units over lodgepole pine bark units 19:8 (59:25%). Beetle mortality occurred in five of 32 trials from ponderosa pine developed host (16%). The natal source species, either ponderosa or lodgepole pine, did not have an effect on the choice of species selected ($X^2_1=0.458$, $P=0.50$). In two-thirds of the trials, beetles preferred ponderosa pine over lodgepole pine ($Z=-2.3$, $p=0.019$).

2.5. Reproductive success

The average brood per female in the choice log sections did not differ between natal hosts or choice hosts ($F_{1,20}=0.4$, $P=0.55$; $F_{1,20}=0.88$, $P=0.36$; Table 2.2). Brood per female from ponderosa pine chosen host logs was 11.3 (9.5, 13.6; mean $\pm 1/2$ LSD) and 10.2 from lodgepole pine logs (8.8, 11.9; Figure 2.9). Oviposition gallery length did not differ between natal host species or choice host species ($F_{1,20}<0.01$, $P=0.99$; $F_{1,20}=1.31$, $P=0.26$; Table 2.2). In ponderosa

pine as the chosen host, oviposition gallery length was 27.6 cm (26.4, 28.9; mean \pm 1/2 LSD) while in lodgepole pine oviposition gallery length was 26.2 cm (20.0, 27.5; Figure 2.10).

Discussion

The results of this series of experiments did not offer support for Hopkins' Host Selection Principle, suggesting that the two alternate hosts are susceptible to beetles that developed in either host, and that under controlled conditions where quantitative tree defenses are largely removed, the beetles prefer to breed in ponderosa pine. First, we did not find any evidence of two reproductively isolated populations of mountain pine beetle from lodgepole and ponderosa pines in sympatry as would be suggested by differences in emergence times or sex ratios. Second, our controlled-field experiments revealed ponderosa pine cut-logs were preferred almost 2:1 over lodgepole pine cut-logs in both years, regardless of natal host species. As further support, our laboratory bioassays revealed near identical host selection behavior, with female beetles exhibiting a 2:1 preference for ponderosa pine over lodgepole pine. This experiment offers a line of support that the effect of any aggregation pheromone based secondary host selection was not a major factor influencing the results of the controlled-field-choice experiments. Finally, we found no differences in brood per female or gallery length per female between the chosen hosts of lodgepole and ponderosa pines, indicating similar reproductive success in both host species. We found approximately twice as many mountain pine beetle chose ponderosa pine cut-logs, which resulted in approximately twice the brood production from chosen ponderosa pine host material versus lodgepole pine. Overall, our findings suggest ponderosa pine forests along the Front Range in Colorado could be vulnerable to elevated levels of mountain pine beetle-caused mortality where mountain pine beetle populations develop in adjacent lodgepole pines.

Sympatric host races and sex ratios

Our findings of overlapping mountain pine beetle emergence phenology do not support the hypothesis of host race separation. Rather, the likely mechanisms behind the similar synchronized temporal emergence between lodgepole and ponderosa pine natal hosts are through adaptive seasonality, controlled through degree days required for life stage development (Bentz *et al.*, 1991; Logan and Bentz, 1999; Safranyik *et al.*, 2010). Sex ratios during the five weeks of greatest emergence were weighted towards female emergence, which was twice that of males earlier in the flight period. Ratios subsequently equilibrated as the flight period waned. Female mountain pine beetle initiate gallery construction and “pioneer” or forage for susceptible hosts, hence, life strategies favoring female emergence early in the flight period would provide an advantage for successful host selection (Reid, 1962). Our findings of how the sex ratios in both hosts changed over time are in accordance with previously reported ratios for the mountain pine beetle over the course of their emergence period (Rasmussen, 1980, DeLeon, 1934).

Hopkins' Host Selection Principle

By controlling the natal host species in our field experiments, we were able to identify whether the beetles' natal host species influenced their subsequent host selection behavior. Results of our field choice experiments did not support Hopkins' Host Selection Principle due to an overall mountain pine beetle preference (almost 2:1) for ponderosa pine over lodgepole pine cut-logs, regardless of the natal host species in which the beetles had developed. Our findings agree with those of Richmond (1933), where mountain pine beetle preferred ponderosa pine over lodgepole pine approximately 2:1 when the quantitative host defenses (oleoresin flow) were debilitated by offering cut-logs. Our findings also support those of Wood (1963) in *Ips* beetle host selections of homogenized phloem discs prepared from cut trees, whereby natal host development did not

control subsequent parental host selection. As highlighted by Barron (2001), the mechanisms underlying host preference are still largely in question and unresolved. However, we found mountain pine beetle in the southern Rocky Mountains preferred ponderosa pine over lodgepole pine when the quantitative host defenses were largely removed from experimentation, which suggests that Corbet's proposition (1985), that host selection is driven by a chemical legacy transferred from larval stages and learned by adult stages is less likely to be involved.

Our finding that many more mountain pine beetle entered the arenas from lodgepole pine natal hosts than ponderosa hosts, but ponderosa pine cut-logs were subsequently selected significantly more than lodgepole pine cut-logs, suggests that mountain pine beetle has a strong affinity for ponderosa pine over lodgepole pine when the host defenses such as oleoresin flow that are related to an intact tree are debilitated by cutting the tree into sections. Significantly more brood developed from ponderosa pine logs than lodgepole pine as a result of selection preference for ponderosa pine. No significant differences in brood per female or gallery length were detected between lodgepole pine and ponderosa pine, which suggests nutrient requirements for acceptance of host material by the breeding parents and development of offspring are similar between lodgepole and ponderosa pine. The behavior of female host selection in our bioassays mirrored that of the selection preference in our controlled-field experiments, lending support for host attraction via olfactory recognition of pine terpenes or gustatory acceptance rather than a secondary pheromone induced response. In merging these lines of evidence, mountain pine beetle preference for ponderosa pine was twice that of lodgepole pine when quantitative host defenses were rendered impotent.

Based on our findings, the mountain pine beetle epidemic in the Front Range of Colorado will most likely impact both lodgepole and ponderosa pine. In Colorado, ponderosa pine forests

occupy 1.3 times that of lodgepole pine forests, and the Front Range offers near contiguous stands of susceptible ponderosa pine hosts. Even aged ponderosa pines stands with tree diameters greater than 25 cm with a basal area of more than 34 m²/ha are considered high risk (Stevens *et al.*, 1980). Impacts along Front Range ponderosa pine stands could be widespread and locally intense where mean diameters and stand basal areas meet or exceed these stocking levels coupled with conditions in which the quantitative host defenses have been weakened or compromised. Warm temperatures coupled with low precipitation provided regional synchrony in habitat susceptibility and facilitated spread of the epidemic within the region of our study (Chapman *et al.*, 2012).

Several limitations of our study design are worthy of discussion. First, our controlled field choice experiments using cut-log offerings may have altered the phloem terpene blend encountered by foraging beetles. Monoterpenes oxidize rather quickly, which in turn may have altered host acceptance behavior as the terpenes were oxidized over the seven days of offering. We attempted to account for this shortcoming by using cut logs large enough to reduce the oxidation effect throughout the logs. Second, flight of adult mountain pine beetles may increase responsiveness to host stimuli (Shepherd, 1966). Our controlled-field-choice experiments allowed the recently emerged beetles to fly once they entered the arenas, though the proportion that actually flew prior to host selection is unknown. However, nearly all beetles infested the logs so we inferred that under these conditions of our experiment, flight was not an issue. Third, in our bioassays we used bark/phloem units from both lodgepole and ponderosa pines that had the furrows, fissures, and leverage points from bark flakes that beetles need to gain penetration into the outer bark (Shepherd, 1965). We used bark/phloem units from multiple trees to introduce as much variation as likely naturally occur, though the influence of variation of these features between units on

beetle host selection was not tested. Additionally, many investigations have shown the preformed chemical composition of individual trees varies greatly, and within tree host volatiles vary (e.g. Smith 1977; Smith 2000; Davis and Hofstetter, 2011; Latta *et al.*, 2000, Sturgeon, 1979; Thoss and Byers, 2006; Clark *et al.*, 2010) which may have implications for bark beetle fecundity (Davis and Hofstetter, 2011). In an attempt to account for the variation within and between individuals (both chemical and phenotypical regarding bark and fissure presence), we used bark/phloem units from 24 trees located at two locations within the lodgepole pine ecotone. Fourth, each infested cut-log was reared in a temperature controlled environment, which may have selected for various microbial symbionts in our rearing studies. Optimal growth temperatures vary between blue staining fungi carried by the mountain pine beetle (Six and Bentz, 2007). We reared the brood in temperature controlled environments that overlap with fungal optimal temperatures, so as to not favor one symbiont over another. These conditions should not have affected comparisons between the brood rearings, though they may be different from the conditions encountered by beetles developing in a montane environment.

Host defenses and future research needs

Differences in host preference by the mountain pine beetle, when two or more alternate species are available, may be attributed to the tolerance or avoidance of tree defenses that are either quantitative and/or qualitative (the amount and chemical composition of oleoresin, respectively). Deterrents to host selection via secondary host defenses may be the single most important factor in insect host selection (Schoonhoven, 1992). Oleoresin flow from pine hosts is considered a primary defense mechanism against bark beetle attack, followed by triggered histological (auto-necrosis) mechanisms to contain the penetration of attack (Franceschi, 2005; Raffa and Berryman, 1982). The quantitative host defenses of constitutive oleoresin flow, initiated under

natural attack by bark beetle-caused severed resin canals, were largely debilitated through our controlled-field experiments. However, secondary host defense compounds within the oleoresins were likely still present (though oleoresins were unable to mire attacking beetles due to the lack of turgor pressure after we had cut the logs). We found mountain pine beetle fecundity and oviposition gallery lengths did not differ between lodgepole and ponderosa pine hosts, which suggests once selection occurs, the greatest hurdle to overcome is host defenses. Although the number of subsequent brood was not different between the two hosts, a separate investigation showed that body size of individual mountain pine beetles was larger in insects that developed in ponderosa pine natal and choice logs (West *et al.*, in prep). The ecological relevance of larger body size in individuals has not been fully resolved, though larger size may reflect a greater cache of lipids available for use against host defenses, may aid females in initiating a breeding site (Reid and Baruch, 2010), or may contribute to greater dispersal distances for foraging adults (Graf *et al.*, 2012).

Only a few studies of bark beetle-host interactions have supported Hopkins' Host Selection Principle, whereby alternate host species were not largely infested (Baker *et al.*, 1971) or one particular host species was attacked in greater proportion than an alternate host species (Dean, 2007). The influence of quantitative and qualitative host defenses in these instances was unaccounted for, although defenses could have played a part in controlling host selection behavior. Further work is needed to resolve the implications of quantitative and qualitative host defenses between lodgepole pine and ponderosa pine and mountain pine beetle-host selection interactions (West *et al.*, in prep.; Chapter 3).

In conclusion, these studies found mountain pine beetle can infest a different host species after developing within another natal host species. Beetles preferred ponderosa 2:1 over lodgepole

pine when the defenses of the trees were largely debilitated. Reproductive success per female was similar in the two hosts. These studies do not support Hopkins' Host Selection Principle and in fact demonstrate a preference for ponderosa pine under primary host selection by the mountain pine beetle. The overall effect of host defenses on host preference has not been resolved, though we suspect host defenses may have a large effect on overall beetle mass attack success. Our findings suggest mountain pine beetle host biotypes are unlikely based on emergence phenologies in these study areas. Taken together, our results suggest both lodgepole and ponderosa pines are likely to incur mountain pine beetle-caused mortality during the ongoing epidemic in Colorado's Front Range, especially in conditions in which tree defenses may be weakened.

Table 2.1. Emerged mountain pine beetles (meter² of each tree) and the number of trees with emergence per week from lodgepole pine (*P. contorta*) and ponderosa pine (*P. ponderosa*) between 2590 and 2650 m of elevation. Collections were conducted near Molly Lake area (2010) and Pennock Pass areas (2011) on the Arapaho-Roosevelt NF, CO. A total of 60 trees were randomly selected in mid-June each year; 30 traps were attached to lodgepole pine and 30 to ponderosa pine.

Year:2010		25-Jun	2-Jul	9-Jul	16-Jul	23-Jul	30-Jul	6-Aug	13-Aug	21-Aug	27-Aug	3-Sep	17-Sep	24-Sep	1-Oct
<i>P. contorta</i>	Number trees	-	1	9	18	25	28	28	24	20	14	5	3	3	-
	Total mpb	0	5	81	135	1157	7282	7185	2653	662	188	27	48	27	0
<i>P. ponderosa</i>	Number trees	2	5	8	14	18	22	26	26	20	9	8	3	4	-
	Total mpb	11	27	97	145	850	2239	1954	829	328	97	48	16	22	0

Year:2011		25-Jun	2-Jul	9-Jul	15-Jul	22-Jul	29-Jul	5-Aug	12-Aug	21-Aug	28-Aug	6-Sep	20-Sep	27-Sep	18-Oct
<i>P. contorta</i>	Number trees	-	-	-	1	20	25	27	26	12	4	3	2	2	-
	Total mpb	0	0	0	5	1227	5721	4801	1232	156	43	32	11	16	0
<i>P. ponderosa</i>	Number trees	-	-	4	5	21	25	27	25	19	6	4	3	1	2
	Total mpb	0	0	22	32	312	1528	1512	474	301	91	54	27	11	43

Table 2. 2. Effect significance from four models (y=response) describing mpb host selection, offspring production, offspring per female, and gallery length per female between lodgepole and ponderosa pine in controlled-field-choice experiments (2010-11; $\alpha=0.05$).

