Legacy Effects of Mountain Pine Beetle Outbreak: Defense, Growth and Survival of Residual Lodgepole Pine Trees in Alberta

By

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Abstract

Periodic mountain pine beetle outbreaks have affected millions of hectares of lodgepole pine forests in western North America. Within these stands often some pine trees remain alive. In addition to causing direct pine mortality, outbreaks also have short- and long-term legacy effects on both above and below ground communities, resulting in altered stand conditions following outbreak. Currently, the effects of the altered stand conditions on the survival of the remaining residual live pine trees are unknown. Giving the observed low natural pine regeneration in the post-outbreak stands in Alberta, these residual pine trees have become a critical component of recovery of lodgepole pine forests. In my PhD thesis, I studied various aspects of the residual pine trees in post-outbreak stands in Alberta. In particular, I first determined whether the survival of residual trees can be explained by their growth and resin duct-based anatomical defenses. Then, I evaluated the potential risks of several species of bark and woodboring beetles to residual pine trees. For the relationship among resin ducts, growth and tree survival, I first compared the resin duct characteristics and radial growth between residual and beetle-killed trees and characterized the resin ducts and radial growth of residual trees before and after outbreak. The results showed that residual trees had larger resin ducts than beetle-killed trees before outbreak and continued having so in post-outbreak stands. Tree radial growth (ring width) was not associated with tree survival. Next, I examined if outbreaks altered the historical growth-defense relationship of residual trees. I found that after outbreak, residual trees prioritized resin duct defenses over growth by increasing resin duct production and area occupied (per mm^2 of radial growth), compared prior to the outbreak. I subsequently determined the impacts of multiple stand factors on the relationship between growth and resin duct characteristics of residual trees before and after outbreak. Results showed increasing resin duct density of healthy (asymptomatic) residual trees with increasing percent lodgepole pine mortality, suggesting inducibility of resin ducts in lodgepole pine trees. Interestingly, the stand density of live overstory trees (including pine and non-pine species) before outbreak was related to the resin duct characteristics of residual trees before outbreak. These results suggest that intra- and inter-specific competition can affect anatomical defenses of lodgepole pine trees.

To quantify the populations of bark and woodboring beetles and their insect predators in post-outbreak stands, I set up three types of passive insect traps seven years after the last mountain pine beetle outbreak in post-outbreak stands. The results from trap data showed that the percent lodgepole pine mortality were not associated with the abundance of bark and woodboring beetles. In addition, there were no difference among the three different categories of residual trees (healthy, declining, or attacked by mountain pine beetle but survived) in terms of the abundance of bark and woodboring beetles or their predators. These outcomes might be a result of low abundance of suitable host trees for bark and woodboring beetles, intra- and inter-specific competition within and between bark and woodboring beetle species, and predation. Overall these results demonstrate that post-outbreak stands did not promote bark and woodboring beetle populations. Through my thesis, I conclude that residual trees may be more resistant to the future mountain pine beetle attacks and bark and woodboring beetles do not pose threats to these residual trees in post-outbreak stands. Leaving residual trees, instead of salvage harvesting, could be critical for the establishment of lodgepole pine forests that show greater resistance to mountain pine beetle attacks in western Alberta.

Preface

This thesis includes three studies (Chapters 2-4). Chapter 3 was published, and Chapter 2 and 4 were submitted as peer-reviewed manuscripts. These chapters represent collaborative work with Dr. Nadir Erbilgin at the University of Alberta. For all studies, I designed the experiments, processed samples, collected and analyzed data, and wrote the thesis and manuscripts. Dr. Erbilgin contributed to the experimental design and revised the thesis and manuscripts in both content and composition. During my studies, I acquired all necessary permits based on the provincial regulations.

Chapter 2 was submitted as Zhao and Erbilgin "A hint left by mountain pine beetle: larger resin ducts enhance tree resistance" to Frontiers in Plant Sciences. I designed the experiments, collected data, processed samples, analyzed data, and wrote the manuscript. Dr. Erbilgin contributed to the experimental design and revised the manuscript in both content and composition.

Chapter 3 was published as Zhao, Klutsch, Cale and Erbilgin (2019) "Mountain pine beetle outbreak enhanced resin duct-defenses of lodgepole pine trees" in Forest Ecology and Management, 441: 271-279. I designed the experiments, collected data, processed samples, analyzed data, and wrote the manuscript. Drs. Klutsch and Cale provided help on data analysis. Dr. Erbilgin contributed to the experimental design and revised the manuscript in both content and composition.

Chapter 4 was submitted as Zhao, Classens and Erbilgin "Post-mountain pine beetle outbreak stands did not promote the populations of secondary bark and woodboring beetles" to Environmental Entomology. I designed the experiments, collected data, processed samples, analyzed data, and wrote the manuscript. Classens was involved in insect identification. Dr.

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Chapter 1. Introduction

During the last decades, the frequency and severity of bark beetle (Coleoptera: Curculionidae) outbreaks have increased across the globe (Logan et al. 2003, Kirilenko and Sedjo 2007, Raffa et al. 2017). For example, mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins), which is native to western Canada and the United States, has recently expanded its distribution into higher elevations, northern latitudes, and eastern longitudes which were previously considered not suitable for MPB survival (Cudmore et al. 2010, Cullingham et al. 2011, Raffa et al. 2017). Millions of pine trees, mainly lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm. ex S. Watson), were killed by MPB in British Columbia (Safranyik and Carroll, 2006) and in its newly expanded range in Alberta (Government of Alberta 2006-2017). However, in many stands some residual mature lodgepole pine trees remain alive after MPB outbreaks (Erbilgin et al. 2017a). In addition to causing direct mortality of pine trees, MPB outbreaks also substantially changed below and above ground biotic communities as well as altered abiotic conditions (McIntosh and Macdonald 2013, Treu et al. 2014, Cigan et al. 2015, Karst et al. 2015, Pec et al. 2015). It is unknown how these changes that occurred in post-outbreak stands can affect the residual pine trees, particularly their ability to defend, grow and survive. In addition, a lack of natural pine regeneration in beetle-killed pine stands further exacerbates the long-term impact of MPB in these stands (Astrup et al. 2008, Teste et al. 2011, McIntosh and Macdonald 2013). Residual overstory lodgepole pine trees (residual trees, hereafter) may become the only natural live seed source for recovering lodgepole pine forests in post-outbreak stands. Therefore, understanding the physiological mechanisms underlying the survival of these residual trees during outbreak and their future health conditions in post-outbreak stands would improve our knowledge of the short- and long-term legacy of climate change-induced insect outbreaks and the sustainability of pine forests in North America.

Herbivory pressure is thought to continuously shape plant defenses in a co-evolutionary process (Franceschi et al. 2005, Erbilgin 2019). Conifers have evolved comprehensive and interlinked anatomical and chemical defense mechanisms in response to bark beetle attacks (Franceschi et al. 2005, Keeling and Bohlmann 2006, Kolosova and Bohlmann 2012, Erbilgin 2019). When bark beetles penetrate the host tree bark, the viscous oleoresins containing toxic compounds are released to expel or kill the attacking beetles and to seal the beetle entry wounds. These oleoresins function as both physical and chemical defenses. While most studies have focused on the chemical defense mechanisms in conifers (Appel 1993, Turtola et al. 2003, Franceschi et al. 2005, West et al. 2016, Erbilgin et al. 2017a, Erbilgin 2019), relatively fewer studies have investigated the resin duct-based anatomical defenses and their potential role in tree resistance to bark beetles (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015, Mason et al. 2019).

In many conifer species, particularly in the genera of *Pinus* and *Picea*, resin ducts are responsible for production, storage, and translocation of oleoresins within a tree (Lombardero et al. 2000, Franceschi et al. 2005, Luchi et al. 2005, Niederbacher et al. 2015, DeRose et al. 2017). Resin ducts are critical components of tree defenses against bark beetles (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015). However, resin duct characteristics can be affected by tree genetics (Westbrook et al. 2013, 2015) and environmental conditions (Rigling et al. 2003, Slack et al. 2017). Whether the characteristics of resin ducts can be used to predict the lodgepole pine resistance to MPB in Alberta is unknown.

The growth-defense relationship in plants has received growing attention and several hypotheses have been proposed to explain the relationship between these two under different growing conditions and herbivory pressures. According to the growth-differentiation balance hypothesis, trees should prioritize defenses over growth when suboptimal growing conditions occur. Along with growth, the production of anatomical defense structures in conifers, such as resin ducts, requires metabolic energy in terms of carbohydrates and nutrients (Stamp 2003). Environmental conditions can affect both constitutive and induced resin duct characteristics through affecting the carbohydrates and nutrients (Rigling et al. 2003, Franceschi et al. 2005). Since pine trees preserve historical records of resin ducts and radial growth over time, resin duct characteristics and radial growth rate can be very well suited to study carbon allocation patterns between defense and growth. Relatively few studies have used resin ducts to investigate the growth-defense relationship in pine trees in North America (Kane and Kolb 2010, Hood and Sala 2015). Kane and Kolb (2010) suggested that conditions benefitting tree growth could increase resin duct production. In contrast Hood and Sala (2015) indicated that slow growing pine trees could invest more in resin duct defenses per unit area of radial growth. These different results from earlier studies demonstrate variability of the growth-defense relationship in pine trees across their natural range.

The altered stand conditions reported in post-outbreak stands may impact growing conditions of pine trees (Murphy et al. 1999, Alfaro et al. 2010, Amoroso et al. 2013, Hawkins et al. 2013). For example, the reduced demands for nutrients and water from live trees and the accumulated pine litter on forest floor greatly increased the soil nutrient input and soil moisture (Cigan et al. 2015) which might provide better growing conditions for residual trees. However, the decline in species richness and community composition of ectomycorrhizal fungi can affect water and nutrient uptake and further impact seedling growth and survival, as lodgepole pine trees and ectomycorrhizal fungi are obligate symbionts (Karst et al. 2015). Changes in tree growth may affect growth-defense relationship in residual trees. These changes might further affect the susceptibility of residual pine trees to bark beetles.

Mountain pine beetle, as one of the most aggressive bark beetle species in North America, interacts with many subcortical insects including other bark and woodboring beetle species (Safranyik and Carroll 2006). In fact, studies have reported that these subcortical associates increase their populations following MPB outbreaks and cause substantial mortality of the remaining live pine trees in post-outbreak stands (Amman and Safranyik 1985, Wood et al. 1987, Rankin and Borden 1991, Safranyik and Carroll 2006, Boone et al. 2008). These beetle associates are considered as 'secondary beetles' (Safranyik and Carroll 2006). In the historical range of MPB, studies have shown that secondary beetle species typically colonize stressed or dying trees and usually compete with MPB for subcortical resources when MPB populations are at endemic phase (i.e., low population) (Rankin and Borden 1991, Safranyik et al. 1999). Currently it is not clear how populations of these secondary beetles respond to the large volume of dead or dying lodgepole pine trees in post-outbreak stands in Alberta.

Along with host tree resistance (Raffa and Berryman 1983, Franceschi et al. 2005, Boone et al. 2011), the predators of secondary beetles can regulate populations of both MPB and secondary beetles (Boone et al. 2008, Tabacaru and Erbilgin 2015). The main coleopteran predators of bark and woodboring beetles are clerid beetles. These predatory beetles are habitat specialists that feed under bark and prey upon bark and woodboring beetles (Reid 1957, Erbilgin and Raffa 2000, Boone et al. 2008). In the naïve lodgepole pine forests of Alberta, it is unknown how populations of predator beetles respond to MPB outbreak and changes in the populations of their subcortical preys.

In this doctoral thesis, my aim was to determine how residual overstory lodgepole pine trees respond to MPB outbreak and to evaluate whether secondary beetles following MPB outbreak may pose risk to residual pine trees in post-MPB stands in Alberta. Specifically, I investigated the following three objectives: 1) whether resin duct defenses and radial growth were associated with the survival of residual trees during MPB outbreaks; 2) whether MPB outbreak affected the resin duct defenses and radial growth of residual trees; and 3) whether MPB outbreak promoted the populations of secondary beetles in post-MPB stands. Such understanding can provide insight of pine health conditions and potential directions for future forest management in post-MPB stands.

In Chapter 2, I compared the resin duct defenses and radial growth between MPB-killed and live residual lodgepole pine trees and characterized the resin ducts and growth of the residual trees before, during, and after outbreak. I built logistic models to predict the probability of survival of trees during MPB outbreaks using the resin duct characteristics and radial growth of MPB-killed and residual trees. I found that residual trees had larger resin ducts prior to outbreaks and continued having so after outbreak in post-MPB stands. In addition, tree radial growth (ring width) was not associated with tree survival. Overall, these results demonstrate that resin duct size of lodgepole pine trees can be an important component of tree defenses against MPB attacks and suggest that lodgepole pine trees with large resin ducts are likely to show resistance to future bark beetle attacks.

In Chapter 3, I compared the growth-defense relationship of lodgepole pine trees between pre- and post-outbreak periods by examining radial growth rate (ring width) and xylem resin duct characteristics. I also tested the effects of percent host tree mortality and forest stand density on the growth-defense relationship of residual pine trees. I found that resin duct densities of residual pine trees were higher and resin ducts occupied a larger proportional area in the xylem in the post-outbreak period than in the pre-outbreak period. Following outbreak, the percent host mortality showed a positive relationship with the resin duct density of all healthy residual trees, while stand density did not alter most of the resin duct characteristics. I conclude that MPB outbreak can alter the growth, defense, and their interactions of residual pine trees, especially in stands with greater levels of pine mortality. This study also suggests that residual trees might be more resistant to future bark beetle attacks.

In Chapter 4, I set up three types of passive (without attractants) insect traps (flight intercept, landing, and emergence) in 27 sites with different levels of pine mortality to investigate the population growth of three functional groups of insects (bark beetles, woodboring beetles, and their coleopteran predators) in the summer of 2017. I attached both landing and emergence traps to trees in different health conditions (healthy, declining, or attacked by mountain pine beetle but survived) and hung flight intercept traps between trees in the same site. I found that the abundance of insect species in each functional group was not related to the percent pine mortality. Furthermore, the health conditions of trees did not influence the abundance of insects. Overall, these results are likely due to low abundance of suitable hosts for beetle colonization, increased resistance of the residual trees, intra- and inter-specific competition among bark and woodboring beetles, and predation. I conclude that seven years after, post-outbreak stand conditions do not promote the population growth of bark and woodboring beetles in the novel habitat of MPB in Alberta.

Chapter 2. A hint left by mountain pine beetle on anatomical defenses of lodgepole pine: larger resin ducts enhance tree survival

2.1 Introduction

Climate change has increased frequency and severity of insect outbreaks across many forest types (Logan et al. 2003, Kirilenko and Sedjo 2007, Raffa et al. 2017). In North America, periodic mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae) outbreaks had historically occurred in the western United States and Canada (British Columbia), but recently shifted to higher elevations (Cudmore et al. 2010, Raffa et al. 2017) and novel habitats in northern latitudes and eastern longitudes (Cullingham et al. 2011, Erbilgin et al. 2014), which was originally considered climatically not suitable to the beetles. The most recent MPB outbreaks in Canada have affected millions of hectares of pine forests, mainly lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm. ex S. Watson) trees and caused substantial changes in above and below ground biotic communities and abiotic conditions (Griffin and Turner 2012, Hawkins et al. 2013, McIntosh and Macdonald 2013, Treu et al. 2014, Karst et al. 2015, Pec et al. 2017). Yet, some individual trees survived these outbreaks in some stands (Erbilgin et al. 2017a, Six et al. 2018). Although studies have suggested possible roles of chemical and anatomical defenses of surviving trees under bark beetle attacks (Kane and Kolb 2010, Ferrenberg et al. 2014, Erbilgin et al. 2017a), the mechanisms underlying survival of these pine trees are still less understood.

Conifer trees have developed constitutive and inducible defense mechanisms that incorporate both anatomical structures and toxic chemicals against bark beetle attacks (Franceschi et al. 2005, Erbilgin et al. 2006, Keeling and Bohlmann 2006, Kolosova and Bohlmann 2012, Erbilgin 2019). During the initial host colonization by bark beetles, constitutive defenses are the first line of defense which can substantially lower the probability of successful beetle colonization. However, if constitutive defenses are not successful to cease the continuous beetle attacks, additional induced defenses are activated to protect the trees from bark beetle colonization. Toxic and sticky resins are the primary constituents of both constitutive and induced defenses of conifers. As a part of anatomical defenses, resin ducts are responsible for production, storage, and translocation of resins to the site of beetle entry and can be found in both the secondary phloem and xylem in pines (Franceschi et al. 2005). Similarly, to the chemical defenses, resin ducts can also be induced in pines by beetle attacks (Hudges et al. 2004).

Historically, studies on pine-bark beetle interactions have mainly focused on tree chemical defenses (e.g.: Erbilgin et al. 2017a, Raffa et al. 2017, Six et al. 2018, Erbilgin 2019), while the role of anatomical defenses, particularly resin duct characteristics, in pine defenses against bark beetles has received relatively less attention (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015, Mason et al. 2019). In contrast to the chemical defenses, resin ducts remain in the tree xylem for a longer period of time, providing valuable information regarding the history of tree defenses (Ferrenberg et al. 2014, Mason et al. 2019). Earlier studies reported differences in the anatomical defenses of beetle-killed and residual pine trees in the historical range of MPB (Kane and Kolb 2010, Ferrenberg et al. 2014). Hood and Sala (2015) suggested that increasing resin flow was positively related to resin duct characteristics and that resin ducts could be indicators of enhanced tree resistance. However, similar studies are currently lacking in the newly expanded range of MPB in Alberta. Furthermore, whether the pine response of anatomical defenses observed in the historical range are comparable to the responses of pine trees in the expanded range is unknown.

Mountain pine beetle outbreaks have altered forest stand conditions, including soil moisture, soil nutrients, soil microbial communities, and above ground plant communities (Griffin and Turner 2012, Hawkins et al. 2013, McIntosh and Macdonald 2013, Pec et al. 2015, 2017), and in many cases, these changes have lasted for years (Cigan et al. 2015, Karst et al. 2015, Dhar et al. 2016). Earlier studies reported accelerated radial growth of residual overstory pine trees after outbreaks in the historical range of MPB (Murphy et al. 1999, Alfaro et al. 2010, Amoroso et al. 2013, Hawkins et al. 2013). However, it is unknown how naïve lodgepole pine trees in Alberta responded to the altered stand conditions caused by beetle outbreak. Specifically, the consequences of MPB outbreaks on the anatomical defenses and radial growth of residual lodgepole pine trees, especially whether outbreaks have altered the anatomical defenses of pine trees, remains to be investigated.

In this study, I was particularly interested in determining whether 1) radial growth and resin duct defenses were associated with the survival of residual lodgepole pine trees during MPB outbreak, 2) the historical radial growth and resin duct defenses of residual pine trees were affected by MPB outbreak, and 3) radial growth and resin duct defenses were associated with the current health conditions of residual pine trees. In the first objective, I compared the growth rate and resin duct characteristics between beetle-killed and residual pine trees to determine the role of growth and defenses in their survival. I expected that residual pine trees would have more or larger anatomical defense structures, but less radial growth rate compared to MPB-killed trees as residual trees might allocate more resources on defense over growth. For the second objective, I compared growth rate and resin duct characteristics of residual pine trees between pre- and post-outbreak periods. Considering the reduced competition among overstory pine trees in the post-outbreak stands, I expected that residual pine trees would produce more carbon resources which may lead to greater allocation to anatomical defense but cause delayed growth release after outbreak, compared to before outbreak. For the last objective, I compared growth rate and resin duct characteristics among different health conditions of residual pine trees within the most recent 20 years of growth. I expected that residual pine trees with healthy appearance would grow faster and have stronger anatomical defenses than trees showing symptoms of declining.

2.2 Materials and Methods

2.2.1 Study site description and tree selection

I selected 31 sites affected by MPB in western Alberta and elevation of these sites ranged from 600 m to 1000 m (App. Fig. 1). In each site, mature lodgepole pine trees (dead or alive) over 15 cm at diameter breast height (DBH) constituted more than 50% of mature overstory canopy trees, and mortality rate of lodgepole pine trees due to MPB ranged from 2% to 83% (App. Tables 1 & 2). I set up two plots (each 20 m x 20 m) per site with different percent lodgepole pine mortality. Plot centers were at least 100 m away from each other in each site. I confirmed trees killed by MPB by the signs of beetle attacks, including pitch tubes, brood beetle emergence holes, and extensive beetle galleries under bark (Erbilgin et al. 2017a). I considered all live mature lodgepole pine trees in each plot as residual trees and classified them into three categories based on their apparent health conditions: **healthy** (no obvious symptoms of any pathogen or insect attacks), **declining** (presence of insect, other than MPB, and pathogen attacks, showing dying branches, bark lesions, sparse crown, yellow-red needles), and **survived** (healthy but with symptoms of unsuccessful MPB attacks) trees. I determined unsuccessful MPB attacks on survived trees based on the failed beetle reproduction, i.e., presence of short maternal galleries, absence of oviposition and larval galleries, and absence of beetle emergence holes (Erbilgin et al. 2017a).

2.2.2 Data collection and preparation

In each plot, I selected up to three MPB-killed and six residual (two from each of three categories) trees. All trees selected were from the dominant or codominant crown class. In total, I selected 140 beetle-killed and 210 residual (76 healthy, 62 declining, and 72 survived) trees. The number of trees in each residual category was not equal because some plots did not have enough trees in each category. I cored the selected residual trees once at DBH on the south side using a 12 mm increment borer in May 2016. The last complete ring formed on these cores occurred in 2015. From beetle-killed trees, I took a single wedge at the same height and side as the residual trees (Fig 2.1). Each wedge contained at least 15 years of growth counting back from the year of death. In addition, I recorded DBH of all residual pine trees sampled. However, I did not measure the DBH of beetle-killed trees because their bark had fallen off due to insect infestations and fungal infections.

I glued both core and wedge samples on wooden mounts and dried them for two weeks at room temperature. I sanded the samples with progressively finer sand paper using belt and hand sanders, and then scanned each sample to create high-resolution digital images (1,200 dpi). I measured ring width (mm) from bark to pith by WinDendro^{TM} (Regent Instruments 2008) on all samples. If the pith was not present, measurements ended at the earliest ring formed. I developed a master chronology based on the ring width of the cores taken from 76 healthy residual trees. The master chronology was used to identify any missing or false rings on the cores before a calendar year was assigned to a particular ring and to determine the year of death of beetle-killed trees by cross-dating using COFECHA (Grissino-Mayer 2001). However, because about 40% of wedges contained less than 30 years of tree rings, the master chronology was not applicable to all wedge samples. In these cases, I compared specific ring characteristics such as contrast between early and late woods, width ratio between early and late wood per ring, or ring width on cores from 1960 to 2015. For example, tree rings were distinctively wider and had darker latewood in 1998 than in 1997 and the two years that followed 1998 (year of 1999 and 2000). I used all this information to determine the year of death of beetle-killed trees (App. Fig. 2). Since the majority of sites experienced beetle mortality over a period of multiple years, the earliest tree death recorded in the site was considered the year of outbreak started in this site (Table 2.1).

I determined resin duct production, total resin duct area and resin duct size within a fixed sampling width (9 mm) of each annual ring using ImageJ (Schneider et al. 2012): **resin duct production** (number of resin ducts per 9mm width in a given year on an increment core or wedge [no. yr^{-1}]), **total resin duct area** (sum of resin duct area per 9mm width in a given year on an increment core or wedge [mm^2 yr^{-1}]), **resin duct size** (mean size of resin ducts per 9mm width in a given year on an increment core or wedge [mm^2 yr^{-1}]). I also developed two resin duct characteristics standardized to sampled area as **resin duct density** (total number of resin ducts per year divided by the ring area (9mm * ring width) for a given year [no. mm^{-2} yr^{-1}]) and **relative resin duct area** (percent area occupied by resin ducts per year within the ring area for a given year [% yr^{-1}]) on each core or wedge.

I used **ring width** (mm yr^{-1}) and **basal area increment** (BAI, [mm^2 yr^{-1}]) to represent tree radial growth. BAI of residual trees was calculated using tree radii and ring width data, under the assumption that tree rings are concentric circles. Tree radius was calculated by dividing tree DBH by two. Since DBH of beetle-killed trees were not measurable in the field, I only incorporated BAI of residual trees in my data analyses.

2.2.3 Data analysis

I conducted separate analyses for each research question as described below. All statistical analyses were done in R (R Core Team 2018). I visually assessed the normality and homogeneity of variance of residuals for all models. Log transformations were applied when necessary. I then visually assessed the normality and homogeneity of variance of residuals of post-transformation models.

2.2.3.1 Can radial growth and resin duct development explain why residual trees were not killed during MPB outbreak?

I pooled data from all three residual tree categories together to test the differences in radial growth (ring width) and resin duct characteristics between residual and MPB-killed trees. I accepted the year of the earliest tree death caused by MPB as the first year of MPB outbreak started in each site. I divided cores from residual trees into two sections: the pre-outbreak and the post-outbreak. Then I further divided the pre-outbreak section on each core into three overlapping periods: 3-, 5-, and 10-year pre-outbreak growth period starting from the first year before outbreak started. Similarly, for MPB-killed trees, I selected three overlapping pre-death growth periods (3, 5, and 10 years) on wedges counting back from the year of tree death, but the year of death was excluded because a complete tree ring (latewood) might have not formed (Cailleret et al. 2017). I calculated the means of each of the five resin duct characteristics and ring width for each pre-outbreak and pre-death growth period separately, and then compared the means from the growth period having the same time length between residual and MPB-killed trees via linear mixed-effects models, using plots nested in sites as a random effect with R package *lme4* (Bates et al. 2015). When tree categories (residual and MPB-killed) had a significant effect on ring width or resin duct characteristics (α=0.05), I used lsmeans to conduct pairwise post-hoc tests to look for differences between tree categories using R package *lsmeans* (Lenth 2016).

I used mixed effects logistic regression models to determine the probability of tree survival during MPB outbreak using R package *lme4* (Bates et al. 2015). The response variables for these models were the tree status (alive or dead) and the explanatory variables included ring width and the five resin duct characteristics. Alive trees included all three categories of residual trees sampled, while dead trees were MPB-killed trees. I took the following steps to select the best explanatory variables for model fitting. In **Step 1**, I used principle component analysis (PCA) to visualize differences among MPB-killed, healthy, declining and survived trees in terms of the resin duct characteristics and ring width (Fig. 2.2) using R package *vegan* (Oksanen et al. 2011) (Fig. 2.2). The two-dimension PCA plot illustrated the means of each of the explanatory variables of each growth period (3-, 5-, and 10-year pre-outbreak period) separately by vector arrows. Since the angles of vector arrows indicated the correlation among variables (when angle is less than 90 degree, the smaller the angle indicates the higher correlation), I classified vector arrows pointing to close directions into the same group (Ramette 2007). This process separated the 18 explanatory variables into three distinct groups: (1) resin duct production and total resin duct area; (2) resin duct density and relative resin duct area; (3) resin duct size and ring width (Fig. 2.2). In **Step 2**, I examined the correlations among all variables using Pearson correlation test using R package *Hmisc* (Harrell 2018) (Table 2.2). P values lower than 0.05 indicated significant correlation between any two variables. In **Step 3**, I collected the possible predictor combinations for the mixed effects logistic regression model using the variables that were separated into different groups according to the PCA plot (the results of Step 1 above) and that were not correlated to each other (the results of Step 2 above). This process yielded a total of 33 mixed effects logistic regression models (Table 2.3). However, as ring width was strongly correlated with all resin duct characteristics, it was excluded from all predictor combinations based on the result of Pearson correlation test. This might bring some bias that ring width was not associated to tree survival. Therefore, instead of using P value of the correlation test, I used correlation coefficients to determine the correlation between ring width and each resin duct characteristic. If ring width had correlation coefficient value less than 0.5 with each explanatory variable in the model, I added ring width into the model. In **Step 4**, I selected the optimal set of variables within each model using Akaike Information Criterion (AIC). The model with lower AIC was considered to have the optimal set of variables (Burnham and Anderson 2002). Models with different set of variables within 2 points of AIC were considered equally supported. In this case, the model containing fewer variables was considered as the optimal model. In **Step 5**, I compared different models by calculating AIC, the area under receiver operating curves (AUC), and the mean percentage of live and dead trees correctly classified (threshold = 0.5) of each model. The models with lower AIC and higher AUC usually fit data better than the models with higher AIC or/and lower AUC (Hosmer and Lemeshow 2000, Burnham and Anderson 2002). I calculated AUC using R package *pROC* (Robin et al. 2011).

2.2.3.2 Were growth and resin duct development of residual trees affected by MPB outbreak?

To compare growth rate and resin duct characteristics of residual trees before and after outbreak, cores from residual trees were divided into two sections as the pre-outbreak and the post-outbreak. The post-outbreak section on each core was further divided into three overlapping growth periods: 3 years, 5 years, and the total (period from the first year after outbreak to the year before sampling [2015]). Because the year of initial outbreak was different among sites, the total post-outbreak period of residual trees was also different among sites, ranging from 8 to 10 years. I first calculated the mean of each resin duct characteristics, ring width, and BAI for each of the three post-outbreak periods. Then I used the mean of each resin duct characteristic, ring width and BAI for 10-year pre-outbreak period (the same 10-year pre-outbreak period on residual trees as section 2.2.3.1) to compare with the mean of the same variable of each post-outbreak period for each category of residual trees (healthy, declining and survived) separately using linear mixed-effects models, with trees nested in plots, plots nested in sites as a random effect. I calculated tree DBH in each growth period and used such DBH as a covariate to account for the influence of tree size changing over time when I compared resin duct production, total resin duct area, resin duct size, and ring width between pre- and post-outbreak periods. When time period (pre and post-outbreak period) had a significant effect on resin duct characteristics, ring width or BAI ($\alpha=0.05$), I used lsmeans to conduct pairwise post-hoc tests to look for differences between time periods. The temporal variation of ring width and BAI were visualized by box plots by calendar years from 1996 to 2015.

2.2.3.3 Can growth and resin duct development explain differences in health conditions of residual trees in post-MPB stands?

I selected four overlapping periods on each increment core: 3-, 5-, 10-, and 20-year counting back from the last completed ring formation in 2015. I then calculated the mean of each resin duct characteristic, ring width, and BAI for each period on each core. For each period, I compared resin duct characteristics, ring width, and BAI among the three categories of residual trees using linear mixed-effects models with plots nested in sites as a random effect. I used tree DBH as a covariate to account for the influence of different tree sizes in the models that compared resin duct production, total resin duct area, resin duct size and ring width among the three categories of residual trees. When tree categories (healthy, declining and survived) had a significant effect on resin duct characteristics, ring width or BAI ($\alpha=0.05$), I used lsmeans to conduct pairwise post-hoc tests to look for differences among tree categories (R package *lsmeans*).

2.3 Results

As stated above, I accepted the year the earliest tree death occurred in a site as the year the MPB outbreak started in that particular site. Even though this approach was necessary to standardize the year the outbreak started in each site, this approach might cause undesirable errors in my analysis and interpretation. For example, if the year of outbreak started in one site was 2006 and it was 2008 in the other site, the 5-year pre-outbreak period conducted in my analysis would contain different calendar years (2005-2001 for one site vs. 2007-2003 for the other site; the year of outbreak started was excluded). Because ring width and BAI could be different year to year due to environmental factors, I expected to observe higher variance of errors on ring width and BAI in my analysis. Such situation applied to all results in section 2.3.1 and 2.3.2.

2.3.1 Can growth and resin duct development explain why residual trees were not killed during outbreak?

Overall, the means of ring width of beetle-killed and residual trees showed no difference in any of the three pre-outbreak periods (Fig. 2.3a). In contrast, all resin duct characteristics showed significant differences in 3-, 5-, and 10-year pre-outbreak periods between killed and residual trees (Table 2.4). Mean resin duct production of killed trees was 119-123% greater than residual trees before outbreak (Fig. 2.3b). Likewise, killed trees had higher total resin duct area (46-53%), resin duct density (105-131%), and relative resin duct area (57-78%) than residual trees before outbreak (Figs. 2.3d, e, f). However, residual trees had significantly larger (14-24%) resin ducts than killed trees (Fig. 2.3c). All resin duct characteristics and ring width were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

Overall, resin duct characteristics were more important to estimate the survival probability of pine trees than ring width in mixed effects logistic regression models. Overall, models classified residual trees (82-88%) better than MPB-killed trees (36-80%). Resin duct production and resin duct size were frequently included in these selected models (Table 2.3). Models that included both resin duct production and resin duct size had lower AIC (< 320) and higher AUC (≥ 0.89) and performed better in classifying killed (67-80%) and residual (83-88%) trees, compared to the models that included only one of the two resin duct characteristics or none (AIC > 350, AUC ≤ 0.85). The best model included the 10-year mean resin duct production (RDP10Y) and the 10-year mean resin duct size (RDS10Y) having the lowest AIC (277.05) and the highest AUC (0.91) (Table 2.3; Fig. 2.4). Both resin duct production and resin duct size were significantly correlated to the survival probability of pines under MPB attack. The equation of lodgepole pine survival probability (P) under MPB attack for this model is:

$$P = \frac{1}{1 + e^{-(-0.7172-1.2379 \times RDP10Y + 396.3965 \times RDS10Y + \lambda + \varepsilon)}}$$

Where λ = random effects

ε = model residuals

2.3.2 Were growth and resin duct development of residual trees affected by post-MPB stand conditions?

Among residual trees, declining and survived trees showed declined ring width and BAI during the first five years after outbreak, compared to before outbreak (Fig. 2.5). The ring width of healthy trees did not show any changes after outbreak, while both declining and survived trees had reduced ring width in the first five years after outbreak compared to before outbreak (Fig. 2.5a). Healthy trees were the only category of residual trees that increased BAI after MPB outbreak, compared to before outbreak. The BAI of declining trees did not respond to the beetle outbreak, while survived trees showed lower BAI in the first 3 years after outbreak (Fig. 2.5b). For all residual trees, the annual BAI and ring width showed an increasing trend starting from 2014 (Figs. 2.6 & 2.7). DBH as a covariate in the models that compared ring width between pre- and post-outbreak periods was not significant (P>0.05). Both ring width and BAI were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

In general, almost all of the resin duct characteristics had higher value after outbreak than before outbreak in the residual trees (Figs. 2.8a, b, c, d & e). After outbreaks, healthy and survived trees had higher resin duct production in the 5-year and total growth periods, while declining trees showed higher resin duct production only in the total growth period (Fig. 2.8a), compared to the pre-outbreak growth period. Total resin duct area in healthy trees increased in the 5-year and total growth periods, while both survived and declining trees had higher total resin duct area in the total growth period compared to before outbreak (Fig. 2.8b). Enlarged resin ducts were found on healthy and survived trees in the total growth period compared to before outbreak. Declining trees had smaller resin ducts in the 5-year growth period compared to pre-outbreak growth period, but the resin ducts in the total growth period returned to the similar size as the pre-outbreak period (Fig. 2.8c). Both healthy and survived trees showed significantly higher resin duct density and larger relative resin duct area in both the 5-year and the total growth periods, compared to before outbreak. Declining trees had higher resin duct density and larger relative resin duct area only in the total post-outbreak growth period compared to the pre-outbreak period (Figs. 2.8d, e). DBH as a covariate was significant in the models that compared resin duct production of healthy trees between the 3-year post-outbreak and the pre-outbreak growth periods, total resin duct area of healthy trees between the 3-year post-outbreak and the pre-outbreak growth periods and total resin duct area of healthy trees between 5-year post-outbreak and pre-outbreak growth periods. All resin duct characteristics were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

2.3.3 Can growth and resin duct development explain differences in health conditions of residual trees in post-MPB stands?