Effect	Response Variables					
	y=Female choice	y=Emerged Brood	y=Brood per Female	y=Gallery Length per Female	Numerator df	Denominator df
Natal species	0.002	0.011	0.094	0.591	1	20
Choice log species	0.004	0.009	0.053	0.073	1	20
Natal species*Choice species	0.719	0.679	0.770	0.090	1	20
Year	0.017	0.041	0.162	0.561	1	20
Natal species*year	0.369	0.534	0.740	0.518	1	20
Choice log species*year	0.595	0.808	0.987	0.238	1	20
Natal species*Choice log species* year	0.900	0.965	0.773	0.661	1	20

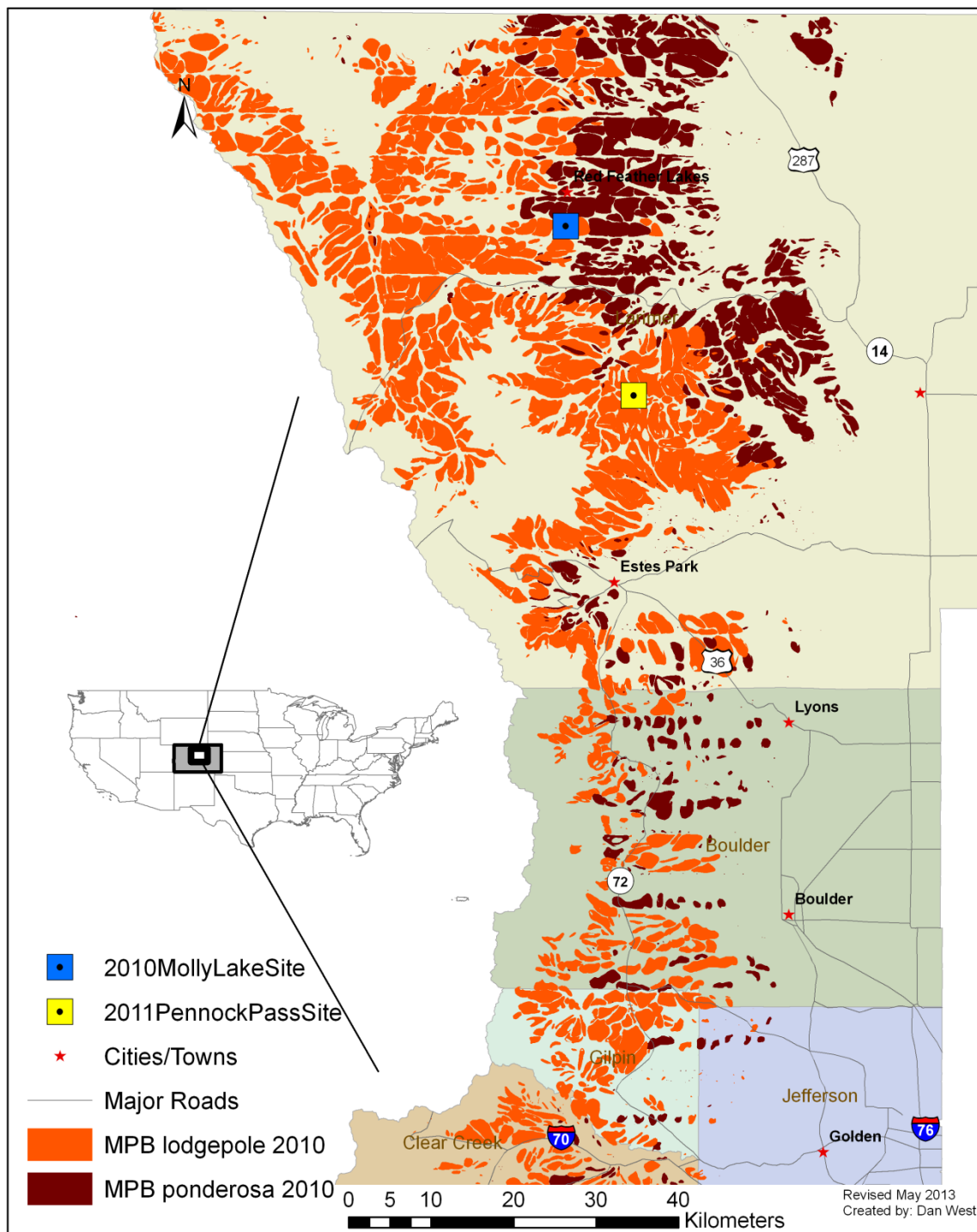


Figure 2. 1. Mountain pine beetle-caused mortality in lodgepole and ponderosa pines from 2010 Aerial Detection Survey along the Front Range, CO with lodgepole-ponderosa pine ecotone site locations for 2010 and 2011.



Figure 2. 2. Host Selection experiment with two ponderosa pine natal hosts providing freshly emerged mountain pine beetle to offered lodgepole pine and ponderosa pine cut-logs. Choice logs were replaced weekly (July and August) for five weeks in 2010 and 2011.



Figure 2. 3. Host Selection experiment with offered lodgepole pine and ponderosa pine cut-logs to freshly emerged mountain pine beetle from either lodgepole pine or ponderosa pine natal hosts. Choice logs were replaced weekly (July and August) for five weeks in 2010 and 2011.



Figure 2. 4. Bioassays for mountain pine beetle host selection between fresh bark/phloem units from lodgepole and ponderosa pines. Individual females from either lodgepole pine or ponderosa pine were offered a choice between the two alternate host bark/phloem units (32 cm²). N=64; half from each natal host.

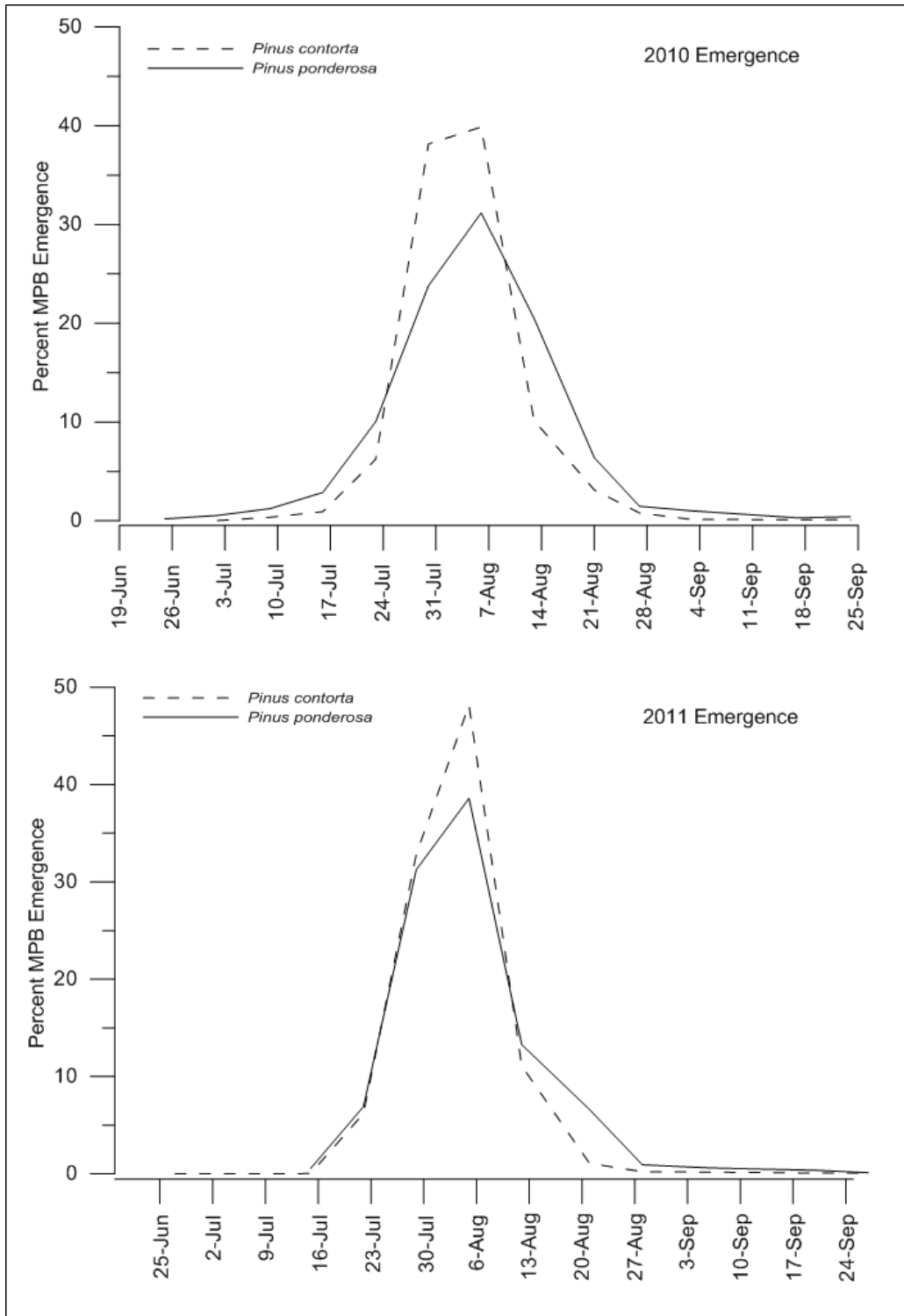


Figure 2. 5. Percent of total emerged mountain pine beetle from naturally infested lodgepole pine and ponderosa pine in 2010 and 2011. Trees were randomly located in the lodgepole-ponderosa ecotone between 2590 to 2650 m of elevation on the Arapaho-Roosevelt NF, CO. N=60 emergence cages (0.18 m²) split between species per year.

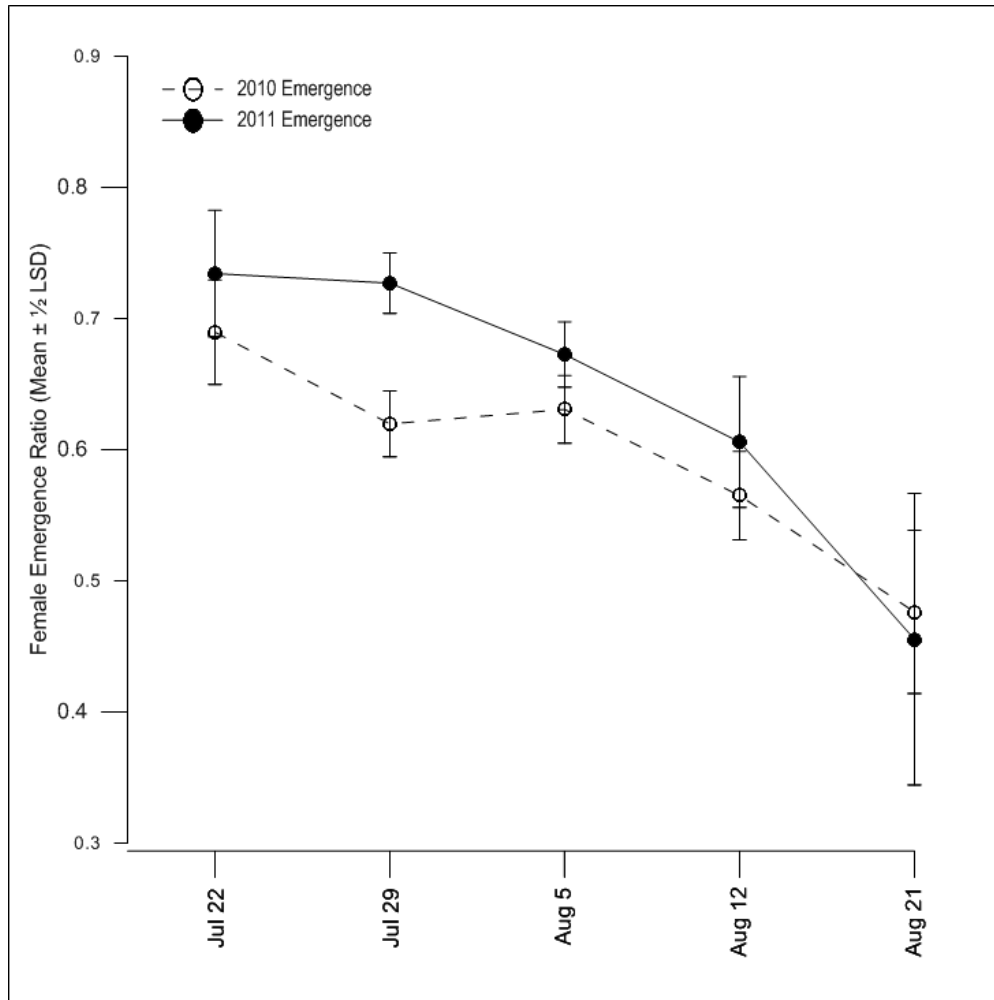


Figure 2. 6. Female to male ratio of mountain pine beetles emerged from combined lodgepole pine (n=60 trees) and ponderosa pine (n=60 trees) in 2010 and 2011 (Mean \pm $\frac{1}{2}$ LSD; N=120 trees split between species per year; emergence cages covered 0.18 m²/tree). Trees were randomly located in the lodgepole-ponderosa ecotone between 2590 to 2650 m of elevation on the Arapaho-Roosevelt NF, CO.