I found no differences in ring width among the three categories of residual trees for the recent 3-, 5-, 10- and 20-year growth periods. In contrast, BAI varied among the categories of residual trees (Fig. 2.9). Both healthy and survived trees had consistently larger (healthy: 43-108%;

survived: 54-106%) BAI than declining trees in all four periods. DBH as a covariate was significant in models that compared ring width among the categories of residual trees ($P<0.05$). Both ring width and BAI were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

For resin duct characteristics, I only found statistical differences in resin duct size (Fig. 2.10) and total resin duct area (Fig. 2.11) among the three residual tree categories. Healthy trees had larger (28-44%) resin duct size compared to declining trees in all four growth periods investigated. The resin duct size of healthy trees was larger (16%) than survived trees in the 10-year growth period, while survived trees had larger (15%) resin duct size than declining trees in the same growth period. In the other time periods, size of resin ducts of survived trees was not significantly different compared to the other two tree categories. Healthy trees had larger (36-44%) total resin duct area than declining trees in the 3-, 5- and 10-year growth periods, while survived trees did not show any differences in total resin duct area compared to the other two categories in any growth periods. All three categories of trees had similar total resin duct area in the 20-year growth period. DBH as a covariate was not significant in most of the models that compared resin duct production, resin duct size and total resin duct area among three residual tree categories ($P<0.05$), but not in the models that compared resin duct production in the 10- and 20-year growth periods among the three tree categories ($P>0.05$). All resin duct characteristics were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

2. 4 Discussion

Through this study, I showed that resin duct characteristics can be critical components of pine defenses and survival before, during, and after MPB outbreak. Overall, beetle-killed trees had smaller resin ducts than residual trees before outbreak, and residual trees continued to have larger resin ducts after outbreak. By increasing the resin flow, larger resin ducts likely substantially reduced the probability of successful beetle colonization on residual trees by providing sticky physical barriers, sealing beetle entry wounds, and releasing toxic compounds (Franceschi et al. 2005, Erbilgin et al. 2017a). Thus, these results are in agreement with earlier studies emphasizing the importance of anatomic defenses in the survival of conifers under the attacks of tree-killing bark beetle species (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015, Mason et al. 2019).

Resin duct size likely plays a critical role in pine survival during MPB outbreak in the current study. Surprisingly the number of resin ducts did not seem to be as important as resin duct size, since trees with relatively more but smaller resin ducts were killed during outbreak. Furthermore, the logistic model of tree survival probability showed that trees with larger but fewer resin ducts had higher probability of survival during MPB outbreak, which indicated a possible trade-off between size and number of resin ducts in pine trees. Finally, trees with larger resin ducts were healthy compared to the declining trees which had relatively smaller resin ducts. All these results indicated that trees with larger resin ducts could be more resistant to beetle attacks.

I provided three possible reasons to explain the importance of resin duct size to pine survival observed in this study. First, larger resin ducts likely result in storage and biosynthesis of a higher volume of resin, thereby increasing resin accumulation within the tree (Hood and

Sala 2015). Consequently, such trees could form a stronger constitutive defense line against bark beetles. Second, larger resin ducts can rapidly deploy a much higher volume of resin flow to the beetle attack points, which would increase the likelihood of entrapment of beetles at the host entrance during the initial host colonization (Schopmeyer et al. 1954, Hood and Sala 2015, Cale et al. 2017, Erbilgin et al. 2017a). With the radius of resin ducts increases one unit, the resin flow volume could increase the fourth power. Thus, linking resin duct characteristics to resin flow can be critical to assess tree resistance to bark beetle attacks (Schopmeyer et al. 1954). Third, it is likely that the size of resin ducts is under genetic control and potentially heritable. In this study, the resin duct size of declining trees fluctuated after MPB outbreak. Such fluctuation of resin duct size may indicate a specific range of resin duct size in trees that controlled by tree genetics. While some resin duct characteristics (density and production) are influenced by tree genetics in some pine species (Moreira et al. 2015, Westbrook et al. 2015), the heritability of resin duct size remains unclear. If the resin duct size of healthy and survived trees after outbreak returns to the level of the pre-outbreak period needs future examination. Nevertheless, survival of pine trees with larger resin ducts suggests that bark beetle outbreaks likely drive selection for better-defended lodgepole pine phenotypes (Balogh et al. 2018).

I consistently found higher levels of characteristics of anatomical defense structures in residual pine trees following outbreak, relatively to the pre-outbreak periods. This is the first report of increased anatomical defenses in pines after bark beetle outbreaks. Two possible characteristics of post-outbreak stand conditions may explain these results. First, the post-MPB stand conditions may not be suitable for tree growth (Karst et al. 2015, Pec et al. 2017). Based on the growth-differentiation balance hypothesis, plants favor growth over defense when resources are abundant, whereas defense is usually favored under limited resources (Herms and Mattson

1992). The reduced growth in residual trees right after outbreak in the current study may reflect the poor growing conditions in these stands for the residual pine trees. Second, it has been widely documented that plants under herbivory insect attacks can release volatile organic compounds which can alarm neighbouring trees (Baldwin and Schultz 1983, Engelberth et al. 2004), which might have led to the production of additional resin ducts in the healthy trees. This explanation warrants further studies in the field. In addition, although residual trees had higher resin duct production, total resin duct area, resin duct density and relative resin duct area after MPB outbreak, compared to before outbreak, the level of these resin duct characteristics of residual trees in the post-outbreak growth period was significantly lower than MPB-killed trees. Furthermore, residual trees after outbreak maintained larger resin ducts than MPB-killed trees (App. Table 3). Overall, the residual trees in post-MPB stands are likely to be resistant to future beetle attacks.

Overall the ring width was similar between beetle-killed and residual trees and the ring width was not associated with pine survival in the top model. However, ring width is correlated with the size of trees. With the same ring width, trees with larger DBH might grow more annual BAI compared to the smaller trees. Therefore, using DBH as a covariate to account for the influence of tree size in models that compared ring width between residual and MPB-killed trees would be an appropriate approach. However, since I could not accurately measure the DBH of killed trees (because of fallen off barks), this created some potential problems for data analysis and interpretation. To resolve this issue, I tested whether DBH of killed and residual pine trees were different by using the DBH of the killed trees with intact bark. These results suggested that beetle killed trees had larger DBH than residual trees (App. Fig. 3), indicating dead trees likely grew faster than residual trees before MPB outbreak (Cooper et al. 2018), which might result in producing less defense compounds. These results are in disagreement with earlier reports suggested that tree growth might not be a good indicator of pine resistance to bark beetles (Kane and Kolb 2010, Ferrenberg et al. 2014). Such disagreements may reflect differences in stand conditions, such as percent tree mortality, tree species, tree ages, forest structure and composition and climate (Campbell et al. 2007, Kane and Kolb 2010, Hawkins et al. 2013, Pec et al. 2015, 2017). For example, in my study, residual trees showed increasing growth rate at least five years after outbreak, while earlier studies reported accelerated radial growth as early as three years since beetle outbreak started (Murphy et al. 1999, Hawkins et al. 2013, Dhar et al. 2016).

In conclusion, in this study, I showed that anatomical defense structures are critical components of lodgepole pine survival during beetle outbreak. Furthermore, these defense structures in residual trees have likely continued playing major roles in defending bark beetle attacks in post-outbreak stands. In particular, resin duct size appears to be an important indicator of tree resistance to bark beetles. Although it is not clear whether resin duct size is heritable, if so, I expect that the next generation of lodgepole pine forests in western Alberta would be resistant to future MPB attacks. Therefore, keeping, and not harvesting, these residual trees could help land managers to assure the future sustainable lodgepole pine forests in Alberta.

Table 2.1 Number of study sites by starting year of *Dendroctonus ponderosae* outbreak and number of residual *Pinus contorta* var. *latifolia* trees sampled in Alberta (Canada).

Initial attack year	**2006**	**2007**	**2008**	**2010**	**Total**
Number of sites	22	4	4	1	31
Number of trees sampled	144	38	22	6	210

Table 2.2 Correlation among growth and resin duct characteristics of residual *Pinus contorta* var. *latifolia* trees over 3, 5, and 10 years before *Dendroctonus ponderosae* outbreak and trees killed by *D. ponderosae* over 3, 5, and 10 years before tree death in post-outbreak stands in Alberta.

	3-year measurement						***5-year measurement***						***10-year measurement***				
	RW	RDP	RDA	RDS	RDD	RRDA	RW	RDP	RDA	RDS	RDD	RRDA	RW	RDP	RDA	RDS	RDD
3-year measurement																	
RDP	0.40 ***																
RDA	0.55 ***	0.84 ***															
RDS	0.34 ***	***0.09***	0.39 ***														
RDD	-0.38 ***	0.29 ***	***0.05***	-0.20 ***													
RRDA	-0.38 ***	0.26 ***	0.12 *	***-0.02***	0.91 ***												
5-year measurement																	
RW	0.99 ***	0.43 ***	0.57 ***	0.34 ***	-0.36 ***	-0.36 ***											
RDP	0.43 ***	0.93 ***	0.79 ***	***0.06***	0.23 **	0.20 ***	0.46 ***										
RDA	0.57 ***	0.79 ***	0.94 ***	0.35 ***	***0.00***	***0.05***	0.60 ***	0.85 ***									
RDS	0.40 ***	***0.06***	0.38 ***	0.90 ***	-0.27 ***	***-0.10***	0.40 ***	***0.06***	0.39 ***								
RDD	-0.40 ***	0.19 ***	***-0.02***	-0.23 ***	0.92 ***	0.81 ***	-0.40 ***	0.24 ***	***0.00***	-0.29 ***							
RRDA	-0.41 ***	0.18 ***	***0.04***	***-0.07***	0.83 ***	0.90 ***	-0.40 ***	0.23 ***	***0.07***	-0.11 *	0.90 ***						
10-year measurement																	
RW	0.93 ***	0.43 ***	0.55 ***	0.34 ***	-0.32 ***	-0.32 ***	0.96 ***	0.47 ***	0.60 ***	0.42 ***	-0.35 ***	-0.35 ***					
RDP	0.41 ***	0.87 ***	0.74 ***	***0.05***	0.20 ***	0.17 **	0.45 ***	0.95 ***	0.81 ***	***0.05***	0.22 *	0.21 ***	0.48 ***				
RDA	0.54 ***	0.75 ***	0.88 ***	0.31 ***	***0.01***	***0.05***	0.57 ***	0.81 ***	0.95 ***	0.34 ***	***0.00***	***0.07***	0.60 ***	0.86 ***			
RDS	0.39 ***	***0.08***	0.38 ***	0.80 ***	-0.27 ***	-0.11	0.41 ***	***0.08***	0.40 ***	0.90 ***	-0.29 ***	-0.12 *	0.43 ***	***0.09***	0.41 ***		
RDD	-0.42 ***	0.14 **	***-0.05***	-0.25 ***	0.83 ***	0.75 ***	-0.42 ***	0.17 **	***-0.04***	-0.33 ***	0.89 ***	0.82 ***	-0.41 ***	0.21 ***	***0.00***	-0.32 ***	
RRDA	-0.40 ***	***0.10***	***-0.01***	***-0.09***	0.68 ***	0.77 ***	-0.40 ***	0.13 *	***0.00***	-0.17 **	0.73 ***	0.85 ***	-0.39 ***	0.17 **	***0.06***	-0.13 *	0.89 ***

Pearson correlation coefficient was given to represent the correlation between two variables. Significant correlations indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ based on Pearson correlation test. RW: ring width (mm yr^{-1}); RDP: resin duct production (no. yr^{-1}); RDA: resin duct area (mm^2 yr^{-1}); RDS: resin duct size (mm^2 yr^{-1}); RDD: resin duct density (no. mm^{-2} yr^{-1}); RRDA: relative resin duct area (% yr^{-1}).

Table 2.3 The 33 models predicting the survival probability of *Pinus contorta* var. *latifolia* trees using Akaike's Information Criterion (AIC), area under receiver operating curve (AUC), and internal validation results.

	Models	AIC	AUC	Killed trees correctly classified (%)	Residual trees correctly classified (%)
1	RDP10Y + RDS10Y	277.05	0.91	78.57	87.25
2	RDP5Y + RDS10Y	281.27	0.91	80.00	87.75
3	RDP5Y + RDS5Y	296.64	0.91	76.43	85.78
4	RDP10Y + RDS5Y	302.54	0.89	75.71	85.78
5	RDP5Y + RDS3Y + RW5Y	303.19	0.90	72.86	86.27
6	RDP3Y + RDS10Y	304.41	0.90	69.29	86.76
7	RDP10Y + RDS3Y + RW10Y	305.88	0.89	76.43	83.33
8	RDP5Y + RDS3Y + RRDA10Y	306.28	0.90	75.00	85.78
9	RDP3Y + RDS5Y	314.55	0.89	68.57	86.76
10	RDP3Y + RDS3Y + RRDA10Y	315.79	0.89	69.29	85.29
11	RDP3Y + RDS3Y	319.32	0.89	67.14	88.24
12	RDP3Y + RRDA10Y	358.94	0.85	61.43	86.76
13	RRDA3Y + RDS5Y + RW5Y	375.20	0.84	60.71	84.80
14	RDS3Y + RRDA5Y + RW10Y	380.65	0.83	57.86	83.82
15	RDA5Y + RDD5Y	381.93	0.83	56.43	83.33
16	RDS3Y + RRDA3Y + RW3Y	382.53	0.83	58.57	84.31
17	RDA3Y + RDD5Y	383.29	0.83	55.00	84.31
18	RDD5Y + RDA10Y	383.55	0.83	55.71	84.31
19	RDD3Y + RDA5Y	390.65	0.81	35.00	84.80
20	RDA5Y + RDD10Y	392.30	0.82	57.86	84.31
21	RDD3Y + RDA10Y	392.69	0.81	52.14	82.35
22	RDA3Y + RDD10Y	395.48	0.82	58.57	84.31
23	RDA10Y + RDD10Y	398.00	0.81	55.00	83.82
24	RDA3Y + RDD3Y	398.83	0.80	51.43	84.31
25	RDS3Y + RRDA10Y + RW10Y	408.23	0.81	57.14	82.84
26	RDA5Y + RRDA5Y	421.17	0.76	42.14	84.31
27	RRDA5Y + RDA10Y	422.22	0.77	39.29	82.35
28	RDA3Y + RRDA5Y	422.59	0.76	44.29	83.33
29	RRDA3Y + RDA5Y	423.10	0.75	42.86	84.8
30	RRDA3Y + RDA10Y	424.23	0.76	42.86	82.84
31	RDA5Y + RRDA10Y	434.08	0.75	40.00	84.31
32	RDA3Y + RRDA10Y	436.94	0.74	39.29	84.31
33	RDA10Y + RRDA10Y	437.88	0.75	36.43	83.33

RW: ring width (mm yr^{-1}); RDP: resin duct production (no. yr^{-1}); RDA: total resin duct area (mm^2 yr^{-1}); RDS: resin duct size (mm^2 yr^{-1}); RDD: resin duct density (no. mm^{-2} yr^{-1}); RRDA: relative resin duct area (% yr^{-1}). 3Y: mean of resin duct and growth characteristics in 3-year pre-outbreak growth period; 5Y: mean of resin duct and growth characteristics in 5-year pre-outbreak growth period; 10Y: mean of resin duct and growth characteristics in 10-year pre-outbreak growth period.

Table 2.4 Comparisons of mean resin duct characteristics between residual *Pinus contorta* var. *latifolia* trees over 3, 5, and 10 years before *Dendroctonus ponderosae* outbreak and *D. ponderosae*-killed *P. contorta* var. *latifolia* trees over 3, 5, and 10 years before death in Alberta. Medians and 25% and 75% quartile of raw data were listed in the table.

Variables	Median (25%, 75% quartile)		F-value	P
	Residual trees	Killed trees		
Resin duct production (no. yr^{-1})				
3-year	1.667 (1.000, 2.333)	3.667 (2.667, 5.417)	126.08	<0.001
5-year	1.600 (1.000, 2.600)	3.800 (2.800, 5.600)	199.22	<0.001
10-year	1.600 (1.000, 2.600)	3.850 (2.875, 5.250)	201.54	<0.001
Resin duct density (no. mm^{-2} yr^{-1})				
3-year	0.439 (0.222, 0.673)	0.863 (0.575, 1.431)	91.847	<0.001
5-year	0.441 (0.237, 0.703)	0.787 (0.599, 1.498)	104.34	<0.001
10-year	0.471 (0.233, 0.796)	0.865 (0.577, 1.330)	88.169	<0.001
Relative resin duct area (% yr^{-1})				
3-year	0.663 (0.353, 1.037)	1.054 (0.738, 1.542)	51.299	<0.001
5-year	0.662 (0.416, 1.048)	0.969 (0.773, 1.528)	59.195	<0.001
10-year	0.702 (0.369, 1.106)	1.020 (0.756, 1.469)	45.124	<0.001
Total resin duct area (mm^{2} yr^{-1})				
3-year	0.030 (0.016, 0.044)	0.046 (0.030, 0.069)	33.49	<0.001
5-year	0.028 (0.017, 0.046)	0.047 (0.030, 0.067)	23.682	<0.001
10-year	0.029 (0.017, 0.045)	0.047 (0.033, 0.067)	45.449	<0.001
Resin duct size (mm^{2} yr^{-1})				
3-year	0.014 (0.009, 0.019)	0.012 (0.009, 0.013)	12.248	<0.001
5-year	0.014 (0.009, 0.019)	0.011 (0.009, 0.013)	20.578	<0.001
10-year	0.014 (0.010, 0.018)	0.012 (0.010, 0.013)	25.086	<0.001

Resin duct characteristics were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

Figure 2.1 Front view (a) and side view (b) of a wedge sampled from *Dendroctonus ponderosae*-killed *Pinus contorta* var. *latifolia* tree

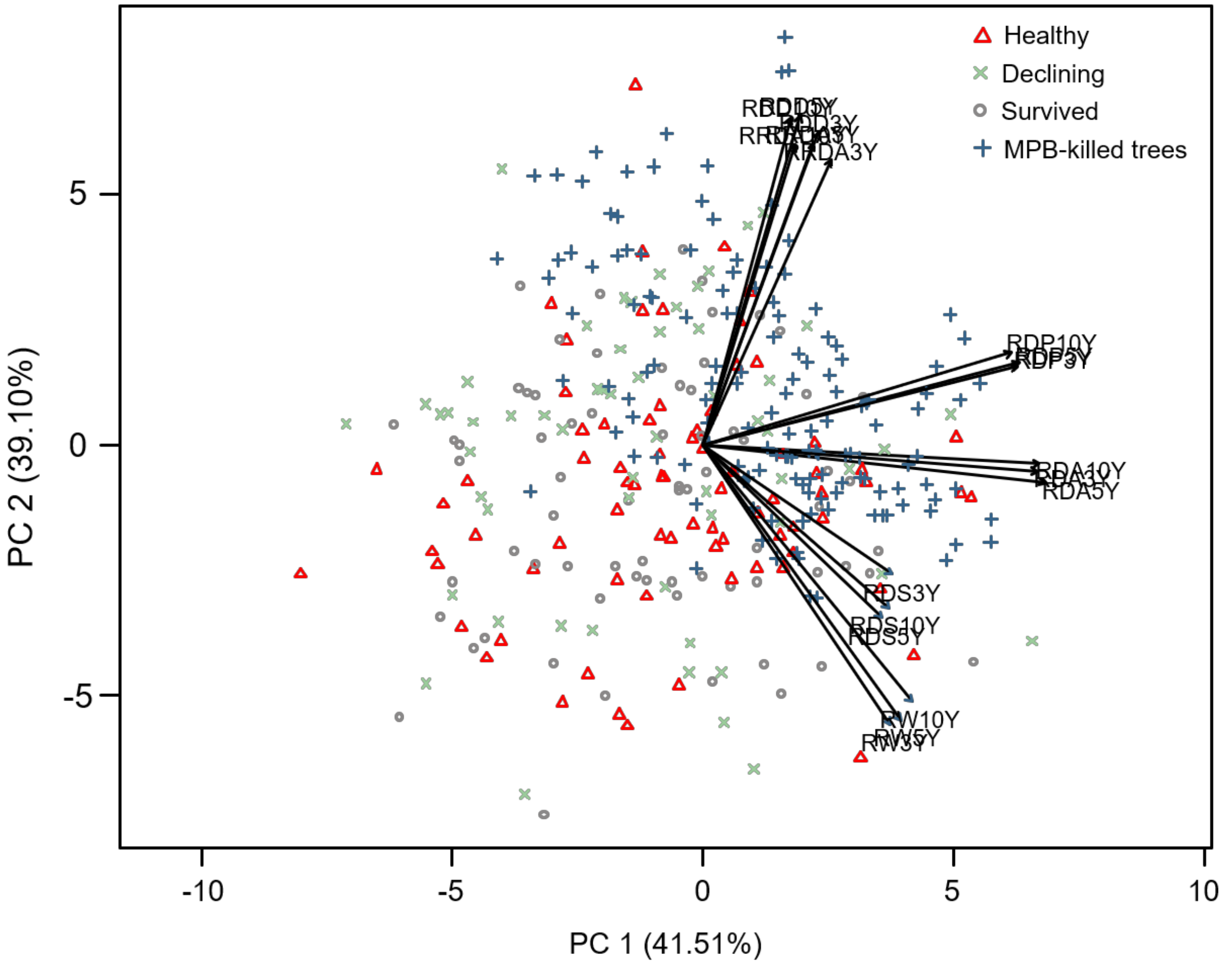


Figure 2.2 Results of principle component analysis (PCA) among healthy, declining, survived and mountain pine beetle (*Dendroctonus ponderosae*, MPB)-killed *Pinus contorta* var. *latifolia* trees in terms of the resin duct characteristics and ring width. Healthy trees are represented as red triangles, declining trees as green cross (×), survived trees as gray circles, and MPB-killed trees as blue cross (+). Vectors indicate the strength and directional influence of resin duct characteristics and growth under deducted dimensions. RW: ring width (mm yr^{-1}); RDP: resin duct production (no. yr^{-1}); RDD: resin duct density (no. mm^{-2} yr^{-1}); RRDA: relative resin duct area (% yr^{-1}); RDA: total resin duct area (mm^2 yr^{-1}); RDS: resin duct size (mm^2 yr^{-1}). 3Y: mean of resin duct and growth characteristics in 3-year pre-outbreak growth period; 5Y: mean of resin

duct and growth characteristics in 5-year pre-outbreak growth period; 10Y: mean of resin duct and growth characteristics in 10-year pre-outbreak growth period.

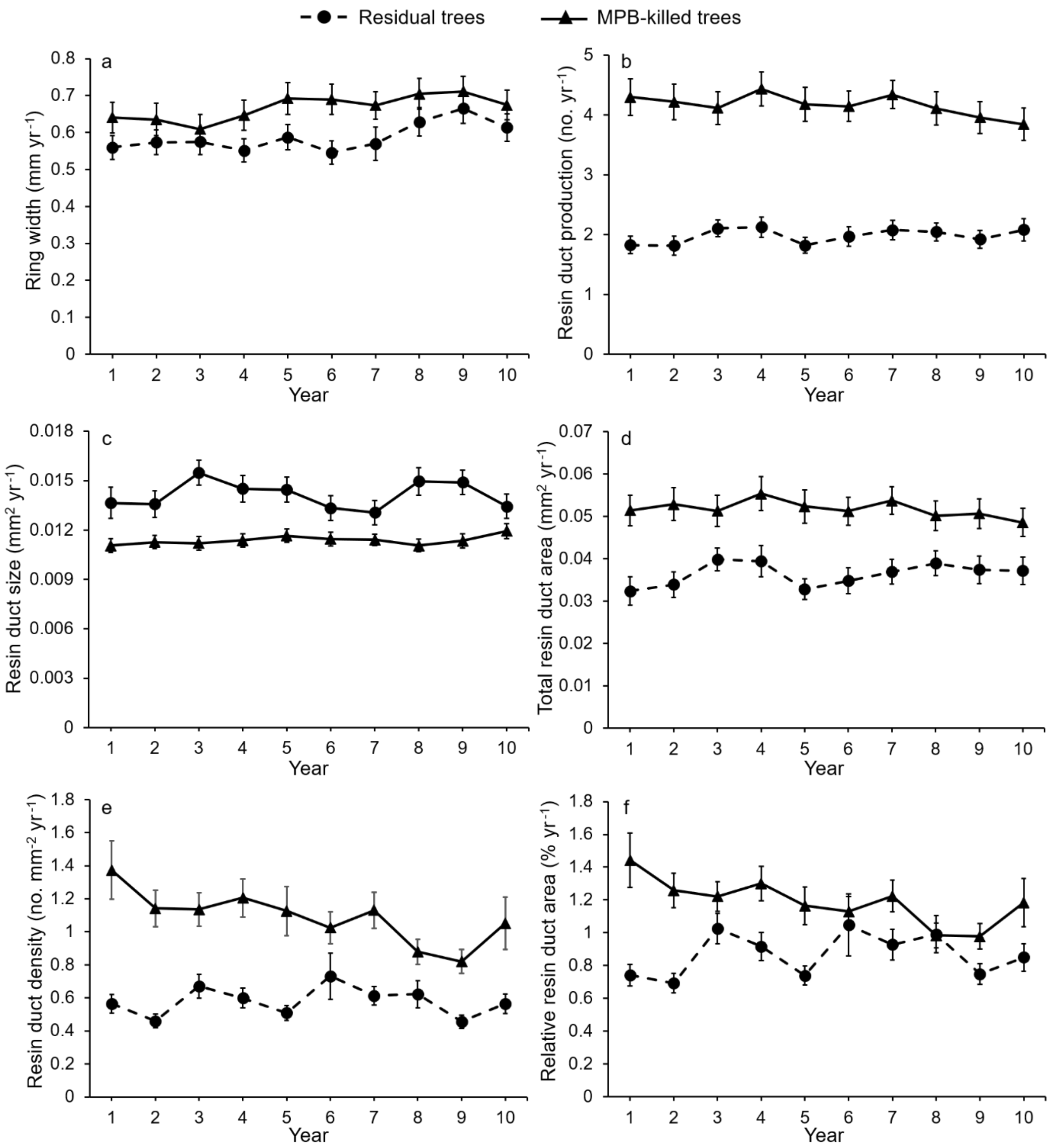


Figure 2.3 Annual variation in the mean of ring width (a), resin duct production (b), resin duct size (c), total resin duct area (d), resin duct density (e) and relative resin duct area (f) of residual *Pinus contorta* var. *latifolia* trees in the 10 years before mountain pine beetle (MPB, *Dendroctonus ponderosae*) outbreak and MPB-killed trees in the 10 years before tree death.

Error bars represent standard error. I did not use the calendar year because the starting year of MPB outbreak varied among sites and the death year of killed trees varied among trees.

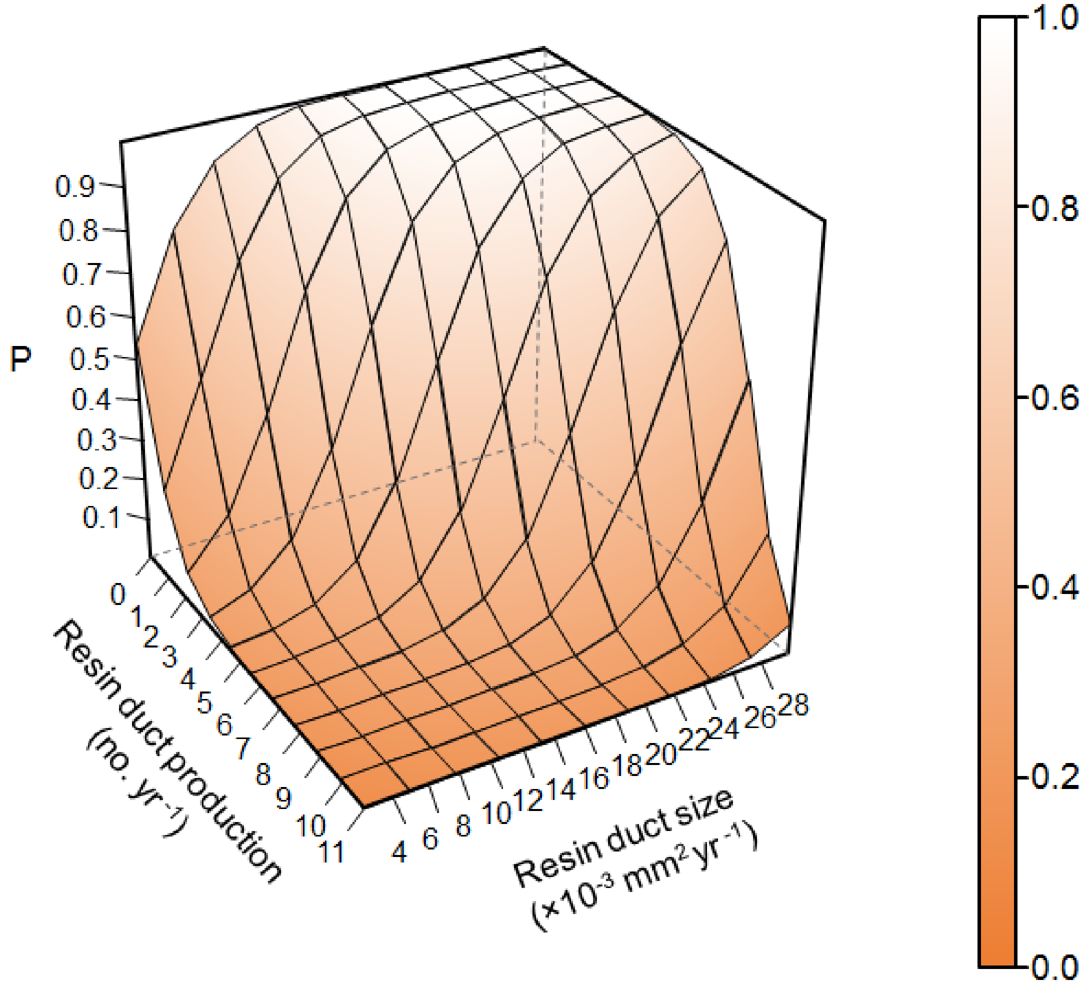


Figure 2.4 Prediction of survival probability of *Pinus contorta* var. *latifolia* trees under *Dendroctonus ponderosae* attacks from the best model in Table 2.3 based on 10-year mean resin duct production and 10-year mean resin duct size of *P. contorta* var. *latifolia* trees in post-outbreak stands excluding random effects. P represents survival probability of *P. contorta* var. *latifolia* trees under *D. ponderosae* attacks.

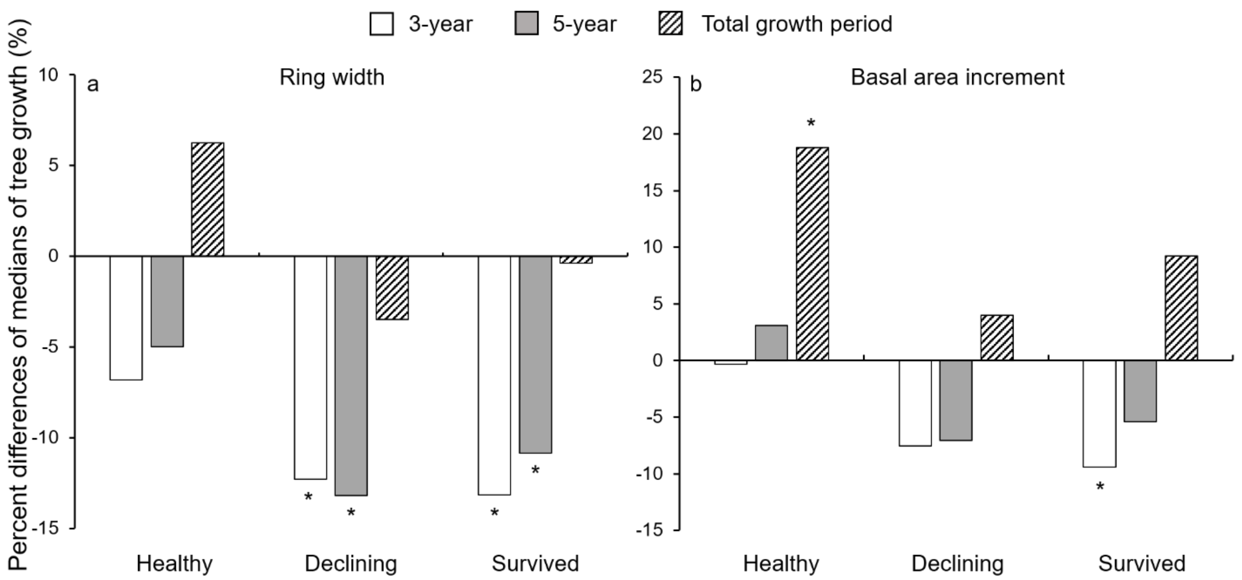


Figure 2.5 Percent differences of medians of ring width (a) and basal area increment (b) between the 10-year growth period before *Dendroctonus ponderosae* outbreak and each of the three growth periods after outbreak (3-, 5-year and total growth period from the outbreak started to the sampled year [2015]) in each of the three categories of residual *Pinus contorta* var. *latifolia* (healthy, declining, and survived) trees. Significant differences (α=0.05) on ring width and basal area increment between each of the three growth periods after outbreak and the growth period before outbreak were indicated by asterisks (*). Percent differences of medians of ring width and basal area increment were further calculated and only used in this figure to represent the differences between each of the three growth periods after outbreak and the growth period before outbreak. Tree diameter at breast height of each growth period was calculated and was used as a covariate in the models comparing ring width between pre-outbreak growth period and each of the three post-outbreak growth periods in each category of residual trees. Both ring width and basal area increment were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

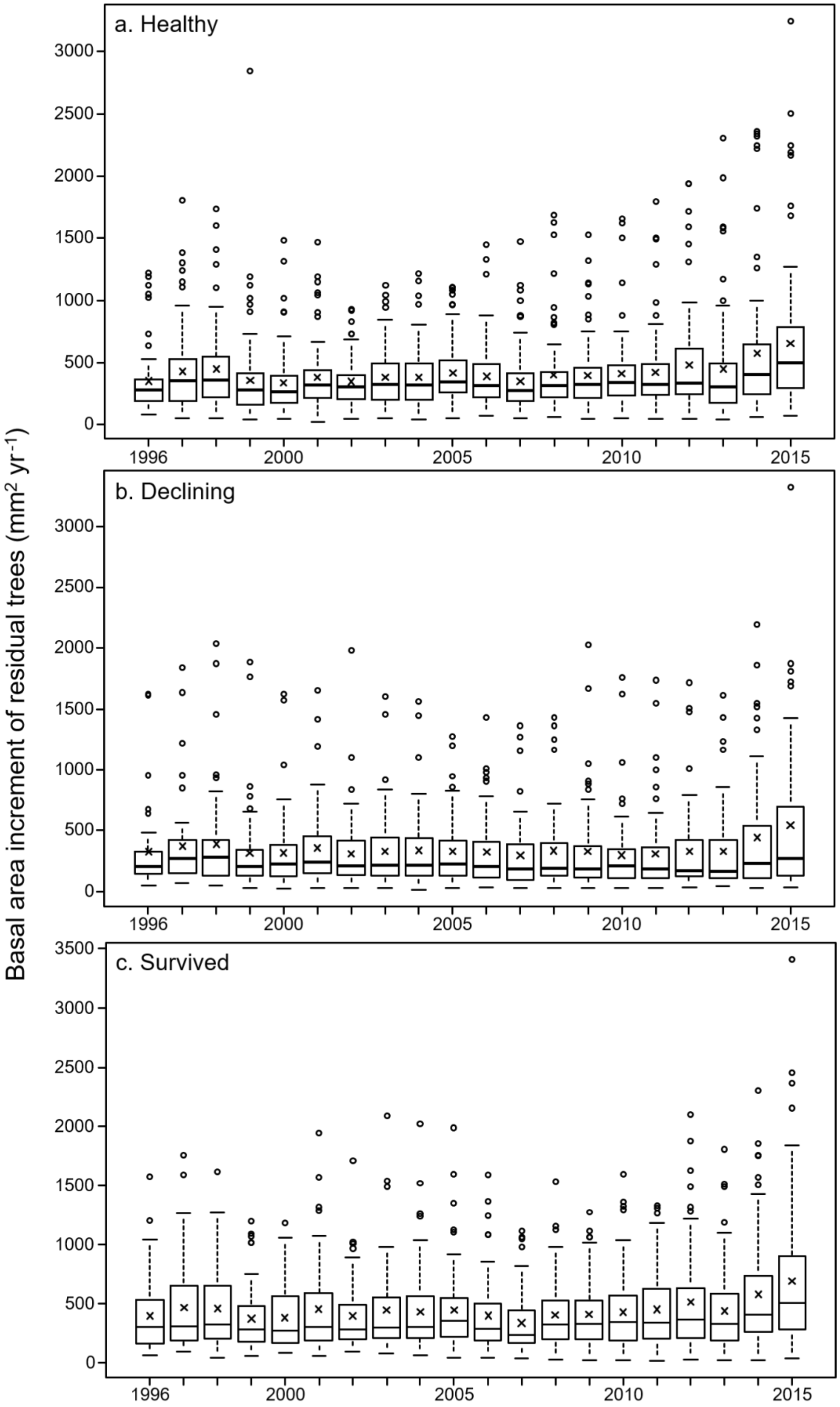

a. Healthy
b. Declining
c. Survived
Basal area increment of residual trees (mm² yr⁻¹)
0
500
1000
1500
2000
2500
3000
3500
1996
2000
2005
2010
2015

Figure 2.6 Temporal variation in basal area increment from 1996 to 2015 in three categories of residual *Pinus contorta* var. *latifolia* (healthy [a], declining [b], and survived [c]) trees. In each box plot, the thick line is the median, × mark indicates the mean, the box represents the 1st and 3rd quartiles, whiskers are Tukey’s 1.5 interquartile range, and the open circles indicate outliers.