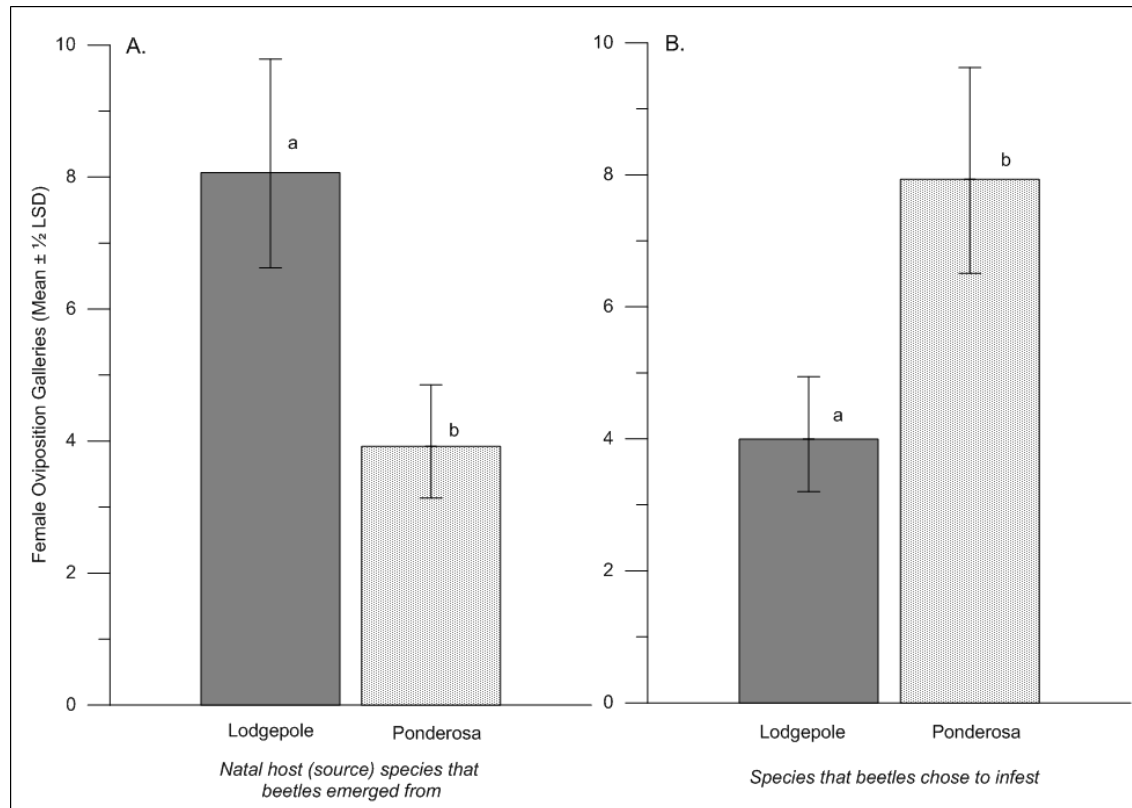


Figure 2. 7. Mountain pine beetle oviposition galleries created on log sections. A. Beetle source species: Mean number of oviposition galleries constructed on log sections by mountain pine beetles that had parental lines of either lodgepole and ponderosa pine host trees (source species). N=240 log sections (120 sections from arenas with lodgepole as natal source of beetles, 120 from arenas with ponderosa as natal source of beetles). (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$). B. Beetle choice species: Mean number of mountain pine beetle constructed oviposition galleries in log sections of lodgepole or ponderosa pine chosen by beetles from both natal tree species. N=240 log sections (120 sections of ponderosa as choice species, 120 sections of lodgepole as choice species) (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$).

*A significant year effect was detected though not depicted in the figure. More insects emerged from source trees in 2010 than 2011, though year effect has little ecological relevance.

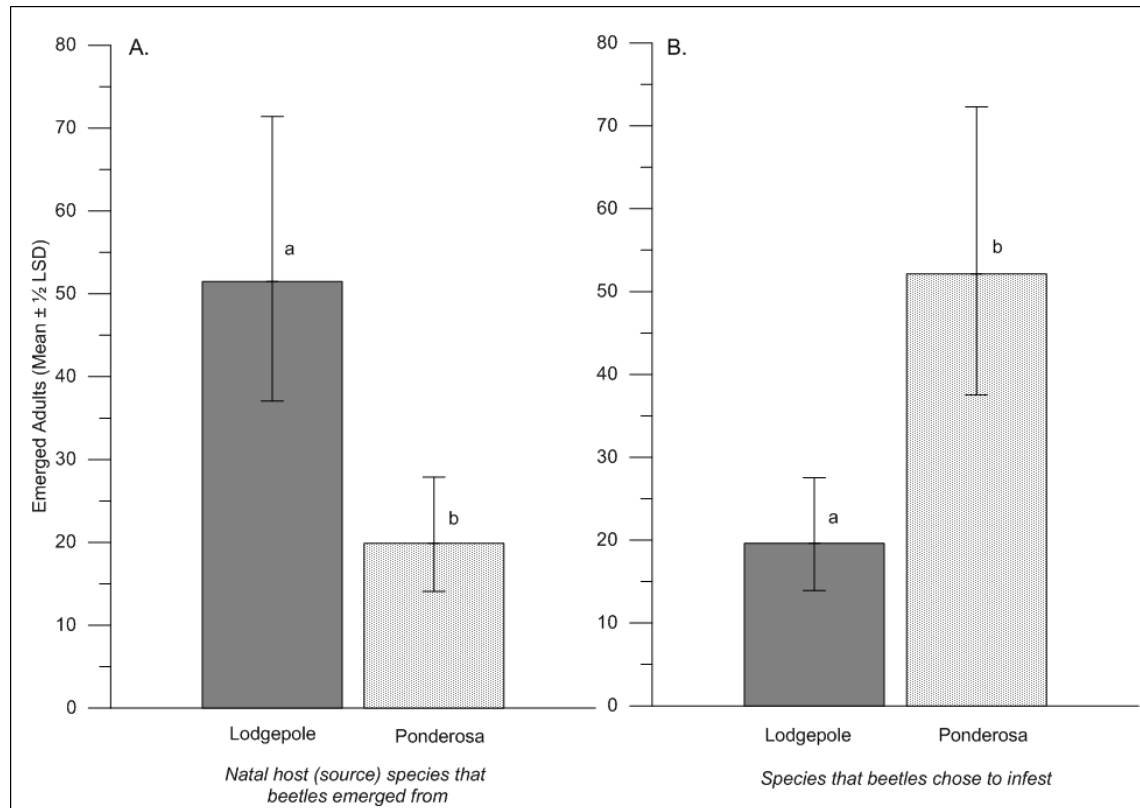


Figure 2. 8. Mountain pine beetle brood produced from log sections. A. Beetle source species: Mean number of mountain pine beetle brood produced in log sections that had parental lines of either lodgepole and ponderosa pine host trees (source species). N=240 log sections (120 log sections from arenas with lodgepole as natal source of beetles, 120 log sections from arenas with ponderosa as natal source of beetles). (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$). B. Beetle choice species: Mean number of mountain pine beetle brood produced in log sections of lodgepole or ponderosa pine chosen by beetles from both natal tree species. N=240 log sections (120 sections of ponderosa as choice species, 120 sections of lodgepole as choice species) (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$).

*A significant year effect was detected though not depicted in the figure. More insects emerged from source trees in 2010 than 2011, though year effect has little ecological relevance.

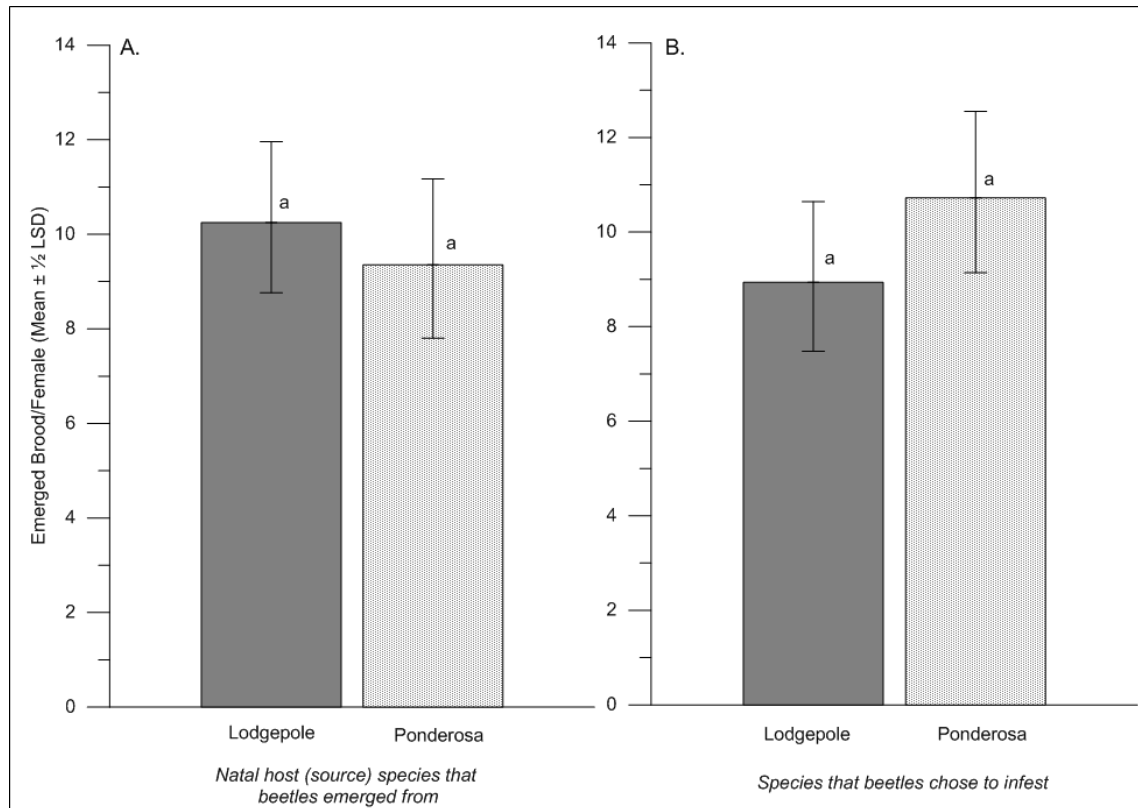


Figure 2. 9. Mountain pine beetle brood per female. A. Beetle source species: Mean number of mountain pine beetle brood per female produced in log sections that had parental lines of either lodgepole and ponderosa pine host trees (source species). N=240 log sections (120 log sections from arenas with lodgepole as natal source of beetles, 120 log sections from arenas with ponderosa as natal source of beetles). (Mean \pm ½ LSD). Differing letters indicate statistical separation ($\alpha=0.05$). B. Beetle choice species: Mean number of mountain pine beetle brood produced in log sections of lodgepole or ponderosa pine chosen by beetles from both natal tree species. N=240 log sections (120 sections of ponderosa as choice species, 120 sections of lodgepole as choice species) (Mean \pm ½ LSD). Differing letters indicate statistical separation ($\alpha=0.05$).

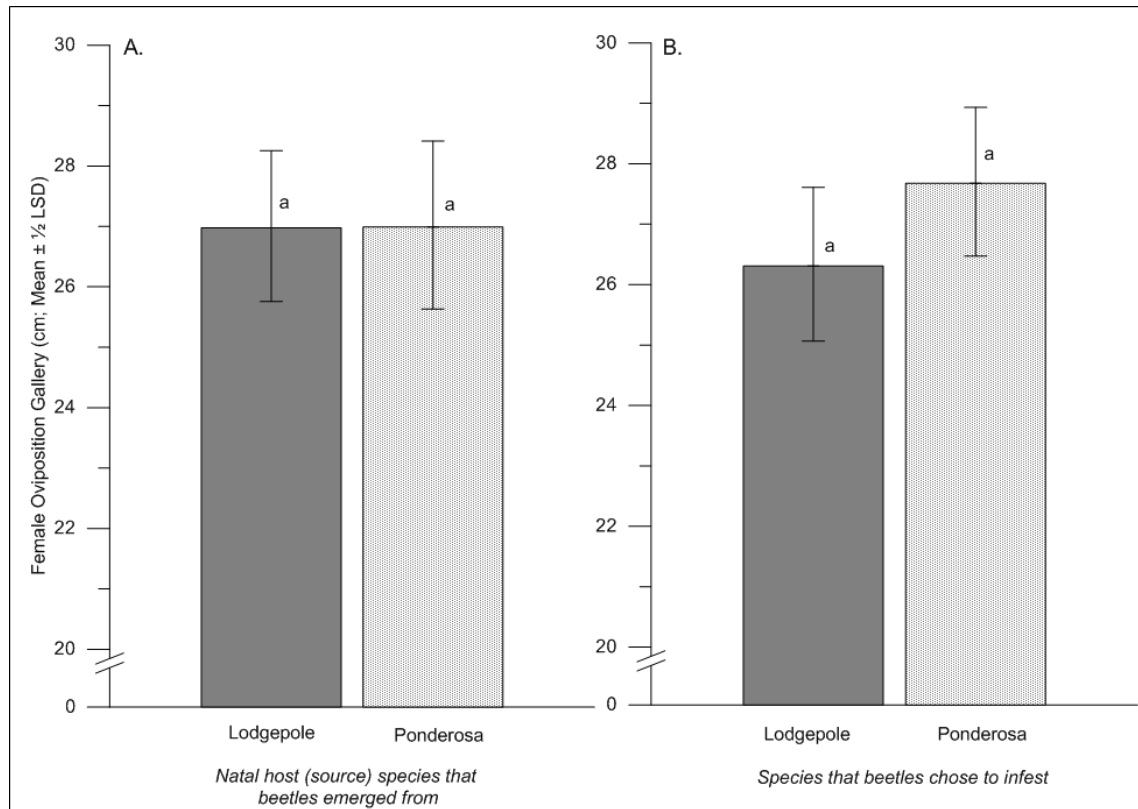


Figure 2. 10. Average mountain pine beetle oviposition gallery length. A. Beetle source species: Mean length of mountain pine beetle oviposition galleries in log sections that had parental lines of either lodgepole and ponderosa pine host trees (source species). N=240 log sections (120 log sections from arenas with lodgepole as natal source of beetles, 120 log sections from arenas with ponderosa as natal source of beetles). (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$). B. Beetle choice species: Mean length of mountain pine beetle oviposition galleries in log sections of lodgepole or ponderosa pine chosen by beetles from both natal tree species. N=240 log sections (120 sections of ponderosa as choice species, 120 sections of lodgepole as choice species) (Mean \pm 1/2 LSD). Differing letters indicate statistical separation ($\alpha=0.05$).

REFERENCES

- Amman, G.D. 1982. Characteristics of mountain pine beetles reared in four pine hosts. *Environmental Entomology* 11:590-593.
- Baker, B.H.; Amman, G.D.; Trostle, G.C. 1971. Does the mountain pine beetle change hosts in mixed lodgepole and whitebark pine stands. Research Note INT-151. Ogden, UT. U.S. Department of Agriculture, Forest Service, Intermountain Research Station. 7p.
- Barron, A.B. 2001. The life and death of Hopkins' Host-Selection Principle. *Insect Behavior* 14: 725-737.
- Barron, A. B., and Corbet, S. A. (1999). Pre-imaginal conditioning in *Drosophila* revisited. *Animal Behavior* 58: 621–628.
- Bentz, B.J.; Logan J.A.; Amman G.D. 1991. Temperature-dependent development of the mountain pine beetle (Coleoptera: Scolytidae) and simulation of its phenology. *The Canadian Entomologist* 123:1083-1094.
- Breed, M. D., Leger, E. A., Pearce, A. N., and Wang, Y. J. 1998. Comb wax effects on the ontogeny of honey bee nestmate recognition. *Animal Behavior* 55: 13–20.
- Chapman, T.B., Veblen, T.T., Schoennagel, T. 2012. Spatiotemporal patterns of mountain pine beetle activity in the southern Rocky Mountains. *Ecology* 93: 2175-2185.
- Clark, E. L., Carroll, A. L., & Huber, D. P. 2010. Differences in the constitutive terpene profile of lodgepole pine across a geographical range in British Columbia, and correlation with historical attack by mountain pine beetle. *The Canadian Entomologist* 142: 557-573.
- Corbet, S. A. 1985. Insect chemosensory responses: A chemical legacy hypothesis. *Ecological Entomology* 10: 143–153.
- Davis, T. S., & Hofstetter, R. W. 2011. Oleoresin chemistry mediates oviposition behavior and fecundity of a tree-killing bark beetle. *Journal of Chemical Ecology*, 37: 1177-1183.
- Dean, D.K. 2007. Host utilization by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), in mixed stands of limber pine, *Pinus flexilis* James, and lodgepole pine, *Pinus contorta latifolia* Engelm. M.S. Thesis, University of Wyoming. 55 p.
- DeLeon, D., Bedard, W. D., Terrell, T. T. 1934. Recent discoveries concerning the biology of the mountain pine beetle and their effect on control in western white pine stands. *Journal of Forestry* 32: 430-436.
- Franceschi, V.R.; Kronkene, P.; Christiansen, E.; Krekling, T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist* 167: 353-376.
- Graf, M., Reid, M.L., Aukema, B.H., Lindgren, B.S. 2012. Association of tree diameter with body size and lipid content of mountain pine beetles. *Canadian Entomologist* 144: 467-477

- Hopkins, A.D. 1909. Contributions toward a monograph of the scolytid beetles. I. The genus *Dendroctonus*. USDA Tech Ser. No. 17, Part I. 1-164 pp.
- Hopkins, A.D. 1916. Economic investigations of the scolytid bark and timber beetles of North America. U.S. Department of Agriculture Program of Work for 1917. p. 353.
- Hopkins, A.D. 1917. A discussion of C. G. Hewitt's paper on "Insect Behavior." J. Econ. Entomol. 10: 92-93.
- Latta, R. G., Linhart, Y. B., Lundquist, L., & Snyder, M. A. 2000. Patterns of monoterpene variation within individual trees in ponderosa pine. Journal of Chemical Ecology 26: 1341-1357.
- Logan, J.A., Bentz, B.J. 1999. Model analysis of mountain pine beetle (Coleoptera: Scolytidae) seasonality. Environmental Entomology 28: 924-934.
- McCambridge, W.F. 1964. Emergence period of Balck Hills beetles form ponderosa pine in the central Rocky Mountains. Res. Note RMRS-RN-32. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 4 p.
- Mitton, J. B., & Ferrenberg, S. M. 2012. Mountain pine beetle develops an unprecedented summer generation in response to climate warming. American Naturalist 179: E163-71.
- Raffa, K.F.; Berryman, A.A. 1982. Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated microorganisms. Environmental Entomology 11:486-492.
- Raffa, K. F., Aukema, B.H., Bentz, B. J., Carroll, A. L., Hicke, J. A., Turner, M. G., Romme, W. H. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: Dynamics of biome-wide bark beetle eruptions. BioScience 58: 501-518.
- Rasmussen, L.A. 1980. Emergence and attack behavior of the mountain pine beetle in lodgepole pine. Res. Note RMRS-RN-297. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 7 p.
- Ray, S. 1999. Survival of olfactory memory through metamorphosis in the fly *Musca domestica*. Neuroscience Letters 259: 37-40.
- Reid, M.L., Baruch, O. 2010. Mutual mate choice by mountain pine beetles: size-dependence but not size-assortative mating. Ecol Entomol 35:69-76
- Reid, R.W. 1963. Biology of the mountain pine beetle, *Dendroctonus monticolae* Hopkins, in the east Kootenay region of British Columbia. III. Interaction between the beetle and its host, with emphasis on brood mortality and survival. The Canadian Entomologist 95:225-238.
- Reid, R.W. 1962. Biology of the mountain pine beetle, *Dendroctonus monticolae* Hopkins, in the east Kootenay region of British Columbia. II. Behaviour in the host, fecundity, and internal changes in the female. The Canadian Entomologist 94:605-613.

- Richmond, H.A., 1933. Host selection studies of *Dendroctonus monticolae* Hopk. in southern British Columbia. *The Forestry Chronicle* 9:60-61
- Safranyik, L., Carroll, A. L., Régnière, J., Langor, D. W., Riel, W. G., Shore, T. L., Taylor, S. W. 2010. Potential for range expansion of mountain pine beetle into the boreal forest of North America. *The Canadian Entomologist* 142: 415-442.
- Safranyik, L. 1968. Development of a technique for sampling mountain pine beetles in lodgepole pine. Ph.D. thesis, University of British Columbia, Vancouver, BC. 195 p.
- Schoonhoven LM, Blaney WM, Simmonds MSJ. 1992. Sensory coding of feeding deterrents in phytophagous insects. In *Insect-Plant Interactions*, ed. E Bernays, 4:59–79. Boca Raton: CRC
- Schmid, J.M., 1972. Emergence, attack densities, and seasonal trends of mountain pine beetle (*Dendroctonus ponderosae*) in the Black Hills. Res. Note RMRS-RN-211. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 7 p.
- Shepherd, R.F. 1965. Distribution of attacks by *Dendroctonus ponderosae* Hopk. on *Pinus contorta* Dougl. var. *latifolia* Engelm. *The Canadian Entomologist* 97:207-215.
- Shepherd, R.F. 1966. Factors influencing the orientation and rates of activity of *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). *The Canadian Entomologist* 98:507-518.
- Six, D.L., Bentz, B.J. 2007. Temperature determines symbiont abundance in a multipartite bark beetle-fungus ectosymbiosis. *Microbial Ecology* 54: 112-118.
- Smith, R. H. 1977. Monoterpenes of ponderosa pine xylem resin in the western United States. USDA Forest Service Technical Bulletin No. 1532. USDA, Washington, D.C.
- Smith, R.H. 2000. Xylem monoterpenes of pines: Distribution, variation, genetics, function. Gen. Tech. Rept. PSW-GTR-177. USDA Forest Service, Pacific Southwest Research Station, Albany, CA. 454p.
- Stevens, R.H., McCambridge, W.F., Edminster, C.B. 1980. Risk rating guide for mountain pine beetle in Black Hills ponderosa pine. Res. Note RMRS-RN-385. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 2 p.
- Sturgeon, K. B. 1979. Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. *Evolution* 33: 803-814.
- Tishmack, J.; Mata, S. A.; Schmid, J. M.; Porth, L. 2005. Mountain pine beetle emergence from lodgepole pine at different elevations near Fraser, CO, Res. Note RMRS-RN-27. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 5 p.
- Thoss, V., and Byers, J. A. 2006. Monoterpene chemodiversity of ponderosa pine in relation to herbivory and bark beetle colonization. *Chemoecology* 16: 51-58.

Tully, T., Cambiazo, V., and Kruse, L. 1994. Memory through metamorphosis in normal and mutant *Drosophila*. *Neuroscience* 14: 68–74.

van Emden, H. F., Sponagl, B., Wagner, E., Baker, T., Ganguly, S., and Douloupaka, S. 1996. Hopkins' 'host selection principle', another nail in its coffin. *Physiological Entomology* 21: 325–328.

Wood, D.L. 1963. Studies on host selection by *Ips confusus*. *University of California Publications in Entomology* 27: 241-282.

CHAPTER 3

HOST DEFENSE MECHANISMS AGAINST BARK BEETLE ATTACK DIFFER BETWEEN PONDEROSA AND LODGEPOLE PINES

Summary

The initial lines of conifer defense against bark beetle attack are the quantitative and qualitative defenses produced prior to attack. Our objective was to assess the host defenses of lodgepole pine and ponderosa pine from ecotone stands where the mix of hosts provided an opportunity for host transition for the mountain pine beetle. As mountain pine beetle populations approached ponderosa pine-dominated stands from adjacent lodgepole pine stands, we tested two hypotheses: (1) preformed quantitative host defenses (amount of oleoresin flow in 24 hrs) differ between lodgepole and ponderosa pines, and (2) preformed qualitative host defenses (monoterpene constituents) differ between the two pine species. The second hypothesis was tested with particular attention to concentrations of α -pinene and limonene, as these two compounds have behavior eliciting properties in bark beetles. We collected oleoresins at three locations in the southern Rocky Mountains from 12 trees that were paired by species and were of similar size and under similar growing conditions. Our results indicate the amount of preformed oleoresins in ponderosa pine was almost four times that of lodgepole pine. The amount of total qualitative preformed monoterpenes did not differ between the two hosts, though we found differences in all but three host defense monoterpenes. We found greater concentrations of limonene, β -phellandrene, and cymene in lodgepole pines than ponderosa pines. The monoterpenes of β -pinene, 3-carene, myrcene, and terpinolene were all greater in ponderosa pine preformed oleoresins than in lodgepole pine. No differences in concentrations

between the two pines were detected in α -pinene, γ -terpinene, and bornyl acetate. The variation in both quantitative and qualitative preformed oleoresin defenses between these two pine species suggests they may have different susceptibility to infestation by mountain pine beetle, but specific hypotheses addressing the ecological relevance of these differences have not been tested.

Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) exploit the subcortical tissues of woody species for sustenance and reproductive shelter. Only a few of over 600 bark beetle species periodically erupt from innocuous population levels (known as endemic populations) to outbreak or epidemic populations capable of altering forest stand characteristics and composition. For example, the mountain pine beetle (*Dendroctonus ponderosae* Hopk.) is capable of transitioning from endemic populations, to incipient-epidemic, to epidemic conditions within a relatively short time period (Safranyik and Carrol, 2006). Within these population phases, the behavior of the mountain pine beetle can vary depending upon population levels (Boone *et al.*, 2011). As mountain pine beetle populations increase, groups of beetles can successfully attack and kill larger diameter, heavily defended trees. In contrast, endemic beetle populations typically attack trees that are weakened and less able to resist infestation (e.g. lightning struck trees or disease); this pattern serves to maintain populations not capable of overcoming well defended trees (Boone *et al.*, 2011). Eruptive mountain pine beetle-caused mortality in lodgepole pine over the two decades of 1990 through 2010 was more than ten-times greater than any previously recorded (Wulder *et al.*, 2010).

The defense of conifers under attack by bark beetles begins with oleoresin produced in a network of preformed resin ducts in the subcortical tissues of the tree (reviewed in Franceschi *et al.*, 2005). As beetles bore into the bark and subcortical tissues, a flow of preformed oleoresin is

released from the ducts which are under pressure from dense epithelial cells surrounding the ducts. This defense strategy is referred to as the preformed quantitative defenses of the tree. The oleoresins serve as a physical barrier to penetrating organisms by flushing and sealing the wounds to prevent desiccation and prevent insects from penetrating further into tissues (reviewed in Paine *et al.* 1997). The constituents of the oleoresin, known as the qualitative defenses, are also important factors affecting bark-penetrating beetles and their fungal and bacterial symbionts (reviewed in Raffa *et al.*, 2005). The oleoresins are mainly comprised of secondary defensive compounds such as monoterpenes, diterpenes, sesquiterpenes, and stilbene phenolics (Franceschi *et al.*, 2005; Raffa *et al.*, 2005). Monoterpenes are documented as the fraction of resin most deleterious to attacking bark beetles and vary in amounts within and between tree species. In contrast, diterpene and stilbene phenolics appear to possess antifungal properties and not only are present in the preformed oleoresins, but also are induced when the subcortical tissues of the bark are penetrated (Franceschi *et al.*, 2005; Raffa *et al.*, 2005). A comparative study between the quantitative and qualitative defenses of two pine species in Wisconsin found that the preformed (constitutive) monoterpenes released when bark beetles attacked (*Ips* species) red pine (*Pinus resinosa*) and jack pine (*Pinus banksiana*) killed 60% of the beetles, while induced concentrations produced *de novo* had greater efficacy, killing 90% of the beetles (Raffa and Smalley, 1995). Once an attack from bark beetles is underway, the induced concentration of secondary monoterpene compounds in the oleoresins and subcortical tissues is significantly greater than those in the preformed oleoresin. The induced response is elevated when phytopathogenic fungal associates of bark beetles are introduced and the response varies depending on which fungal associates are introduced (Raffa and Smalley, 1995).

Trees in several families of conifers have an ability to produce a greater amount of induced defensive compounds than preformed compounds after wounding or an attack occurs (Lewinsohn *et al.* 1991). Particularly relevant to our study, monoterpene cyclase activity was found to be greater upon wounding in both lodgepole pine and ponderosa pines (Lewinsohn *et al.* 1991). To overcome these induced defenses, bark beetles have evolved a suite of semiochemical communications derived from host monoterpenes (reviewed in Seybold *et al.*, 2006). The monoterpenes are oxidized as the beetles mine subcortical tissues, and subsequently are emitted from the entry wounds and frass (insect feces) as semiochemicals for conspecific attraction, or aggregation pheromones (Seybold *et al.*, 2006). This attraction results in insects attacking focal trees *en masse*, which allows them to overcome the host defenses in a relatively short time period (Seybold *et al.*, 2006). Furthermore, *Dendroctonus* species have evolved in close relationship with their hosts, developing a tolerance for host species defense compounds while lacking a tolerance for non-host volatiles. This tolerance of defensive compounds may explain in part the high level of host specificity seen in many species of bark beetles (Smith, 1963).

The sequence of attack rates by the mountain pine beetle is directly linked to the status of host defenses. Beetle attack rates decrease once preformed oleoresin stores have been depleted, rather than the attack sequence of individual trees being controlled by the concentrations of beetle synthesized anti-aggregation compounds alone (produced mainly from monoterpenes), or epideictic pheromones (Raffa and Berryman, 1983; 1987). The beetle strategy of overcoming host defenses through aggregated attacks *en masse* enables relatively large groups of attacking beetles to overcome individual tree defenses, while minimizing the effect of intraspecific competition. Raffa and Berryman (1987) found an optimal density for a successful mountain pine beetle attack in lodgepole pine was near 62 attacks/m². When attack densities were high

(~80/m²) host defenses were rapidly overwhelmed but subsequent larval development was negatively affected by intraspecific competition. Low attack densities of ~40/m² failed to overcome host defense mechanisms. In ponderosa pine, an alternate host species of the mountain pine beetle, trees with large resin ducts, more resin ducts per mm², and containing more resin ducts per unit area of growth remained unattacked compared to trees with less defensive production (Kane and Kolb, 2010). Preformed oleoresin flow caused by severing the ducts upon subcortical penetration by the beetles not only poses a physical obstacle, but also serves to restrict the release of oxidized host monoterpenes in the form of pheromones. At the entry site, when sufficient oleoresin has been produced, the oleoresin traps pheromones and keeps them from dispersing through the stand, mitigating the effectiveness of pheromones for con-specific recruitment (Raffa and Berryman, 1983).

Conifer monoterpenes are deleterious to bark beetle adults, eggs, and associated fungi in laboratory and field assays (Raffa *et al.*, 2005; Raffa and Berryman, 1987). The toxicity of α -pinene to mountain pine beetles exposed to ecologically relevant doses suggests low concentrations lack a strong insecticidal property while higher concentrations had more than 90% lethality (Reid and Purcell, 2011). However, α -pinene is oxidized by attacking mountain pine beetles to produce an aggregation pheromone, *trans*-verbenol (Seybold *et al.* 2006) and has attraction properties within a range of concentrations (Renwick and Vite, 1970). Thus, increased concentrations of α -pinene from one tree species over another may synergize an aggregated or mass attack for the species containing greater levels of the behavioral terpenoid, provided the attacking beetles can tolerate and synthesize the specific compound concentrations. For example, spruce beetle (*Dendroctonus rufipennis* Kirby) populations avoided trees with high concentrations of α -pinene, the monoterpene of greatest concentration in spruce, though

moderate concentrations were associated with host acceptance and gallery initiation by the insects (Wallin and Raffa, 2004). Similar relationships were reported with *Ips* species and host acceptance within a range of monoterpene concentrations (Wallin and Raffa, 2000). Not only may the host monoterpenes exert negative pressures on host selection, pine bark beetle aggregation pheromones are synergized by the presence of some host monoterpenoids. Seybold *et al.* (2006) review the synergisms between pheromones and kairomones, and highlight a need for further investigations in their complexity with regard for beetle host selection.

The attack of bark beetles can exert selective pressure on tree chemistry over time. For instance, Raffa and Berryman (1982) found no differences in monoterpene compositions between trees that were attacked versus trees that resisted or escaped attack (i.e., trees which survived after exposure to naturally occurring epidemic populations of beetles), though there were greater quantitative defenses in the unattacked trees. Limonene is one of the secondary host defense compounds present in ponderosa pine and lodgepole pine phloem layers and resins, as well as other conifer species (Clark *et al.* 2010; Smith, 1977; Smith, 2000). This monoterpene has toxic properties against several species of bark beetles (Bordasch and Berryman, 1977; Cook and Hain, 1988; Coyne *et al.* 1976), particularly at induced concentrations (Raffa and Berryman, 1987). Levels of induced limonene after initial beetle attack rose to 95% more than that of preformed limonene in lodgepole pine (Raffa and Berryman, 1987). Ponderosa pines from areas that had survived historic outbreaks of the western pine beetle (*D. brevicomis* LeConte) had higher preformed concentrations of limonene than ponderosa pines from areas that had been free of beetle attack and outbreaks (Sturgeon, 1979). In another study, ponderosa pine trees with higher preformed limonene coupled with high oleoresin flow resisted or escaped attack following an outbreak over trees lacking these traits (Smith, 1975).

Another monoterpene of particular interest produced by some pine species is β -phellandrene. In lodgepole pine, β -phellandrene has been found to be the most abundant compound in the oleoresin, but its role as a defensive compound with the mountain pine beetle has yet to be resolved. The concentrations of this monoterpene were low to non-existent in ponderosa pines (Smith 1977, 2000). Furthermore, β -phellandrene appears to have reduced toxicity against bark beetles when compared to other defensive monoterpenes found in relatively small proportions in some oleoresins (Coyne *et al.* 1976). However, β -phellandrene increased the attraction of *Ips pini* (Say) to its pheromone (Miller and Borden, 1990), which suggests this compound may have kairomonal properties and demonstrates the variation in bark beetle adaptation by the beneficial utilization of these compounds.

In Colorado, the major hosts of the native mountain pine beetle are lodgepole pine (*Pinus contorta* Dougl.) and ponderosa pine (*Pinus ponderosa* Lawson), while less abundant limber pine (*Pinus flexilis* James) and bristlecone pine (*Pinus aristata* Englm.) are also host species. Historical outbreaks of the mountain pine beetle failed to spread to adjacent alternate host stands from the host species for which they developed, though current outbreaks of this beetle have successfully caused adjacent alternate host mortality as populations spread through higher elevation lodgepole pine stands, mixed conifer stands that included several species of pine, and lower elevation ponderosa pine-dominated stands ((USDA, Aerial Detection Survey, <http://www.fs.usda.gov/detail/r2/forest-grasslandhealth/>).

Mountain pine beetle host selection behavior between lodgepole pine and ponderosa pine was found to favor ponderosa pine at a rate of 2:1 in choice experiments and laboratory bioassays (West *et al.*, in prep). Those studies were conducted under controlled conditions in the field and laboratory with cut-log and bark/phloem units that offered no oleoresin flow defenses against

attack. Therefore, we designed the following experiments to compare the quantitative and qualitative host defenses between lodgepole pine and ponderosa pine species in the field. Our objective was to assess the host defenses of lodgepole pine and ponderosa pine in mixed pine stands where both alternate host species of the mountain pine beetle were available for attack and beetle populations were building. We tested two hypotheses: (1) quantitative host defenses vary between lodgepole and ponderosa pines, and (2) qualitative host defenses differ between the two pine species.

Methods and Materials

Study Sites. Three sites were selected in 2011 within the lodgepole-ponderosa pine ecotone between 2568-2648 m along the Front Range, Colorado, US. Two sites were located on the Arapaho-Roosevelt NF (Molly Lake area, UTM NAD-83 Easting Northing: 13N 449861 4512902; Pennock Pass Area, UTM 13N 458090 4492379) and one site was in Rocky Mountain NP (Little Horseshoe Park, UTM 13N 448546 4471386). From each site, pairs of trees of lodgepole and ponderosa pines were selected that were within 2.5 cm DBH (diameter at breast height; 1.4 m from forest floor) from each other in the same stand and had no visual sign of insect or disease activity. We sampled a total of 56 pairs of trees with 28 pairs within 36.3 ha at the Molly Lake area, 17 pairs in 24.2 ha at the Pennock Pass area, and 11 pairs within 25.3 ha at the Little Horseshoe Park area; n=112 trees). Trees were sampled with similar site conditions of both host species with similar diameters from the same stands. Oleoresin collections were performed the last week of July and the first two weeks of August while the peak mountain pine beetle emergence was occurring; this allowed us to quantify host defenses at the time when beetle attacks were occurring (West *et al.*, in prep; see Ch 2).

Quantitative host defense mechanisms. Preformed oleoresin was collected from trees at 1.4m from the forest floor for twenty four hours (\pm 1 hr). Outer bark and phloem disks were removed on the north and south aspects of each tree using an 11 mm diameter punch (#14; C.S. Osbourne & Company, Harrison, New Jersey). Both north and south sides of trees were sampled to test for variances between oleoresin flow by aspect since mountain pine beetle attack densities are commonly greatest on the north aspects (Reid, 1963). Glass vials were placed into the wounds and held in place with a band of packing tape around the tree. Oleoresin was allowed to accumulate for 24 hours, then was left in vials, weighed, and stored in freezer storage (-22° C) prior to chemical analyses. Empty vial weights were subtracted from weights of the oleoresin collections in calculating the overall oleoresin weights. Differences in oleoresin flow were tested in response to north and south aspects of both host species by using a two-way ANOVA (Proc GLM; SAS 9.2). Preformed, 24-hour quantitative host defenses were not different between north and south aspects for either tree species (lodgepole: 0.87 mg (0.73, 1.05); mean (Lower half LSD, Upper half LSD); ponderosa: 0.99 mg (0.83, 1.2); $F_{3, 183}=0.52$, $P=0.47$). To test for differences in oleoresin flow (weights) between host species, we averaged the oleoresin collections from the north and south aspects of each tree, and calculated half Least Significant Difference (LSD) values for confidence bounds (Proc GLM; SAS 9.2). \log_{10} -transformations were performed on oleoresin weights to reduce high leverage points. Mean estimates were back-transformed from \log_{10} values to improve interpretation of the results. Upper and lower confidence bounds were calculated through \log_{10} back-transformed values (mean \pm 1/2 LSD). Eight samples leaked during collection and were not used in the analyses (7 collections from lodgepole pine trees, 1 from ponderosa pine).