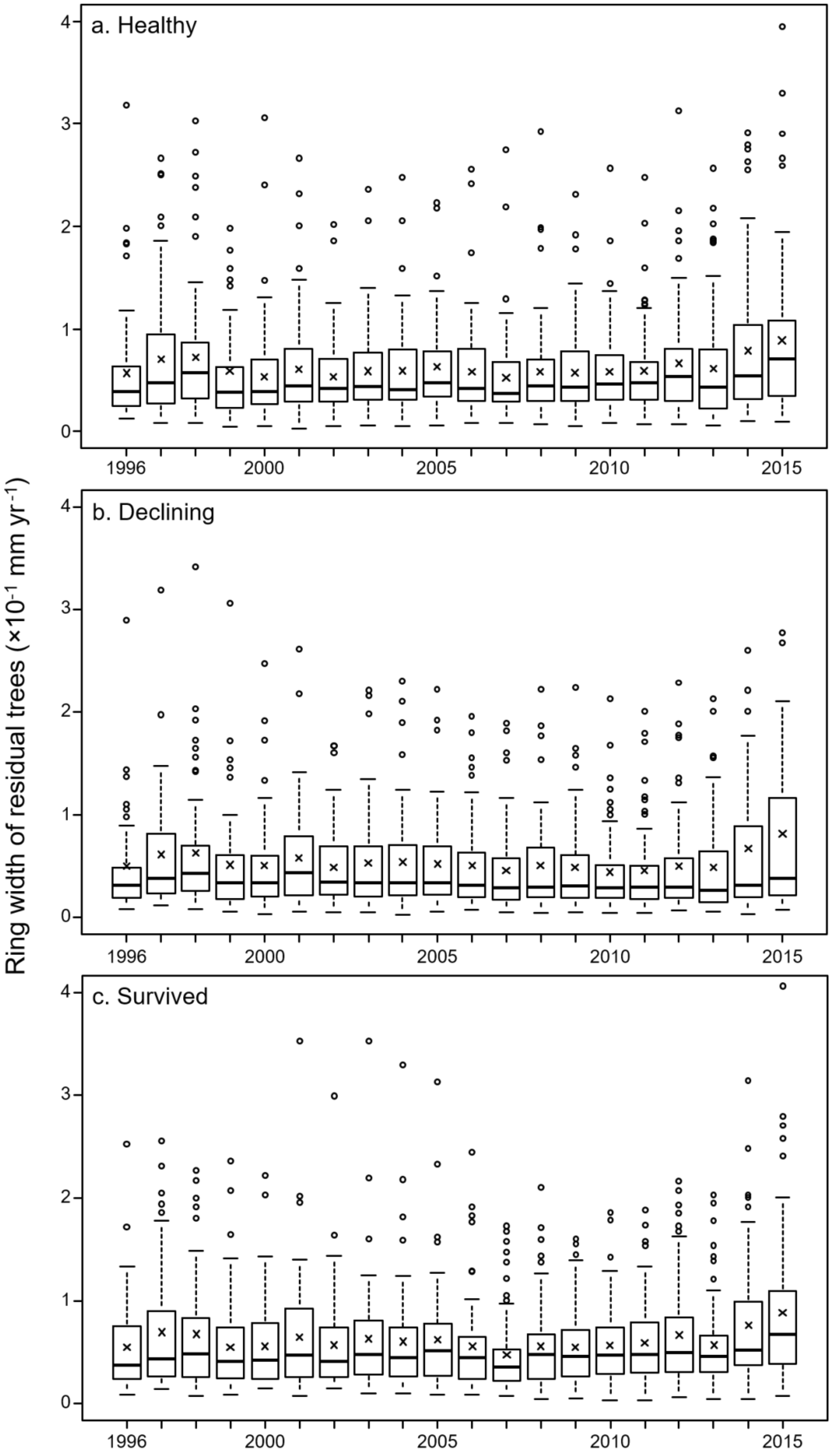

a. Healthy
b. Declining
c. Survived
Ring width of residual trees (×10⁻¹ mm yr⁻¹)
0
1
2
3
4
1996
2000
2005
2010
2015

Figure 2.7 Temporal variation in ring width from 1996 to 2015 in three categories of residual *Pinus contorta* var. *latifolia* (healthy [a], declining [b], and survived [c]) trees. In each box plot, the thick line is the median, × mark indicates the mean, the box represents the 1st and 3rd quartiles, whiskers are Tukey's 1.5 interquartile range, and the open circles indicate outliers.

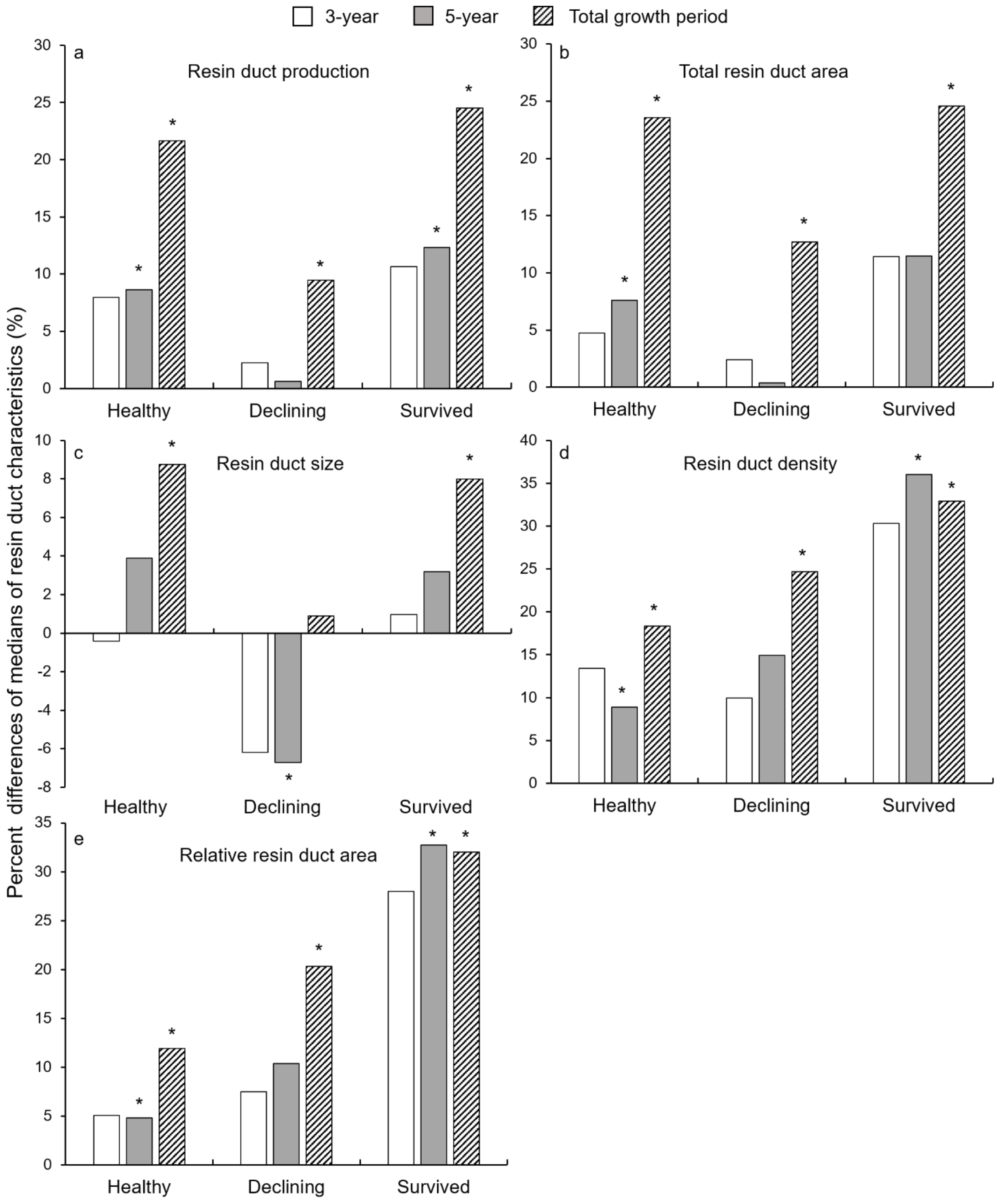


Figure 2.8 Percent differences of medians of resin duct production (a), total resin duct area (b), resin duct size (c), resin duct density (d) and relative resin duct area (e) between the 10-year growth period before *Dendroctonus ponderosae* outbreak and each of the three growth periods

after outbreak (3-, 5- and total growth period from the outbreak started to the sampled year [2015]) in three categories of residual *Pinus contorta* var. *latifolia* (healthy, declining, and survived) trees. Significant differences (α=0.05) on each resin duct characteristic between each of the three growth periods after outbreak and the growth period before outbreak were indicated by asterisks (*). Percent differences of medians of each resin duct characteristic were further calculated and only used in this figure to represent the differences between each of the three growth periods after outbreak and the growth period before outbreak. Tree diameter at breast height of each growth period was calculated and was used as a covariate in the models compared resin duct production, total resin duct area and resin duct size between pre-outbreak growth period and each of the three post-outbreak growth periods in each category of residual trees. All resin duct characteristics were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

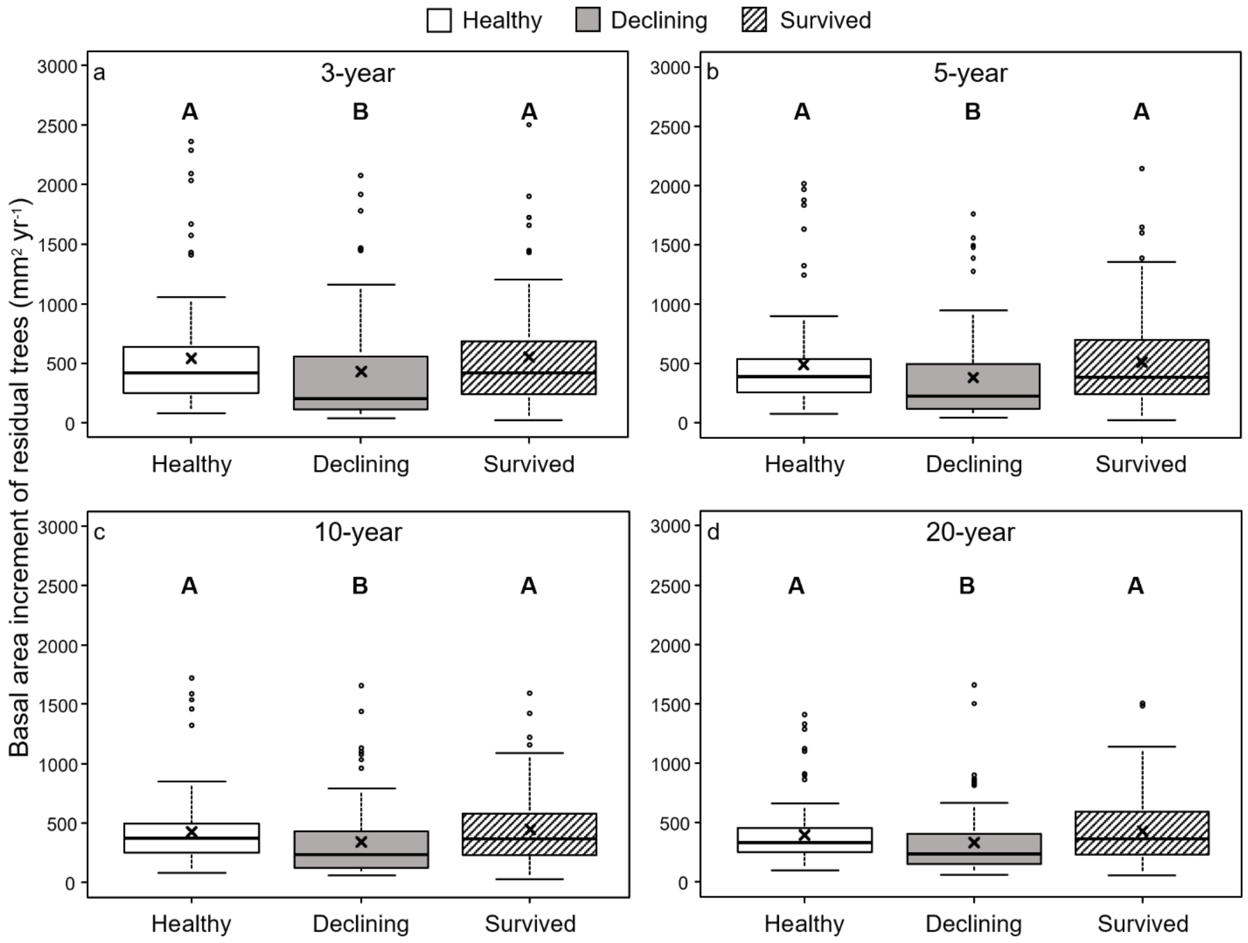


Figure 2.9 Basal area increments of three categories (healthy, declining, survived) of residual *Pinus contorta* var. *latifolia* trees in four most recent growth periods (3, 5, 10, 20 years). Significant differences among three tree categories at each growth period were indicated by different letters based on the results of linear mixed-effects model (α=0.05). In each box plot, the thick line is the median, × mark indicates the mean, the box represents the 1st and 3rd quartiles, whiskers are Tukey's 1.5 interquartile range, and the open circles indicate outliers. Basal area increment was log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

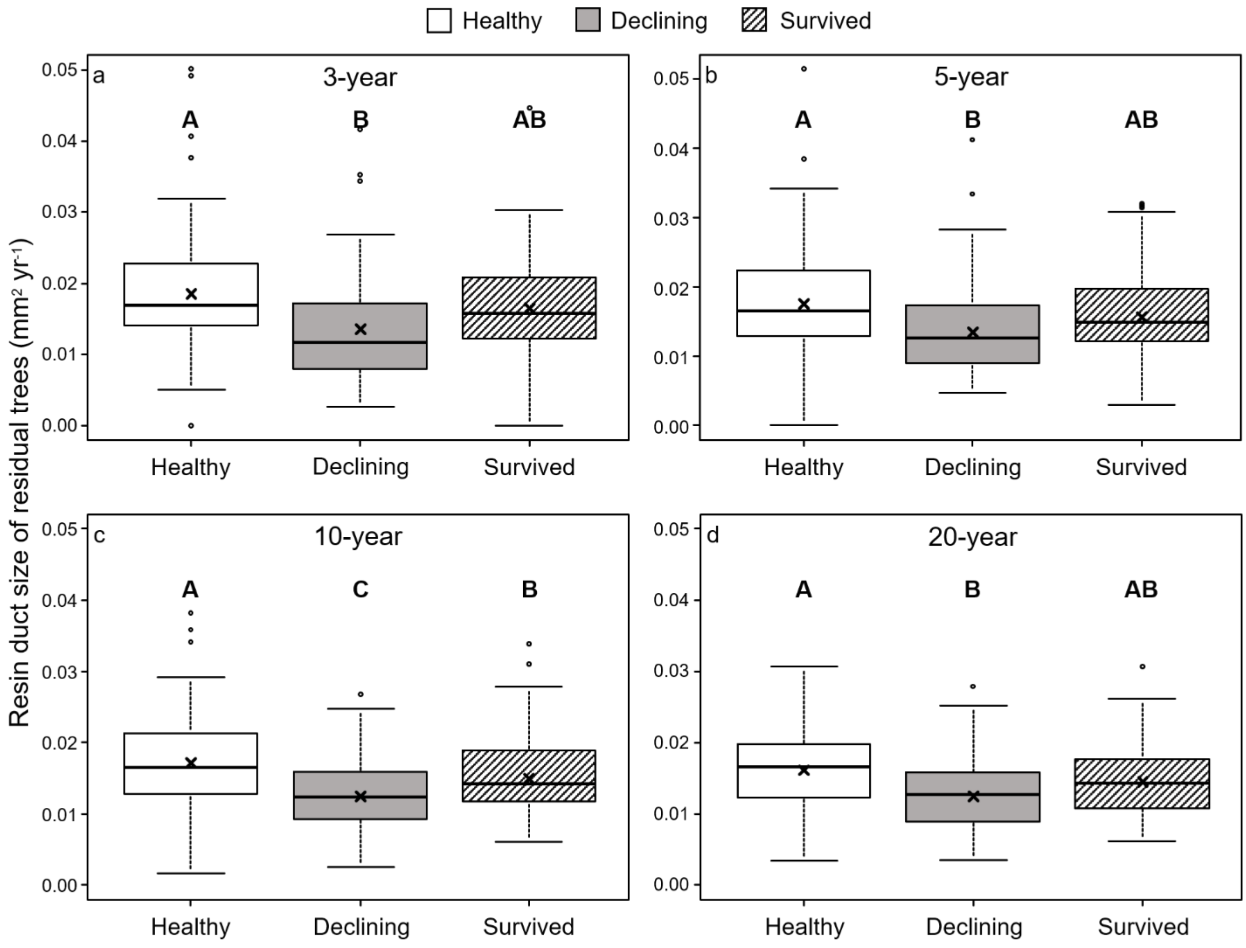


Figure 2.10 Resin duct size of three categories (healthy, declining, survived) of residual *Pinus contorta* var. *latifolia* trees in four most recent growth periods (3, 5, 10, 20 years). Significant differences among three tree categories at each growth period were indicated by different letters based on the results of linear mixed-effects model (α=0.05). In each box plot, the thick line is the median, × mark indicates the mean, the box represents the 1st and 3rd quartiles, whiskers are Tukey's 1.5 interquartile range, and the open circles indicate outliers. Tree diameter at breast height was a covariate in models that compared resin duct size among the three categories of residual trees in each of the four growth periods. Resin duct size was log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