Qualitative defense mechanisms. Preformed monoterpenoids were quantified in 2012 from oleoresins exuded from a random sample of 20 study trees (ten per species). Terpenoid profiles and quantities were analyzed via gas chromatography coupled with flame ionization detection (GC-FID). We prepared a solution of 1 g oleoresin/mL hexane. We transferred 10 μ l of solution into a clean vial and added eicosane (100 μ g in dichloromethane) as an internal standard. The sample was concentrated under a gentle stream of nitrogen until dry, and then re-dissolved in 1 mL hexane. A 0.5 μ l sample was injected onto a FAMEWAX column (30 m, 0.32 mmID, 0.25 μ m df, Cat. No. 12498, Restek, Bellefonte, PA) on a HP 5890 Series II gas chromatograph with FID detector with temperature programming from 40°C (held for 1 min) then increased by 5°C per min to 200°C (held for 2 min). Results were analyzed with Clarity® software (Clarity 2008) and quantified using a calibration curve created for synthetic standards of all compounds (Sigma Aldrich, Inc., St. Paul, MN) with the internal standard. β -phellandrene was identified on a HP 5890 Series II gas chromatograph coupled with a HP 5970 mass selective detector with temperature programming from 40°C (held for 1 min) to 200°C at a rate of 5°C/min. The resulting mass spectra were analyzed visually and with a probability-based computer search of the NIST/EPA/NIH 75K spectral library (Hewlett-Packard, Palo Alto, CA). Total and individual terpenoid concentrations were standardized based on per gram of oleoresin.

Concentrations of each of the 11 terpenoids we identified were compared between species using a multivariate ANOVA (Proc GLM; SAS 9.2; α -pinene, β -pinene, myrcene, 3-carene, α -phellandrene, β -phellandrene, p-cymene, γ -terpinene, terpinolene, and bornyl acetate). When significant differences of terpenoid concentrations between species were detected in the model under Hotellings-Lawley trace tests, Tukey's HSD test was used to identify any significant differences between mean concentrations for that terpenoid between the species. Log_{10} -

transformations were performed on terpenoid concentrations as previously described for the multivariate ANOVA. To investigate potential differences in the total terpenoid and specific individual terpenoids, we used a nonparametric, one factor model (host species: lodgepole pine, ponderosa pine; Proc NPAR1WAY, SAS 9.2) using Wilcoxon Exact tests, treating monoterpene concentrations in response to tree species. The nonparametric tests were used to account for any skewness in the individual terpenoid concentrations. Mean estimates were back-transformed to improve interpretation of the results and confidence bounds were calculated as previously described (mean $\pm 1/2$ LSD).

Chemicals. The following compounds and reagents used in the monoterpene analysis were purchased from Sigma-Aldrich, Inc., St. Paul, MN: p-Cymene (99%, Cat. No. C121452), (-)-bornyl acetate (97%, Cat. No. B55203), γ -terpinene (97%, Cat. No. 223190), 3-carene (95%, Cat. No. 115576), (-)- α -pinene (99%, Cat. No. 305715), R(-)- β -pinene (99%, Cat. No. 112089), myrcene (tech. grade, Cat. No. M100005), and R(-)- α -phellandrene (>95%, Cat. No. 77429). Terpinolene was purchased from TCI America (>85%, Cat. No. T0817).

Results

Mean preformed, 24-hr oleoresin flow from ponderosa pine was nearly four times greater than that of lodgepole pine oleoresin flow (ponderosa: 2.0mg (1.6, 2.5); mean mg (Lower half LSD, Upper half LSD); lodgepole: 0.59 mg (0.48, 0.75); $F_{1,102}=30.17$, $P < 0.0001$; Figure 3.1). No differences in tree diameters were detected between the paired alternate host pine species used in the analyses (lodgepole pines 24.9 cm (24.1, 25.7); mean (Lower half LSD, Upper half LSD); and ponderosa pines 25.5 cm (24.7, 26.2); $F_{1, 102}=0.63$, $P=0.42$).

Total cumulative terpene concentrations did not differ between lodgepole pines and ponderosa pines ($F_{1, 18}=2.79$, $P=0.11$; Figure 3.2). However, we found differences in all but three host defense monoterpenes between lodgepole pines and ponderosa pines (Table 3.1; Figure 3.3). There were greater concentrations of limonene ($F_{1, 18}=5.87$, $P=0.026$), β -phellandrene ($F_{1, 18}=51.2$, $P<0.0001$), and cymene ($F_{1, 18}=13.5$, $P=0.002$) in lodgepole pines compared to ponderosa pines (Figs. 3.3, 3.4). The monoterpenes of β -pinene ($F_{1, 18}=8.09$, $P=0.01$), 3-carene ($F_{1, 18}=20.5$, $P=0.0003$), myrcene ($F_{1, 18}=22.9$, $P=0.0001$), and terpinolene ($F_{1, 18}=10.1$, $P=0.005$) were all greater in ponderosa pine than lodgepole pine preformed oleoresins. Three monoterpene concentrations were not different between lodgepole and ponderosa pines: α -pinene, γ -terpinene, and bornyl acetate (Figure 3.3).

Discussion

Our results support the hypothesis that quantitative host defenses of ponderosa pine differ from lodgepole pine. The qualitative analyses also supported the hypothesis that preformed oleoresin compositions markedly vary between the two pine species from the same ecotone stands, particularly those compounds known to be toxic to bark beetles (e.g. limonene) or those that have been shown to elicit mountain pine beetle aggregation behavior (e.g. β -phellandrene, myrcene). We found that upon wounding, ponderosa pines exuded almost four times the preformed oleoresin compared to lodgepole pines under similar environmental conditions. The implications of this finding may in part explain mountain pine beetle preference for ponderosa pine over lodgepole pine in manipulated choice experiments (Richmond, 1933; West *et al.*, in prep). When exogenous factors of temperature and precipitation cause drought stress in ponderosa pines, the susceptibility of these trees to attack may be enhanced due to reduced quantitative host defenses compared to the defenses of lodgepole pines. Previous studies have

shown that susceptibility to attack increases when drought stress is high (Chapman *et al.*, 2012; Dunn and Lorio, 1993; Lombardero *et al.*, 2000). Further investigation of drought stress and beetle-caused mortality may be warranted between lodgepole pine and ponderosa pine in northern Colorado, given the positive relationship between mountain pine beetle epidemics and drought conditions. Not only might the physical resistance to attack be diminished under drought stress from reduced oleoresin flow, but the ability of the oleoresins to contain the beetle's aggregation pheromone blend produced at the site of attack is also diminished. The density of attack necessary to overcome quantitative host defenses may be reduced when trees are under drought stress (Raffa and Smalley, 1995). When quantitative host defenses were debilitated under experimental conditions, such as cut-log experiments and bioassays, avoidance of defenses is likely less of a behavioral strategy of bark beetles.

Total concentrations of monoterpenes were not different between the preformed oleoresins of lodgepole pine and ponderosa pine. This finding led to further investigations of the qualitative constituents within the oleoresins. The quantity of β -phellandrene from lodgepole pine clearly was a significant proportion of the overall monoterpene composition (61%). However, β -phellandrene can cause a positive flight response (attract) the mountain pine beetle when coupled with an aggregation pheromone blend, though it did not significantly increase trap catches across a multitude of release rates (Miller and Borden, 2000). Since this compound has been shown to elicit attraction in *Ips pini*, and has been shown to elicit antennal responses in the mountain pine beetle (Huber *et al.*, 2000; Pureswaran *et al.*, 2004), further assays may be warranted.

We found preformed lodgepole pine oleoresins contained significantly more limonene (1.5% of total monoterpenes) than ponderosa pine (1.3%) oleoresins. It is unclear whether the small concentration differences of this compound between the two hosts translate into greater chemical

defenses against attack. However, since limonene has been demonstrated to be highly toxic to bark beetles, we suspect an incremental difference may have an ecological effect on attacking beetles (Bordasch and Berryman, 1977; Coyne *et al.* 1976; Raffa and Berryman, 1987).

We did not find any differences in concentrations of the common beetle attractant α -pinene between lodgepole and ponderosa pine preformed oleoresins. Unfortunately, the concentration that elicits mountain pine beetle aggregation behavior is not known for these pine species.

Whether α -pinene in a blend of other monoterpenes can synergize mountain pine beetle attack, or which blends of other monoterpenes synergize host selection, is largely unknown. Further investigations will be needed to clarify its potential role in primary host selection behavior. This is of particular interest since other bark beetle species have an attractive behavioral connection to specific concentrations of α -pinene (Wallin and Raffa, 2004). Our study has identified the range of α -pinene in both lodgepole pine and ponderosa pine oleoresins, setting the stage for further investigations in behavioral correlations with the mountain pine beetle and other pine bark beetles.

From this study we have identified differences in individual qualitative defense compounds and abundance of quantitative preformed oleoresins between lodgepole pines and ponderosa pines in the north central Rocky Mountains. However, whether concentrations of each compound within the oleoresins and/or the amount of oleoresins present during attack are significant factors in mountain pine beetle host selections is unknown at this time. Whether groups of mountain pine beetles switch from attacking individual trees with low concentrations of monoterpenes, which are usually few within the forests, to attacking heavily defended trees with higher concentrations of monoterpenes as populations build in one pine host versus another is unknown, as was previously recorded within lodgepole pine trees by Boone *et al.* (2011). However, West *et al.* (in

prep; Ch. 1 and 2) did record mountain pine beetle attacks in both tree species in mixed stands within the same emergence year, though the defensive chemotypes of attacked trees were not studied.

The success of a bark beetle attack is linked to preformed host defenses (Chapman *et al.*, 2012; Dunn and Lorio, 1993), and those defenses induced by the attack (Raffa and Berryman 1987). We did not attempt to quantify the induced defenses and thus we feel a comparative study of the response to wounding between the two hosts may be of interest to this and future epidemic situations. Gaylord *et al.* (2012) found ponderosa pine may have a weak to nonexistent inducible defense to bark beetle attack, while the findings of Raffa and Berryman (1982, 1987) in lodgepole pine suggest a strong response to wounding.

Although we did not focus on myrcene and terpinolene in particular at the outset of this investigation, these monoterpenes can be attractive or elicit antennal responses in the mountain pine beetle (Borden *et al.*, 2008; Huber *et al.*, 2000; Pureswaran *et al.*, 2004). We found differences in terpinolene between the two host species from the Front Range. Myrcene and α -phellandrene co-eluted in our gas chromatograms, and no attempts to further separate these compounds were made, as our focus was on total monoterpene load, limonene, α -pinene, and β -phellandrene. If further investigations are made in behavioral assays, attempts to separate and quantify these compounds may be of interest.

In conclusion, the results suggest striking differences in oleoresin flow between the two alternate host tree species of the mountain pine beetle. Ponderosa pines produced almost four times the oleoresin flow than lodgepole pines. The influence of these differences in oleoresin flow on beetle attack is unknown, though we suspect have an effect upon landscape scale disturbance

from this insect as stresses of drought have the potential to impact forests, not just stands or individual trees. Ponderosa pines produced greater oleoresin flow, which could be interpreted one of two ways: 1) ponderosa pine is more resilient to beetle attacks during episodic drought since it produces a greater amount of oleoresins, or 2) ponderosa pine is less resilient since it relies on a greater quantity of oleoresins to defend against bark beetle attack. We identified differences in oleoresin constituents that may cause differences in host selection behavior of the beetle between these two pines under similar growing conditions. Further investigations are needed to resolve the importance of oleoresin flow and its constituents against beetle attack in these two pine species. This study provides the groundwork for further investigations in the complexity of host defenses and host selection behavior of the mountain pine beetle.

Table 3. 1. Qualitative constitutive oleoresin composition (micrograms/gram oleoresin) from lodgepole pines (*P. contorta* var. *latifolia*; n=10) and ponderosa pines (*P. ponderosa*; n=10) sampled from Front Range, Colorado. Collections were conducted in July and August of 2011. Monoterpene compositions were identified using Gas Chromatography-Flame Ionization Detection and quantified using Clarity software.