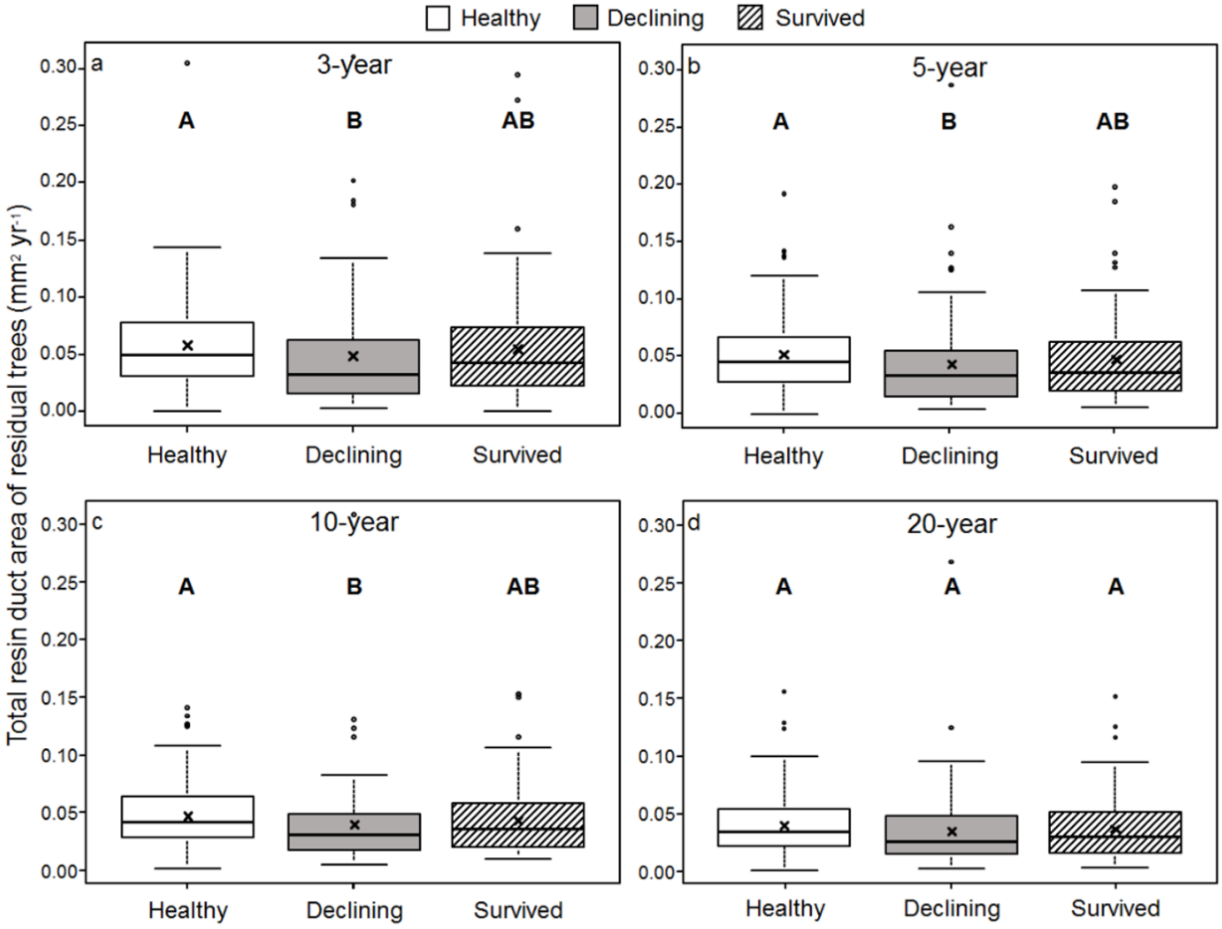


Figure 2.11 Total resin duct area of three categories (healthy, declining, survived) of residual *Pinus contorta* var. *latifolia* trees in four most recent growth periods (3, 5, 10, 20 years). Significant differences among three tree categories at each growth period were indicated by different letters based on the results of linear mixed-effects model ($\alpha=0.05$). In each box plot, the thick line is the median, × mark indicates the mean, the box represents the 1st and 3rd quartiles, whiskers are Tukey's 1.5 interquartile range, and the open circles indicate outliers. Tree diameter at breast height was a covariate in models that compared total resin duct area among three categories of residual trees in each of the four growth periods. Total resin duct area was log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

Chapter 3. Mountain pine beetle outbreak enhanced resin duct-defenses of lodgepole pine trees

3.1 Introduction

The frequency and severity of insect outbreaks have increased during the last several decades due to climate change and other anthropogenic disturbances (Logan et al. 2003, Parmesan and Yohe 2003, Battisti et al. 2006, Kirilenko and Sedjo 2007). For example, the population of mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae) increased to an epidemic level, resulted in millions of hectares of dead lodgepole pine trees (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm. ex S. Watson) in Canada due to changes in climate and anthropogenic activity (Tayler et al. 2006, Bentz et al. 2010, Safranyik et al. 2010). Furthermore, MPB has expanded its host range and geographical distribution to forests at higher elevations, northern latitudes, and eastern longitudes (Cudmore et al. 2010, Cullingham et al. 2011, Erbilgin et al. 2014, Raffa et al. 2017). Even after widespread landscape-scale mortality, a small number of overstory lodgepole pine trees remain alive in post-outbreak stands (Erbilgin et al. 2017a, Six et al. 2018). These residual trees have become a critical component of recovering pine stands and, thus, the persistence of lodgepole pine species in some areas. However, it is difficult to predict the long-term impacts of MPB outbreaks on the remaining residual pine trees. Particularly, it is unknown how the sudden changes in forest stand conditions following pine mortality can affect growth, defense, and their relationship of residual pine trees.

Conifers have evolved a complex of anatomical and chemical defenses to resist attacks from their insect enemies, particularly bark beetles (Franceschi et al. 2005, Keeling and Bohlmann 2006, Kolosova and Bohlmann 2012, Erbilgin 2019). Viscous oleoresins, acting as both physical and chemical defenses, are the first line of tree defense when bark beetles penetrate tree bark. These resins are able to expel or kill the attacking beetles, seal the beetle entry wounds and contain toxic chemicals, (Raffa et al. 2005, Erbilgin 2019). In general, studies have mainly focused on the chemical defenses of pines (Keeling and Bohlmann 2006, Bentz et al. 2017, Chiu et al. 2017, Erbilgin et al. 2017a, 2017b, Raffa et al. 2017, Erbilgin 2019), but recent studies have suggested that resin duct-based anatomical defenses can be critical to tree resistance to bark beetles (Franceschi et al. 2005, Kane and Kolb 2010, Carmona et al. 2011, Ferrenberg et al. 2014, Hood and Sala 2015). Resin ducts are anatomical structures that produce, store and translocate resins (Franceschi et al. 2000, Niederbacher et al. 2015). Therefore, resin ducts are involved in both chemical and anatomical defenses in conifers (Lombardero et al. 2000, Franceschi et al. 2005, Luchi et al. 2005, DeRose et al. 2017) and can be linked to conifer resistance to some biotic agents (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015). However, as with tree chemical defenses, resin duct characteristics also show variations due to tree genetics and growing conditions (Rigling et al. 2003, Rosner and Hannrup 2004, Westbrook et al. 2013, Slack et al. 2017, Balogh et al. 2018).

Like resins, the production of resin ducts in conifers likely has an associated metabolic cost as resources such as carbohydrates are diverted from other tree functions especially growth (Stamp 2003). There are a number of hypotheses proposed to explain the complex interactions between defense and growth in plants. For example, the growth-differentiation balance hypothesis predicts a trade-off between growth and defense depending on the plant growing conditions (Herms and Mattson 1992, Stamp 2003), with suboptimal conditions often favoring defense over growth. Mountain pine beetle outbreaks have caused short- and long-term changes in both aboveground and belowground abiotic and biotic communities as well as substantially altered forest dynamics and species interactions (Dhar and Hawkins 2011, Griffin and Turner 2012, Hawkins et al. 2013, McIntosh and Macdonald 2013, Cigan et al. 2015, Karst et al. 2015, Pec et al. 2015, Dhar et al. 2016). Although some studies reported increasing annual radial growth in pine trees after MPB outbreaks (Murphy et al. 1999, Alfaro et al. 2010, Amoroso et al. 2013, Hawkins et al. 2013), the altered stand conditions might provide unfavorable growing conditions to residual trees. For example, pine trees have obligatory symbiotic relationships with ectomycorrhizal fungi as these fungi provide essential nutrients and water to host pines. Earlier studies showed that the richness and composition of ectomycorrhizal fungi community were altered after MPB outbreak due to the mortality of their hosts (Treu et al. 2014, Pec et al. 2017). Such changes had cascading negative impacts on pine seedling growth (Karst et al. 2015). These sudden changes are also expected to have substantial impacts on residual pine trees. However, studies are lacking whether such changes can also alter resin duct-based anatomical defense, and further affect the historical growth-defense relationship and health conditions of residual pine trees in post-outbreak stands.

My primary research objective was to determine whether residual pine trees favor defense over growth in post-MPB outbreak stands in the northern range of lodgepole pine forests in western Canada. I was particular interested in determining whether 1) MPB outbreak altered the growth-defense relationship of residual pine trees; 2) the growth-defense relationship of residual trees was affected by the percent lodgepole pine mortality; 3) the changes in the density of living trees in MPB-affected stand affect the growth-defense relationship of residual pine trees. In this study, I used the resin duct characteristics standardized to tree growth to represent the growth-defense relationship of pine trees. I expected that residual trees would prioritize one direction (growth or defense) of the growth-defense relationship as the stand conditions changed after outbreak.

3.2 Materials and methods

3.2.1 Study site description, data collection and preparation

I selected 31 lodgepole pine-dominated sites in western Alberta at elevations between 600 m and 1,000 m above sea level. I set up two plots, each 40 m x 40 m in size, in each site to capture the percent variation in lodgepole pine mortality. Plot centers within a site were at least 100 m away from one another. All sites were dominated by mature lodgepole pine trees (>50% of all tree composition) and had varying levels of lodgepole pine mortality due to MPB (ranging from 2% to 83%). Trees killed by MPB were identified based on the external symptoms of beetle colonization, including beetle entrance holes (pitch tubes), brood emergence holes, and extensive beetle galleries under tree bark (Erbilgin et al. 2017a). All live mature lodgepole pine trees (>15 cm diameter at breast height [DBH]) in plots were considered as residual pine trees (residual trees, hereafter).

Residual trees were separated into three categories based on their apparent conditions: healthy (no obvious symptoms of pathogen or insect attacks), declining (showing symptoms such as sparse crowns, yellowish needles, resin dripping on barks, and bark lesions, other than MPB attacks), and survived trees (showing evidence of unsuccessful MPB attacks). Unsuccessful MPB attacks on survived trees were determined based on failed beetle reproduction, as indicated by the presence of short maternal galleries, absence of oviposition and larval galleries, and absence of beetle emergence holes (see details in Erbilgin et al. 2017a). I also determined the density of all mature pine and non-host pine trees, density of MPB-killed trees, density of all mature lodgepole pine trees (including residual and killed trees), and percent lodgepole pine mortality in each site.

In each plot, I selected up to six overstory mature residual trees (two from each of three residual tree categories) to take one increment core (12 mm in width) from each selected tree at breast height (1.4 m) on the south side of the tree in May 2016. Selected trees were either dominant or co-dominant trees in stands. DBH of the selected residual pine trees was recorded. In total, 210 increment cores were taken from 76 healthy, 62 declining and 72 survived trees. To determine when the outbreak started in each site, I collected small wedges from multiple trees killed by MPB at the same height and aspect as the residual trees (up to six MPB-killed trees maximum per plot, total 140 trees).

Both cores and wedges were glued onto wooden mounts and dried for two weeks at room temperature. After drying, the samples were progressively polished by belt and hand sanders using sand paper (120-600 grit) to create a smooth surface for observing and measuring tree rings. Then, a high-resolution digital image (1,200 dpi) was taken for each core or wedge samples. Annual ring width (mm), which represented the annual radial growth rate, was measured from the bark to the pith on all cores. If the pith was not present, measurements ended at the earliest formed ring. A master chronology was built based on the ring width series from healthy residual trees by using COFECHA (Grissino-Mayer, 2001). Missing rings or false rings on cores were corrected using the master chronology, and then calendar years for each ring were added after correction. For wedges, visual cross-dating was applied to determine the year of tree death due to the limited rings available on wedges. Given that MPB outbreaks were relative recent events in all sites sampled (since 2006), the year MPB outbreak-induced tree morality began in each stand was determined based on the year of the earliest tree death in each site. Then I separated the increment cores into two periods: pre-outbreak and post-outbreak periods based on the year of the outbreak began. The pre-outbreak period was considered 10-year prior to the outbreak, whereas the period starting from the first year of outbreak until the year of the latest ring formed (2015) was considered the post-outbreak period.

All resin ducts were examined by ImageJ (Schneider et al. 2012) within a fixed sampling width (9 mm) of each ring. I used resin duct production (number of resin ducts in a 9mm wide sample area in a given year on an increment core [no. yr^{-1}]), resin duct size (average size of resin ducts in a 9mm wide sample area in a given year on an increment core [mm^2. yr^{-1}]), and total resin duct area (sum of resin duct area in a 9mm wide sampled area in a given year on an increment core [mm^2. yr^{-1}]) as non-standardized annual resin duct characteristics. I also calculated two standardized annual resin duct characteristics: resin duct density (total number of resin ducts per year divided by the given year ring area (9mm * ring width) [no. mm^{-2} yr^{-1}]) and relative resin duct area (percent area occupied by resin ducts per year within the given year ring area [% of yr^{-1}]). Tree growth rate was represented by ring width (mm. yr^{-1}) which was measured by WinDendro^{TM} (Regent Instruments, 2008).

3.2.2 Data analysis

Due to differences in the start date of outbreaks among study sites, the total growth period after outbreaks also varied and ranged from eight to ten years. The total pre-outbreak growth period contained 10-year before outbreak. I acknowledge that this approach might bring higher error variance in following analysis as the pre- and post-growth periods might contain different calendar years due to differences in the starting year of outbreaks among sites selected for the study. All statistical analyses were done in R (R Core Team 2018). I visually assessed the normality and homogeneity of variance of residuals for all models. Log transformations were applied when necessary. I then visually assessed the normality and homogeneity of variance of residuals of post-transformation models. In all statistical analyses, alpha value (<0.05) indicates statistical significance. Below, I describe a specific data analysis for each research question to improve the clarity.

3.2.2.1 Has MPB outbreak altered the relationship between growth and resin duct based-anatomical defenses of residual lodgepole pine trees?

I used nine separate linear mixed models to determine the relationship of each non-standardized resin duct characteristic with ring width of each of the three categories of residual trees between the two outbreak periods, using trees nested in plots, plots nested in sites as a random effect in R (R Core Team, 2018) with package *lme4* (Bates et al. 2015). I used DBH as a covariate to account for the influence of tree size when I compared the relationship of non-standardized resin duct characteristics with ring width between pre- and post-outbreak periods. The effect of the interactions between ring width and outbreak periods on each non-standardized resin duct characteristic were tested by two-way ANOVA (type II sum of squares). Total resin duct area and resin duct size were log-transformed to meet the assumptions of the normality of model residuals.

I conducted similar statistical analysis for the standardized resin duct characteristics (resin duct density and relative resin duct area). However, since standardized resin duct characteristics account for the possible effect of differences in ring width per unit area (mm^2), I accepted and used standardized resin duct characteristics representing the growth-defense relationship, without including ring width in the models. Therefore, I compared each standardized resin duct characteristic of each of the three residual tree categories between the outbreak periods as the only fixed effect and trees nested in plots, plots nested in sites as a random effect using six separate linear mixed models. Resin duct density and relative resin duct area were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

Similarly, ring width of each of the three residual tree categories was compared between the outbreak periods using three separate linear mixed models, where outbreak period was a fixed effect, DBH was a covariate and plots nested in sites was a random effect. Ring width were log-transformed to meet the assumptions of the normality of model residuals.

3.2.2.2 Is the relationship between growth and resin duct-based anatomical defenses affected by the percent lodgepole pine mortality?

Six separate linear mixed models were used to determine whether the percent lodgepole pine mortality affected the growth-defense relationship of each of the three categories of residual trees in post-outbreak period, considering the random effect of plots nested in sites. Since the results of the first research question above indicated that non-standardized resin duct characteristics did not explain the growth-defense relationship well (see results and discussion for details), I used only the standardized resin duct characteristics as response variables in my models to investigate the growth-defense relationship as a function of percent lodgepole pine mortality. Ring width of each category of residual trees in post-outbreak period was examined individually as a function of the percent lodgepole pine mortality with DBH as a covariate and plots nested in sites as a random effect by three separate linear mixed models. Resin duct characteristics and ring width were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

3.2.2.3 Do changes in the density of living trees in a stand affect the relationship between growth and resin duct-based anatomical defenses of residual pine trees?

I used the density of all live trees (regardless of species) in a site to determine the forest density. Similar to the analyses described in the second research question (above), I used the standardized resin duct characteristics to represent the growth-defense relationship of three categories of residual trees. Because MPB outbreak altered the forest stand density of live trees, stand density of live trees, as a function of MPB outbreak, is auto-correlated with time periods (pre- and post-outbreak) of MPB outbreak, meaning that I had to take the auto-correlation account in my future data interpretation. Therefore, I tested the relationship between each standardized resin duct characteristic of each category of residual trees and the density of live trees in each outbreak period separately using twelve separate linear mixed models with plots nested in sites as a random effect. To test if there was any legacy effect on the growth-defense relationship of residual trees, I tested the post-outbreak standardized resin duct characteristics in response to the pre-outbreak stand density of live trees by six separate linear mixed models with plots nested in sites as a random effect. Ring width of each tree category in each period of outbreak was tested by six separate linear mixed models, where the density of live trees was the fixed effect, DBH was a covariate and plots nested in sites was a random effect. If the post-outbreak ring width was affected by pre-outbreak density of live trees was tested as well. If significant relationships were found between resin duct characteristics or ring width and the density of live trees in both pre- and post-outbreak periods, I tested the effect of the interactions between the density of live trees and outbreak periods on resin duct characteristics or ring width by linear mixed models with plots nested in sites as a random effect. Similar tests were performed to test if significant relationships were found between pre- and post-outbreak resin duct characteristics or ring width and the pre-outbreak density of live trees. Resin duct characteristics and ring width were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

3.3 Results

Overall, most of the non-standardized resin duct variables (resin duct production, resin duct size and total resin duct area) of residual trees were affected by ring width and outbreak period (Table 3.1), except that the resin duct size in healthy and declining trees did not change between pre- and post-outbreak periods. DBH as a covariate was only significant in the model that tested the relationship of total resin duct area with ring width of survived trees between pre- and post-outbreak periods. The interaction between ring width and outbreak period only significantly affected the non-standardized resin duct characteristics of survived trees: the strength of the positive relationship between growth and non-standardized resin duct characteristics of survived trees increased after MPB outbreak. In particular, all non-standardized resin duct variables showed a positive relationship with ring width in each of the three residual tree categories. Both resin duct production and total resin duct area of all residual trees increased from the pre-outbreak to the post-outbreak period, while only the resin duct size of the survived trees showed a significant increase during the post-outbreak period. The ring width of each category of residual trees did not change between the two periods. DBH as a covariate was not significant in models that compared ring width between the two outbreak periods. For the standardized resin duct characteristics, both resin duct density and relative resin duct area of residual trees increased from the pre- to post-outbreak periods (Table 3.1; Fig. 3.1).

The ring width was not affected by the percent lodgepole pine mortality for any categories of residual trees. DBH as a covariate was not significant in models that compared ring width with percent pine mortality. On the other hand, healthy trees were the only category of residual trees that had a higher resin duct density with the higher percent host tree mortality caused by MPB ($F=5.53$, $R^2=0.098$, $P=0.019$; Fig. 3.2). Both declining and survived trees did not show a significant relationship between resin duct characteristics and the percent host tree mortality.

The density of live trees influenced the ring width, resin duct density and relative resin duct area of residual trees, but the MPB outbreak altered this relationship. During the pre-outbreak period, the density of live trees showed a significant positive relationship with the relative resin duct area of both healthy and declining trees (Table 3.2). There was also a positive relationship between live stand density and resin duct density of the declining trees before outbreak. However, I did not find such relationships during the period following outbreak; the only exception was that the relationship of the stand density with the relative resin duct area of the declining trees was positive. Furthermore, the beta coefficient of the regression between standardized resin duct characteristics and the density of live trees after outbreak tended to decrease compared to pre-outbreak period. Survived trees did not show any relationships between standardized resin duct characteristics and the stand density during either the pre- or post-outbreak period. Survived trees were the only residual tree category that showed a significant negative relationship between the ring width and stand density during the period following outbreak (Table 3.2, Fig. 3.3a & b), while the ring width of healthy and declining trees did not show significant relationships with stand density in pre- and post-outbreak periods. DBH as a covariate was not significant in models testing the ring width as a function of stand density.

I also used pre-outbreak stand density to predict the post-outbreak standardized resin duct characteristics and ring width of each category of residual trees separately. My results showed a similar positive relationship of the relative resin duct area of healthy and declining trees with increasing stand density prior to outbreak (Fig. 3.4a & b). The relationship of pre-outbreak relative resin duct area and pre-outbreak stand density was not significantly different from the relationship of post-outbreak relative resin duct area and pre-outbreak stand density in healthy and declining trees. The post-outbreak ring width was not affected by pre-outbreak stand density in all categories of residual trees. DBH as a covariate was not significant in all models testing the ring width as a function of stand density.

3.4 Discussion

On the basis of changes in resin duct-based anatomical defenses and ring width, MPB outbreak altered the growth-defense relationship of residual lodgepole pine trees. Specifically, all residual trees had a higher resin duct density and relative resin duct area in the post-outbreak period, compared to the pre-outbreak period. Furthermore, percent pine mortality due to MPB outbreak and the density of live trees in pre-outbreak period were associated with changes in the growth-defense relationship of residual trees after outbreak. Growth and defense are two major carbon sinks in trees, and, thus, interactions between them are expected (Herms and Mattson 1992, Stamp 2003). Although studies have commonly reported how forest stand conditions are altered by MPB outbreaks (Teste et al. 2011, Griffin and Turner 2012, Hawkins et al. 2013, McIntosh and Macdonald 2013, Treu et al. 2014, Karst et al. 2015, Pec et al. 2017), the current study is the first to demonstrate that outbreaks can also have an effect on the growth-defense relationship of residual lodgepole pine trees.

Residual trees appeared to prioritize defense over growth during the first 10 years after MPB outbreak. Three aspects support this conclusion. First, I found no changes in the ring width of residual trees after outbreak but observed increases in non-standardized resin duct characteristics of residual trees, suggesting that residual trees might allocate more resources to produce anatomical structures in the first 10 years after outbreak. Second, it has been well documented in angiosperms that neighbouring non-attacked plants could respond to volatile organic compounds released by plants under attack (Baldwin and Schultz 1983, Engelberth et al. 2004), which may induce production of additional anatomical defenses on the neighbouring trees. However, further research is needed to confirm volatile communication between conifers. Finally, I found a positive relationship of anatomical defense structures of healthy residual trees with the percent lodgepole pine mortality in post-MPB outbreak stands, suggesting that these trees favored defense over growth particularly in stands with higher levels of tree mortality. This is the first study reporting a positive correlation between anatomical defenses and the level of tree mortality in pines after MPB outbreak. Such positive correlation could also be a result of MPB selection in stands, as beetles might have killed trees with lower level of anatomical defense during the outbreak. Neither declining nor survived trees showed such response to the percent host tree mortality. I suspect that the changes in anatomical defenses of these trees were a reflection of their history with biotic attacks (e.g., survived trees were recovering from earlier MPB attacks and declining trees were struggling to cope with attacks by other species of bark and woodboring beetles and pathogens), rather than the percent host tree mortality.

Stand density of live trees in post-outbreak stands similarly influenced the growth-defense relationship of residual trees, which favored defense over growth. However, the strength of associations declined in the first 10 years after outbreak compared to the pre-outbreak period. Interestingly, the growth-defense relationship after outbreak was significantly associated with the density of live trees before outbreak. In contrast, the ring width of survived trees declined as the post-outbreak density of live trees increased. One likely explanation is that overstory competition for resources in dense stands such as light, water, nutrients and growing space likely affects tree growth (Barnes et al. 1998, Fettig et al. 2007), but not photosynthesis rate. In this case, trees likely allocated resources preferentially for defense than for growth. This prediction is in agreement with the growth-differentiation balance hypothesis (Herms and Mattson 1992). Likewise, a recent study by Slack et al. (2017) also showed that competition among trees increased the total resin duct area in those trees. Furthermore, the stand conditions altered after outbreak might have also resulted in insufficient resources available for survived trees, which likely allocated resources overwhelmingly for both chemical (Erbilgin et al. 2017a) and anatomical defenses (e.g., the enlarged resin duct size observed in this study). In addition, as the stands recover over time, the density of all live trees (pine and non-pine) in post-outbreak stands is expected to increase. Considering the low natural regeneration of pines in post-outbreak stands in Alberta (McIntosh and Macdonald 2013), the species composition in these formerly pine-dominated stands will likely shift to spruce-pine/aspen-pine mixed stands in the future. It is unknown how these changes in species composition would affect the growth-defense relationship of residual pine trees.

The outcome of investigations on the growth-defense relationship can be influenced by the types of resin duct characteristics analyzed. In this study, I incorporated both non-standardized (e.g., resin duct production) and standardized (e.g., resin duct density) resin duct characteristics to explain the growth-defense relationship. Interestingly, the two types of characteristics showed different outcomes. I found that while the relationship between the non-standardized resin duct characteristics and ring width was not affected by outbreak, the relationship between standardized characteristics was significantly differed between pre- and post-outbreak periods in both declining and healthy trees. These results might be caused by the lack of changes on tree growth. In general, trees with larger growth rate are likely to have more or larger resin ducts (Kane and Kolb 2010; Ferrenberg et al. 2014; Hood and Sala 2015). As the area where resin ducts occur in the xylem, it is reasonable to expect that larger growth rate or basal area increment would likely provide more space to produce more or larger resin ducts. I did not observe significant increases in post-outbreak growth rate in any category of residual trees, suggesting that resin duct production, total resin duct area and resin duct size are likely to be limited by restricted growth rate of healthy and declining trees. On the other hand, the relationship between non-standardized resin duct characteristics and ring width was significant for only survived trees after outbreak, compared to pre-outbreak period. These findings are in agreement with the results of standardized resin ducts, which indicate that survived trees favored defense over growth after MPB outbreak. These responses of survived trees might be induced by MPB attacks.

Natural environments can introduce complexity beyond the conditions of my experiments. For example, I derived my results from dominant or co-dominant pine trees. However, the trees in other crown classes (such as supressed) may have yielded different results from what I have reported, due to differences between dominant/co-dominant and supressed trees in their ability to acquire resources (Rodríguez-García et al. 2014). Furthermore, although I did not observe growth release of residual trees in this study, the assessment was limited to aboveground tree radial growth. Along with tree defense, the other carbon sinks in trees, such as root growth and reproduction, may deplete carbon resources and caused no growth release on tree stem after beetle outbreak (Wiley and Helliker 2012). In addition, as forests are continuously shaped and changed by disturbances and succession, using stand density as a proxy of stand condition might cause confounding on tree ages and ecological characteristics of site (Barnes et al. 1998). Such interconnections also add difficulties to examine the growth-defense relationship of trees. Future studies should further category post-MPB stand conditions before examining the growth-defense relationship of residual trees in post-MPB stands.

In conclusion, I provide empirical evidence that growth-defense relationship in pines can be affected by insect outbreaks. Specifically, overstory residual pine trees favored defense over growth after outbreak and that residual trees had higher resin duct defenses after outbreak than before. In fact, healthy residual trees in stands with the higher level of pine mortality had higher anatomical defenses than those in stands with the relatively lower levels of pine mortality. Although the effects of stand conditions, such as stand density, were lowered by outbreak, the resin duct density and relative resin duct area of residual trees may increase as stand recovers over time. I expect that anatomical defenses of residual pine trees will likely remain at elevated levels, which may result in an increased population of MPB-resistant lodgepole pine phenotypes in mixed stands in the future.

Table 3.1 Relationship of non-standardized and standardized resin duct characteristics with the ring width of *Pinus contorta* var. *latifolia* trees, two *Dendroctonus ponderosae* outbreak periods, and their interactions, in three residual tree categories.

Parameters	Tree Categories	Non-standardized resin duct characteristics			Standardized resin duct characteristics	
		Resin duct production (no. yr^{-1})	Resin duct size (mm^2 yr^{-1})	Total resin duct area (mm^2 yr^{-1})	Resin duct density (no. mm^{-2} yr^{-1})	Relative resin duct area (% yr^{-1})
Ring width (mm yr^{-1})	Healthy	0.40 (**<0.001**)	0.48 (**<0.001**)	0.55 (**<0.001**)		
	Declining	0.40 (**<0.001**)	0.61 (**<0.001**)	0.50 (**<0.001**)		
	Survived	0.31 (**<0.001**)	0.41 (**<0.001**)	0.47 (**<0.001**)		
Time period (post-outbreak)	Healthy	0.12 (**<0.001**)	0.09 (0.090)	0.13 (**<0.001**)	0.11 (**<0.001**)	0.12 (**<0.001**)
	Declining	0.07 (**0.012**)	0.02 (0.555)	0.06 (**0.006**)	0.11 (**<0.001**)	0.10 (**0.002**)
	Survived	0.16 (**<0.001**)	0.11 (**0.038**)	0.15 (**<0.001**)	0.17 (**<0.001**)	0.18 (**<0.001**)
Ring width × Time period (post-outbreak)	Healthy	-0.08 (0.071)	0.19 (0.054)	0.03 (0.580)		
	Declining	-0.01 (0.911)	-0.02 (0.733)	0.06 (0.128)		
	Survived	0.17 (**0.002**)	0.28 (**0.007**)	0.27 (**<0.001**)		

Beta coefficients (P-values) from linear mixed models with P-values in bold are statistically significant ($P<0.05$). Increment cores were separated into two periods based on the start date of *D. ponderosae* outbreak: the pre-outbreak period refers to the period prior to the start date of outbreak, and the post-outbreak period refers to the period starting from the start date of outbreak until 2015 when the cores were taken. Diameter at breast height of trees were used as a covariate in the models that tested the relationship of ring width and non-standardized resin duct characteristics between two outbreak periods in each of the three categories of trees. Positive beta coefficients indicate higher value of the resin duct characteristics in post-outbreak than in pre-outbreak. Since ring width was used to calculate the standardized resin ducts characteristics, I did not conduct statistical analyses of ring width or its interaction with the outbreak periods on standardized resin duct characteristics. Resin duct size, total resin duct area, resin duct density and relative resin duct area were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

Table 3.2 Relationships of stand density of live trees (including pine and non-pine species) with ring width, standardized resin duct characteristics of *Pinus contorta* var. *latifolia* trees in pre- and post-*Dendroctonus ponderosae* outbreak periods.

Density of live trees (no. ha^{-1})	Tree category	Ring width (mm yr^{-1})	Resin duct density (no. mm^{-2} yr^{-1})	Relative resin duct area (% yr^{-1})
Pre-outbreak	Healthy	-0.02 (0.855)	0.23 (0.063)	0.28 (**0.019**)
	Declining	-0.13 (0.205)	0.34 (**0.010**)	0.34 (**0.008**)
	Survived	-0.19 (0.551)	0.15(0.207)	0.08 (0.510)
Post-outbreak	Healthy	-0.09 (0.514)	0.08 (0.529)	0.18 (0.173)
	Declining	-0.09 (0.164)	0.28 (0.063)	0.31 (**0.031**)
	Survived	-0.33 (**0.016**)	0.18 (0.129)	0.05 (0.685)

Beta coefficients (P-values) from linear mixed models were provided in this table. P-values in bold indicates significant relationships between stand density and annual radial growth rate/resin duct characteristics ($P<0.05$). Both resin duct characteristics and ring width were separated into two periods based on the start date of *D. ponderosae* outbreak: the pre-outbreak period refers to the period prior to the start date of outbreak, and the post-outbreak period refers to the period starting from the start date of outbreak until 2015 when the cores were taken. Diameter at breast height of trees were used as a covariate in the models that tested the relationship of ring width with the density of live trees. Both standardized resin duct characteristics and ring width were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

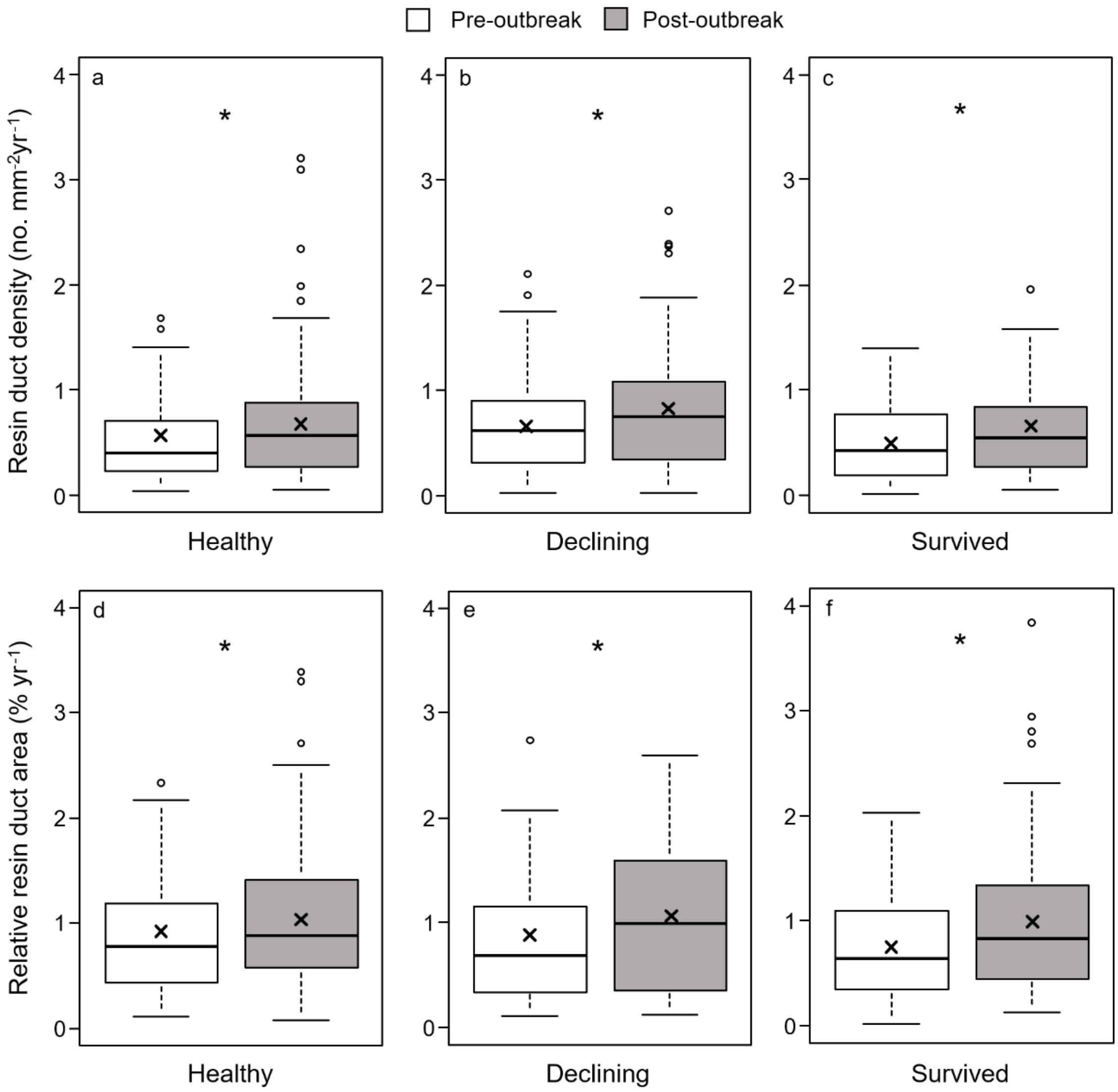


Figure 3.1 Resin duct density (a, b, c) and relative resin duct area (d, e, f) of three categories of residual *Pinus contorta* var. *latifolia* trees between the two periods (pre- and post-outbreak) of *Dendroctonus ponderosae* outbreak. Asterisks indicate significant differences between the two periods in each of the three tree categories as determined from linear mixed models ($\alpha = 0.05$). In each box plot, the thick line is the median, × mark indicates the mean, the box represents the 1st and 3rd quartiles, whiskers are Tukey's 1.5 interquartile range, and the open circles indicate outliers. Both resin duct density and relative resin duct area were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