		α -pinene	β -pinene	3-carene	α -phellandrene & myrcene	limonene	β -phellandrene	γ -terpinene	cymene	terpinolene	bornyl- acetate	total
Retention time (min)		4.6	6.1	7.1	7.46, 7.51	8.2	8.5	9.5	10.0	10.4	18.3	na
Lodgepole	Means	1.08	1.37	3.64	1.31	0.32	12.96	0.05	0.03	0.39	0.16	426.35
	St errors	0.10	0.29	0.63	0.08	0.02	0.85	0.005	0.01	0.05	0.02	23.92
Ponderosa	Means	0.84	3.04	9.56	3.14	0.24	0.25	0.07	0.01	0.66	0.22	360.38
	St errors	0.12	0.66	0.89	0.50	0.08	0.09	0.01	0.002	0.06	0.03	39.30
	p value*	0.11	0.01	0.0003	0.0001	0.026	<0.0001	0.11	0.002	0.005	0.09	0.11

* Tests between host species were conducted using Multivariate Analysis of Variance model treating compounds as a response to species type (Proc GLM; SAS)

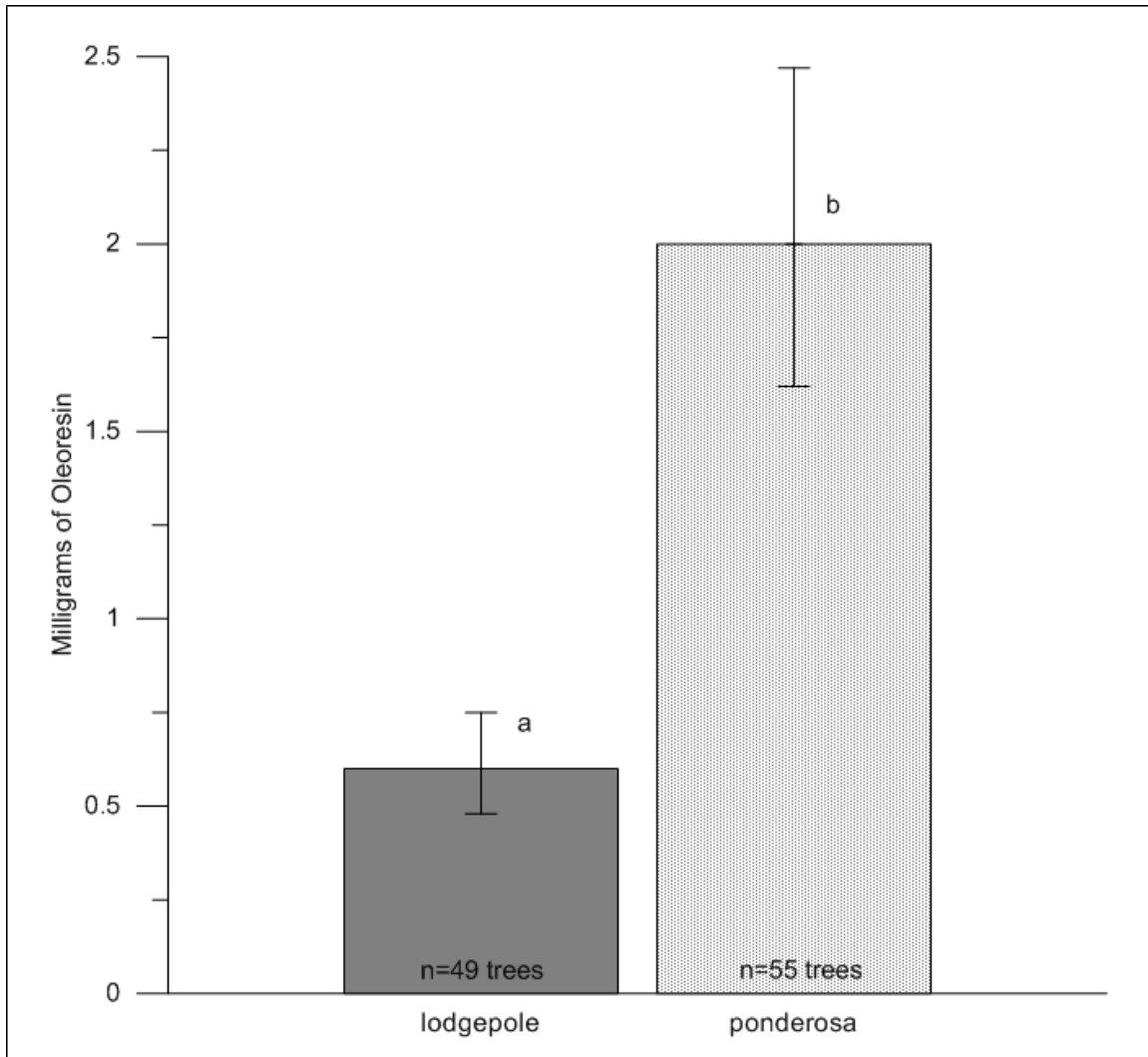


Figure 3. 1. Total preformed (24 hr) quantitative oleoresin (mean milligrams oleoresin \pm $\frac{1}{2}$ Least Significant Differences) from lodgepole pine (*P. contorta* var. *latifolia*) and ponderosa pines (*P. ponderosa*) in the Front Range, CO. Differing letters indicate significance $p < 0.05$ determined from Analysis of Variance (Proc GLM; SAS). Means \pm $\frac{1}{2}$ Least Significant Differences.

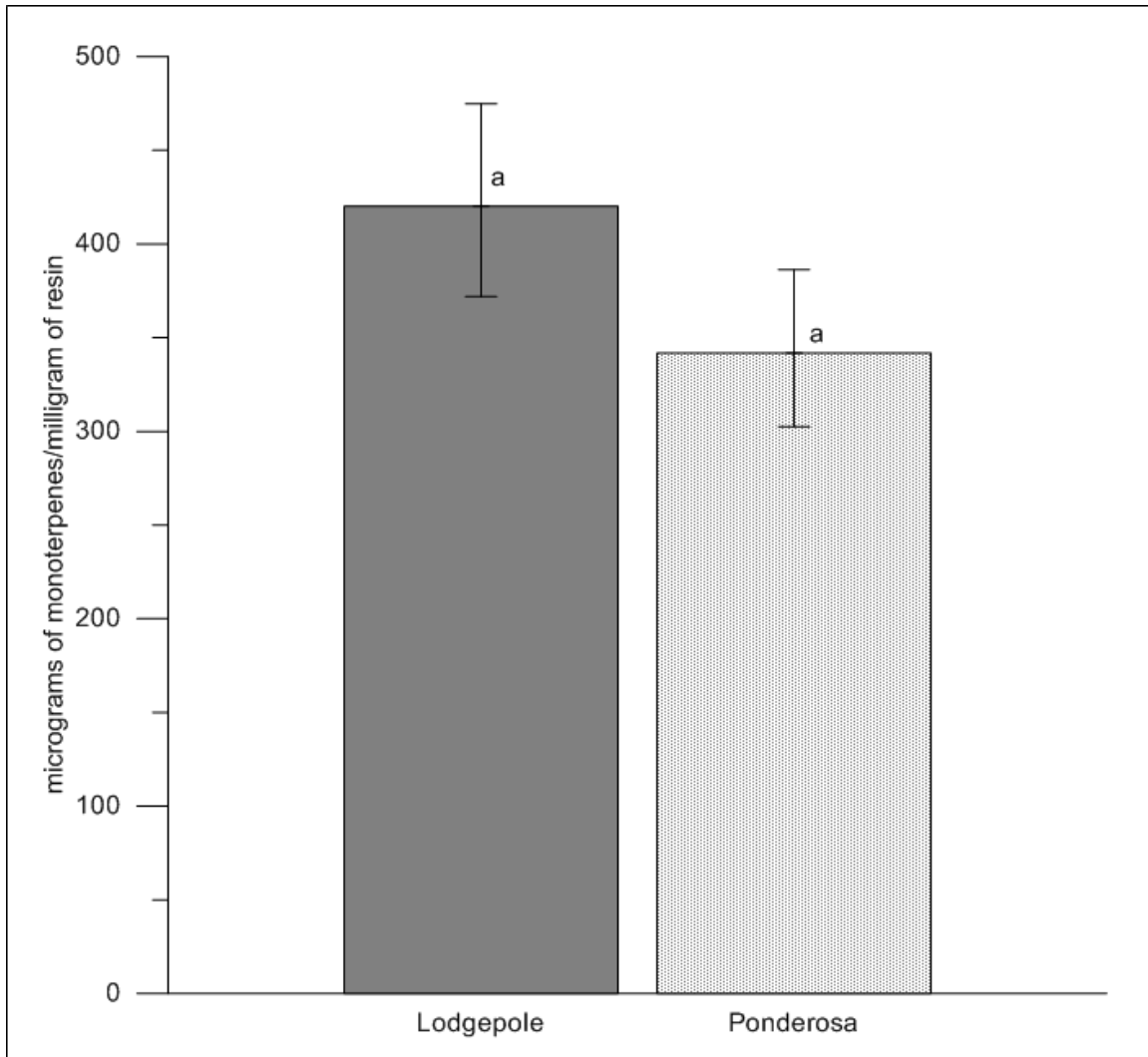


Figure 3. 2. Total cumulative constitutive terpenoid concentrations (mean ug/mg oleoresin \pm $\frac{1}{2}$ Least Significant Differences) from lodgepole pine (*P. contorta* var. *latifolia*; n=10) and ponderosa pines (*P. ponderosa*; n=10) in the Front Range, CO. Differing letters indicate significance $p < 0.05$ determined from a non-parametric ANOVA corrected using Exact Wilcoxon tests (Proc NPAR1WAY; SAS).

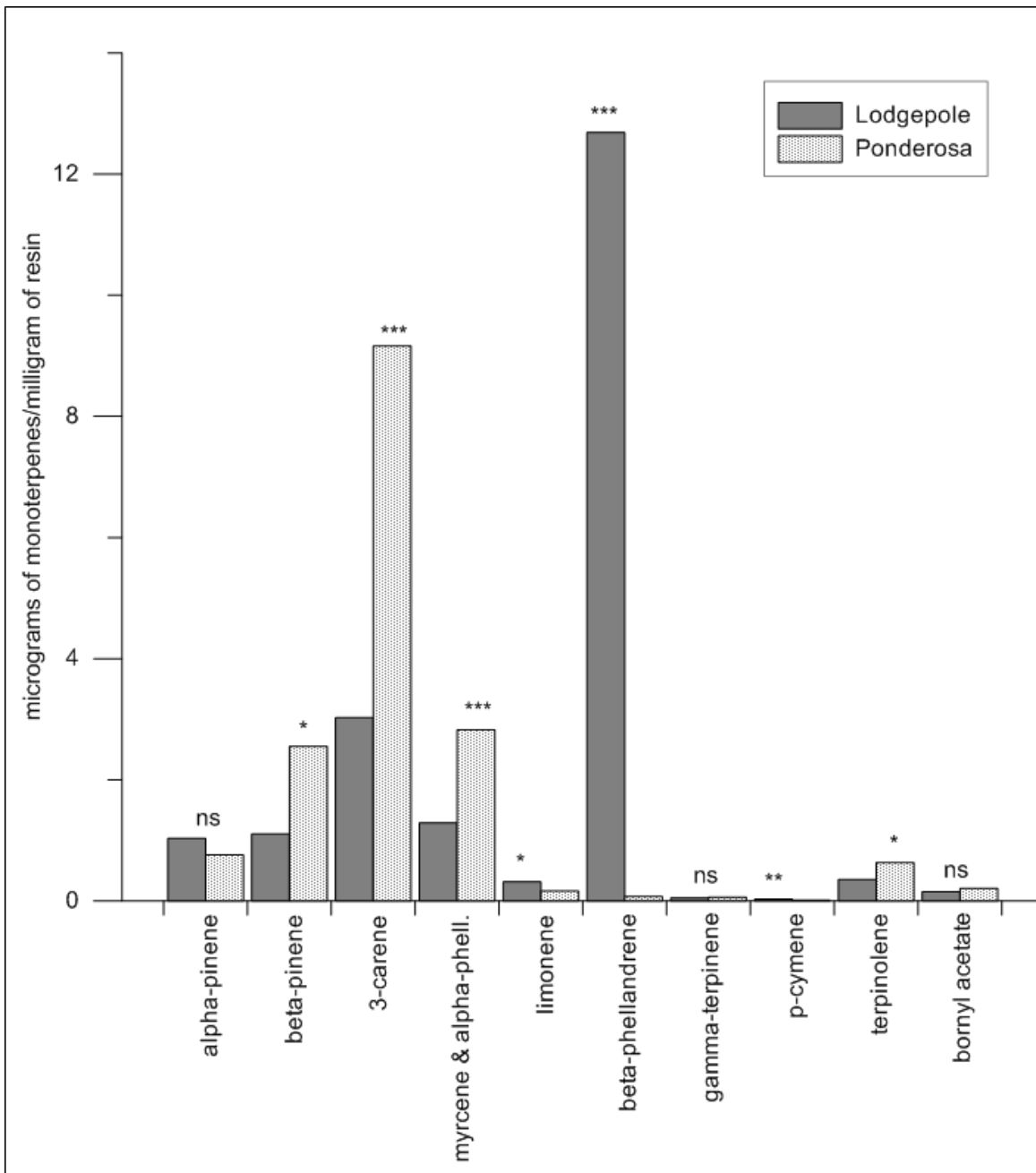


Figure 3. 3. Constitutive oleoresin profile (mean ug/mg) from Front Range, CO. lodgepole pines (*P. contorta* var. *latifolia*; n=10) and ponderosa pines (*P. ponderosa*; n=10) derived from GC-FID. Differences between pine species were determined through a MANOVA model (Proc GLM; SAS). Significance: *P < 0.05, **P < 0.005, ***P < 0.0005, ns P > 0.05.

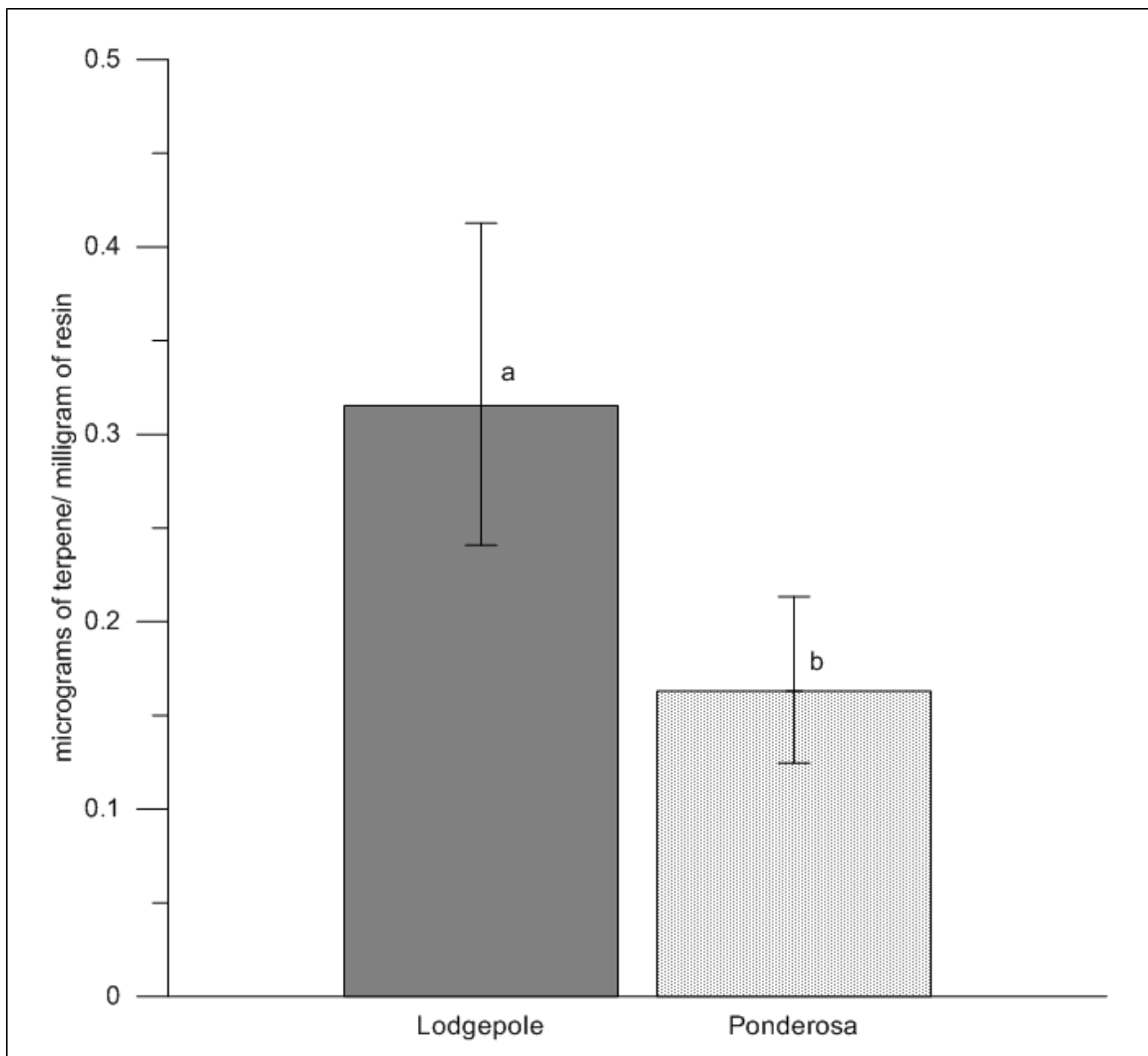


Figure 3. 4. Limonene concentrations (mean ug/mg oleoresin \pm 1/2 Least Significant Differences) from constitutive oleoresin obtained from lodgepole pine (*P. contorta* var. *latifolia*; n=10) and ponderosa pines (*P. ponderosa*; n=10) in the Front Range, CO. Differing letters indicate significance $p < 0.05$ determined from a non-parametric ANOVA corrected using Exact Wilcoxon tests (Proc NPAR1WAY; SAS).

REFERENCES

- Berryman, A.A., Raffa, K.F., Millstein, J.A., Stenseth, N.C. 1989. Interaction dynamics of bark beetle aggregation and conifer defense rates. *Oikos* 56: 256-263.
- Berryman, A.A. 1972. Resistance of conifers to invasion by bark beetle- fungus associations. *Bioscience* 22: 598-602.
- Boone, C.K., Aukema, B.H., Bohlmann, J., Carroll, A.L., and Raffa, K.F. 2011. Efficacy of tree defense physiology varies with bark beetle population density: A basis for positive feedback in eruptive species. *Can. J. For. Res.* 41:1174–1188.
- Bordasch, R. P., and Berryman, A. A. 1977. Host resistance to the fir engraver beetle, *Scolytus ventralis* (Coleoptera:Scolytidae): 2. Repellency of *Abies grandis* resins and some monoterpenes. *The Canadian Entomologist*, 109: 95-100.
- Borden, J. H., Pureswaran, D.S., and Lafontaine, J.P. 2008. Synergistic blends of monoterpenes for aggregation pheromones of the mountain pine beetle (Coleoptera: Curculionidae). *J. Econ. Entomol.* 101:1266–1275.
- Cook, S. P., and Hain, F.P. 1988. Toxicity of host monoterpenes to *Dendroctonus frontalis* and *Ips calligraphus* (Coleoptera, Scolytidae). *J. Entomol. Sci.* 23:287–292.
- Coyne, J.F., and Lott, L.H. 1976. Toxicity of substances in pine oleoresin to southern pine beetles. *J. GA Entomol. Soc.* 11:301–305.
- Dunn, J.P., and Lorio, P.L.Jr. 1993. Modified water regimes affect photosynthesis, xylem water potential, cambial growth, and resistance of juvenile *Pinus taeda* L. to *Dendroctonus frontalis* (Coleoptera:Scolytidae). *Physiological and Chemical Ecology* 22: 948-957
- Franceschi, V.R., Krokene, P., Christiansen, E., and Krekling, T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytol.* 167:353–375.
- Gaylord, M. L., Hofstetter, R.W., Thomas E. Kolb, T.E. and Wagner, M. R. 2012. Limited response of ponderosa pine bole defenses to wounding and fungi. *Tree Physiology* 31: 428-437
- Huber, D.P.W., Gries, R., Borden, J.H., Pierce, H.D Jr. 2000. A survey of antennal responses by five species of coniferophagous bark beetles (Coleoptera:Scolytidae) to bark volatiles of six species of angiosperm trees. *Chemoecology* 10: 103-113
- Klepzig, K.D., Smalley, E.B., and Raffa, K.F. 1996. Combined chemical defenses against an insect-fungal complex. *J. Chem.Ecol.* 22:1367–1388.

- Keeling, C.I., and Bohlmann, J. 2006. Genes, enzymes, and chemicals of terpenoid diversity in the constitutive and induced defense of conifers against insects and pathogens. *New Phytol.* 170:657–675.
- Lewisohn, E., Gijzen, M., Croteau, R. 1991. Defense mechanisms of conifers. Differences in constitutive and wound-induced monoterpene biosynthesis among species. *Plant Physiology* 96: 44-49.
- Lombardero, M.J., Ayres, M.P., Lorio, P.L.J., Ruel, J.J. 2000. Environmental effects on constitutive and inducible resin defences of *Pinus taeda*. *Ecol. Letters* 3: 329-339.
- Miller, D.R., Borden, J.H. 1990. β -Phellandrene: Kairomone for pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). *J Chem Ecol* 16: 2519–2531
- Miller, D.R., Borden, J.H. 2000. Dose dependent and species-specific responses of pine bark beetles (Coleoptera:Scolytidae) to monoterpenes in association with pheromones. *The Canadian Entomologist* 132: 183–195
- Paine, T.D., Raffa, K.F., Harrington, T.C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annu. Rev. Entomol.* 42: 179-206.
- Pearce R.B. 1996. Antimicrobial defences in the wood of living trees. *New Phytologist* 132: 203-233.
- Phillips, M.A., Croteau, R.B. 1999. Resin-based defense in conifers. *Trends in Plant Science* 4: 184-190.
- Pureswaran, D.S., Gries, R., Borden, J.H. 2004. Antennal responses of four species of tree killing bark beetles (Coleoptera:Scolytidae) to volatiles collected from beetles and their host and non-host conifers. *Chemoecology* 14: 59-66
- Raffa, K. F., and Berryman, A. A. 1982. Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated organisms. *Environ. Entomology.* 11:486–492.
- Raffa, K. F., and Steffek, R. J. 1988. Computation of response factors for quantitative analysis of monoterpenes by gas-liquid chromatography. *J. Chem. Ecol.* 14:1385–1390.
- Raffa, K. F., and Smalley, E. B. 1995. Interaction of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle-fungal complexes. *Oecologia.* 102:285–295.
- Raffa, K. F., Aukema, B.H., Bentz, B. J., Carroll, A. L., Hicke, J. A., Turner, M. G., Romme, W. H. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: Dynamics of biome-wide bark beetle eruptions. *BioScience* 58: 501-518.

- Raffa, K.F., Aukema, B.H., Erbilgin, N., Klepzig, K.D., and Wallin, K.F. 2005. Interactions among conifer Terpenoids and bark beetles across multiple levels of scale: An attempt to understand links between population patterns and physiological processes. *Recent Adv. Phytochem.* 39:80–118.
- Reid, R.W. 1963. Biology of the Mountain Pine Beetle, *Dendroctonus monticolae* Hopkins, in the east Kootenay Region of British Columbia. III. Interaction between the beetle and its host, with emphasis in brood mortality and survival. *Canadian Entomologist.* 95:225–238.
- Renwick, J.A.A. and Vite, J.P. 1970. Systems of chemical communications in *Dendroctonus*. *Contributions of Boyce Thompson Inst.* 24:283–292.
- Richmond, H.A. 1933. Host selection studies of *Dendroctonus monticolae* Hopk. in southern British Columbia. *The Forestry Chronicle* 9:60-61.
- Rosner, S., Hannrup, B. 2004. Resin canal traits relevant for constitutive resistance of Norway spruce against bark beetles: environmental and genetic variability. *Forest Ecology and Management* 200: 77-87.
- Safranyik, L., and CARROL, A. L. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. Pages 3–66 in L. Safranyik and B. Wilson, editors. The mountain pine beetle: a synthesis of its biology, management, and impacts on lodgepole pine. Canadian Forest Service, Pacific Forestry Centre, Natural Resources Canada, Victoria, British Columbia, Canada.
- Seybold, S.J., Huber, D.P.W., Lee, L.C., and Bohlmann, J. 2006. Pine monoterpenes and pine bark beetles: A marriage of convenience for defense and chemical communication. *Phytochem. Rev.* 5:143–178.
- Seybold S. J., Bohlmann, J., Raffa, K.F. 2000. Biosynthesis of coniferophagous bark beetle pheromones and conifer isoprenoids: evolutionary perspective and synthesis. *Canadian Entomologist* 132: 697-753.
- Smith, R.H. 1961. Techniques for determining the toxicity of pine resin vapors to *Dendroctonus brevicomis* and *D. Jeffreyi*. *J. Econ. Entomol.* 54: 359-365.
- Smith, R.H. 1963. Toxicity of pine resin vapors to three species of *Dendroctonus* bark beetles. *J. Econ. Entomol.* 56: 827-831.
- Smith, R.H. 1965. Effect of monoterpene vapors on the western pine beetle. *J. Econ. Entomol.* 58: 509-510.
- Smith, R.H. 1975. Formula for describing effect of insect and host tree factors in resistance to western pine beetle attack. *J. Econ. Entomol.* 68: 841-844.
- Vite J.P. 1961. The influence of water supply on oleoresin exudation pressure and resistance to bark beetle attack in *Pinus ponderosa*. *Contributions from the Boyce Thompson Institute* 21: 37-66.

Wallin, K.F., and Raffa, K.F. 1999. Altered constitutive and inducible phloem monoterpenes following natural defoliation of jack pine: Implications to host mediated Interguild interactions and plant defense theories. *J. Chem. Ecol.* 25:861–880.

Wallin, K.F., and Raffa, K.F. 2001. Effects of folivory on subcortical plant defenses: Can defense theories predict interguild processes? *Ecology* 82:1387–1400.

Wulder, M.A., Ortlepp, S.M., White, J.C., Nelson, T., Coops, N.C. 2010. A provincial and regional assessment of the mountain pine beetle epidemic in British Columbia: 1999–2008. *J. Environmental Informatics* 15: 1–13.