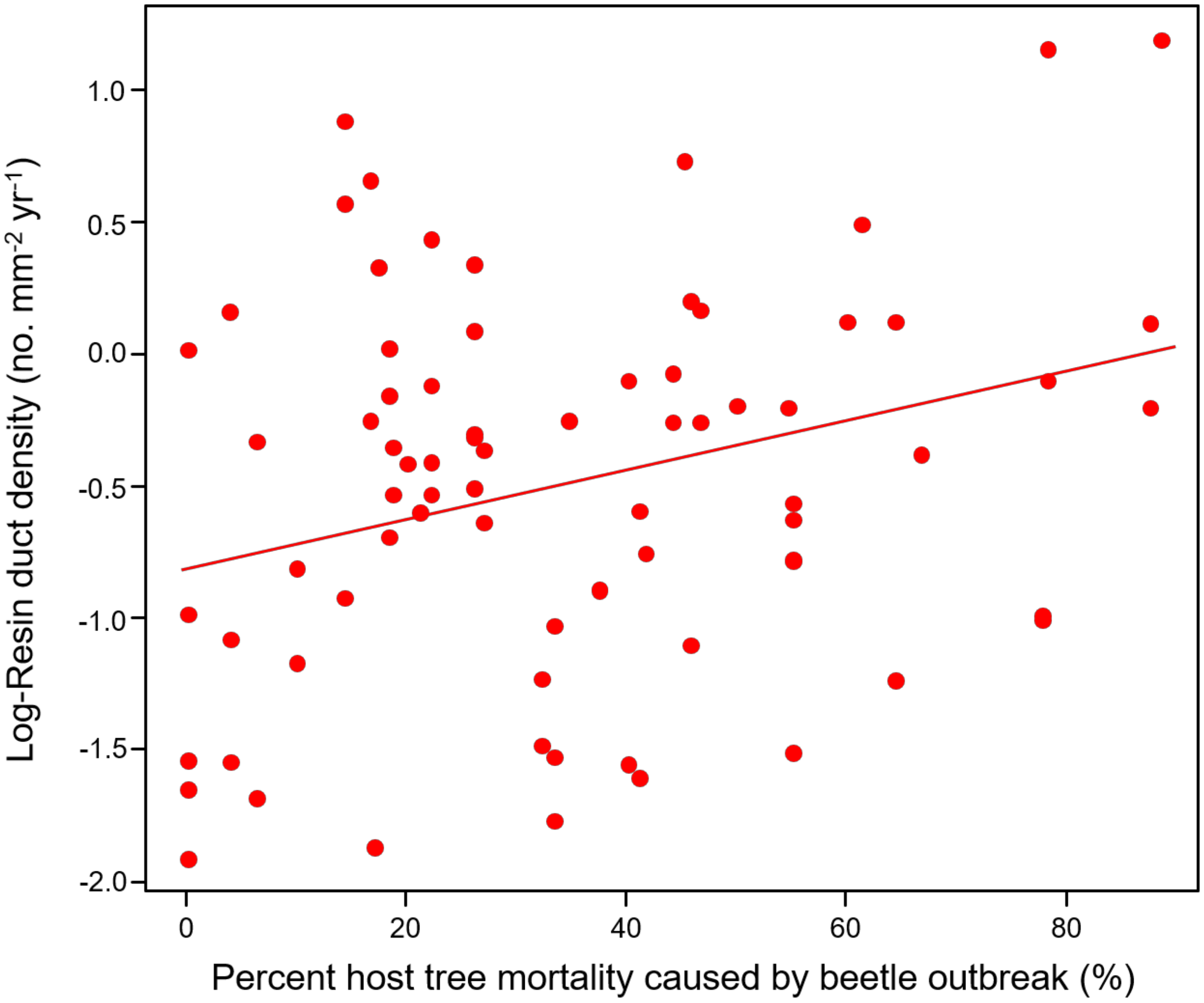


Figure 3.2 Relationship of resin duct density of healthy residual lodgepole pine (*Pinus contorta* var. *latifolia*) trees and percent lodgepole pine tree (host tree) morality out of total lodgepole pine trees after mountain pine beetle (MPB; *Dendroctonus ponderosae*) outbreak. Resin duct density was log-transformed to fit the normality and homogeneity of variance of model residuals.

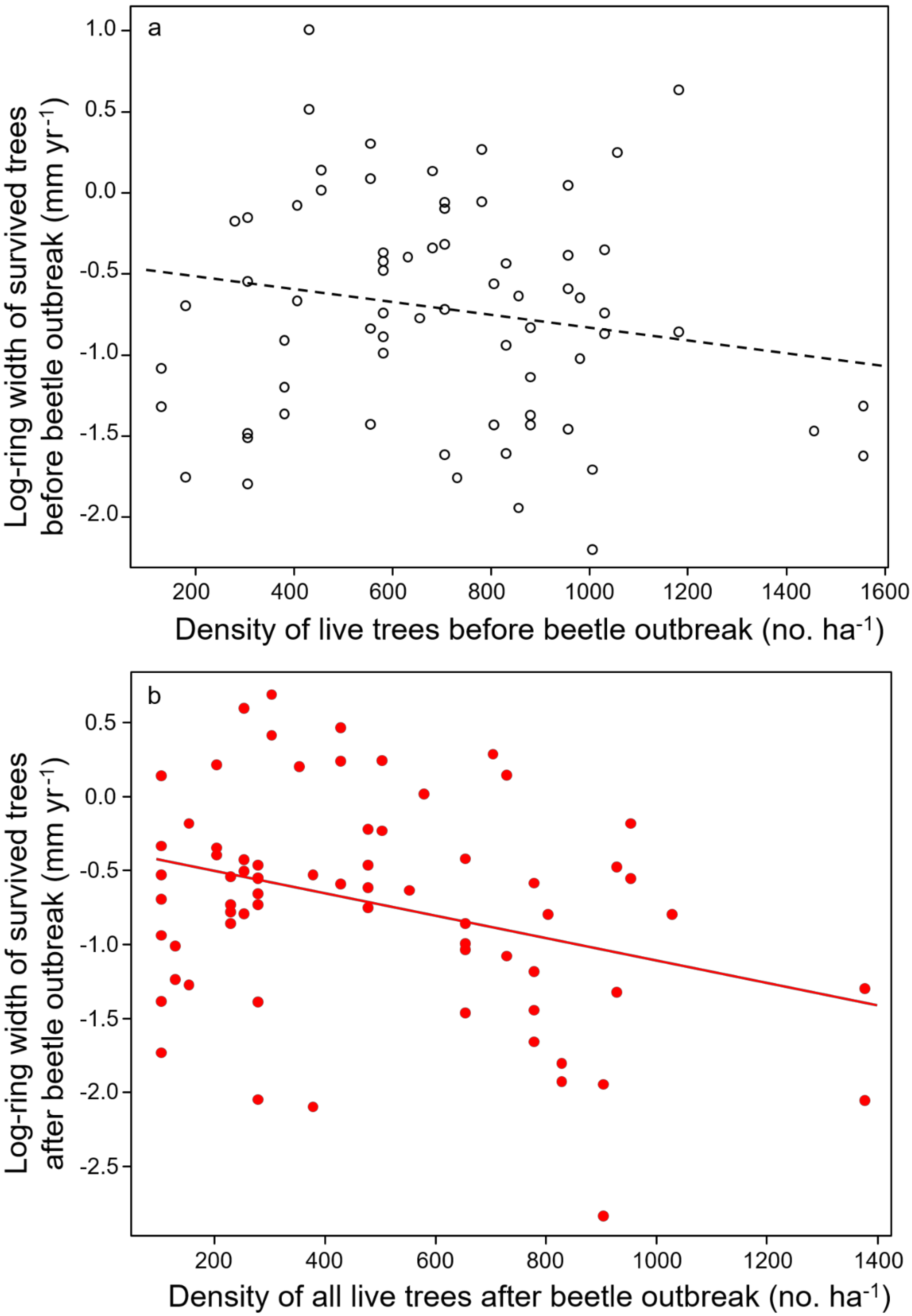

a
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Log-ring width of survived trees before beetle outbreak (mm yr^{-1})
200
400
600
800
1000
1200
1400
1600
Density of live trees before beetle outbreak (no. ha^{-1})
b
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Log-ring width of survived trees after beetle outbreak (mm yr^{-1})
200
400
600
800
1000
1200
1400
Density of all live trees after beetle outbreak (no. ha^{-1})

Figure 3.3 The relationship between ring width of survived *Pinus contorta* var. *latifolia* trees and stand density of live trees before *Dendroctonus ponderosae* outbreak (a) and after *D. ponderosae* outbreak (b). Survived trees showed significant relationship between ring width and stand density of live trees after *D. ponderosae* outbreak. The ring width of survived trees did not show significant relationship with density of live trees before *D. ponderosae* outbreak. Diameter at breast height of trees were used as a covariate in the models that tested the relationship of ring width with the density of live trees. Ring width was log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

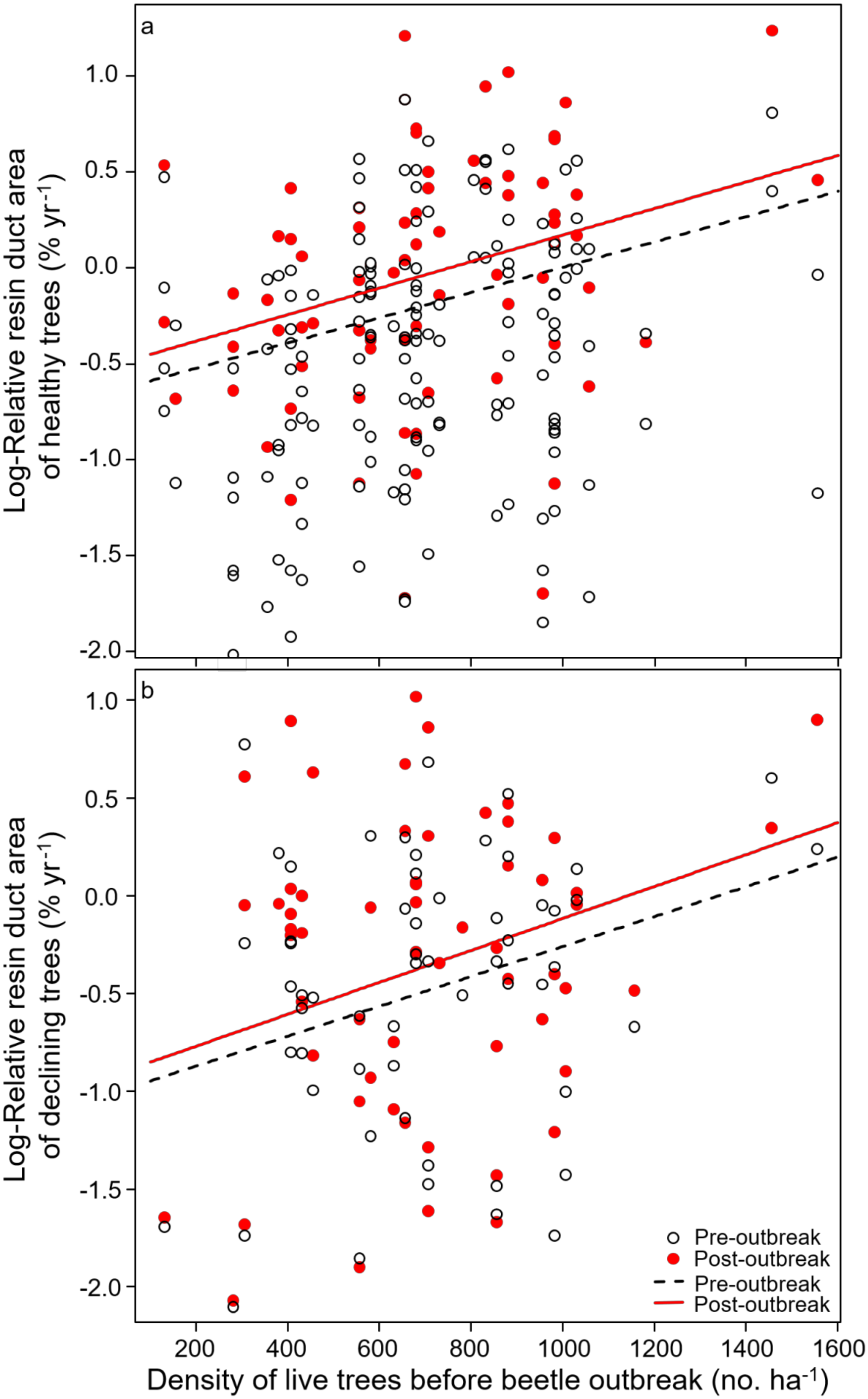

a
Log-Relative resin duct area of healthy trees (% yr^{-1})
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
b
Log-Relative resin duct area of declining trees (% yr^{-1})
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Pre-outbreak
Post-outbreak
Pre-outbreak
Post-outbreak
200
400
600
800
1000
1200
1400
1600
Density of live trees before beetle outbreak (no. ha^{-1})

Figure 3.4 Relationship between relative resin duct area of healthy (a) and declining (b) *Pinus contorta* var. *latifolia* trees during the mountain pine beetle (MPB; *Dendroctonus ponderosae*) pre- and post-outbreak periods and the density of all live trees before outbreak. Dashed lines and empty circles indicate the relative resin duct area in pre-outbreak period, whereas solid lines and solid circles indicate the relative resin duct area in post-outbreak period. Relative resin duct area was log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

Chapter 4. Mountain pine beetle outbreak did not promote the population growth of secondary bark and woodboring beetles

4.1 Introduction

The recent mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins) (Coleoptera: Curculionidae, Scolytinae) outbreak has expanded beyond its historical distribution in lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm. ex S. Watson) forests, and reached into higher elevations, northern latitudes, and eastern longitudes which were previously thought to be unsuitable for beetle survival (Cudmore et al. 2010, Cullingham et al. 2011, Erbilgin et al. 2014, Raffa et al. 2017). In 2006, MPB was reported to have crossed the eastern slope of Rocky Mountains and invaded the lodgepole pine forests outside of its historical range in west-central Alberta. This invasion killed a large number of mature lodgepole pine trees (Government of Alberta 2006-2017) and left a small number of overstory pine trees alive. These residual live overstory pine trees (residual trees, hereafter) have become a critical component for the recovery of pine forests in post-MPB stands. However, due to altered stand conditions (Hawkins et al. 2013, Cigan et al. 2015, Karst et al. 2015, Pec et al. 2017), the survival of these residual trees and their future role in restoring pine forests is not clear. In particular, it is unknown whether the invasion of MPB could promote the population growth of native bark and woodboring beetles and, thus, further threaten the residual trees in the post-outbreak stands.

Bark and woodboring beetle species are some of the most abundant and destructive species causing the mortality of lodgepole pine trees (Wood 1982, Amman and Safranyik 1985, Safranyik and Carroll 2006). Mountain pine beetle is considered as a ‘primary species’ due to its ability to kill healthy trees during periodic outbreaks (Lindgren and Raffa 2013). In contrast, other bark and woodboring beetle species are generally considered as ‘secondary beetles’ because they commonly colonize recently dead or dying trees and tend to increase their populations following outbreaks by the primary bark beetle species. One of the major concerns in post-outbreak stands is these secondary beetles that may use dead or dying trees as ‘sources’ to build-up their populations and then attack healthy pine trees in the same stands (Amman and Safranyik 1985, Safranyik et al. 2000, Safranyik and Carroll 2006), further exacerbating the recovery of pine forests after MPB outbreak.

However, there are a number of factors could influence the population growth of these insects. One possible factor is the suitability of host trees in post-outbreak stands. Studies have found a strong correlation between host plant resistance driven primarily by anatomical (i.e., resin ducts) and chemical (i.e., toxic resins) defenses and host colonization success of bark beetles (Boone et al. 2011, Hood and Sala 2015, Erbilgin et al. 2017a, Erbilgin 2019). However, since MPB outbreak had cascading effects on above and below ground conditions (Hawkins et al. 2013, Cigan et al. 2015, Karst et al. 2015, Pec et al. 2017), how these changes further affect the suitability of residual pine trees, particularly their responses to bark and woodboring beetle attacks are unknown. Likewise, intra- and inter-specific competition is another factor that could affect the population growth of bark and woodboring beetles. In fact, when the primary bark beetle species are at the endemic phase, their interactions with secondary beetles can range from competition to facilitation depending on the availability of suitable host trees (Rankin and

Borden 1991, Boone et al. 2008, Safranyik et al. 2010, Smith et al. 2011). Thus, these intra-and inter-specific interactions may further complicate predicting lodgepole pine forest recovery in post-outbreak stands. Finally, predation can also influence the population growth of bark and woodboring beetles. Among the top predators of secondary beetles are clerid beetles. By preying upon both immature and mature stages of bark and woodboring beetles, they can inflict density-dependent mortality (Reeve 1997, Turchin et al. 1999, Dodds et al. 2001, Erbilgin and Raffa 2002, Boone et al. 2008). All these factors could potentially make post-outbreak stands as 'sink' (stands/individual trees reduce bark beetle population) or 'source' (stands/individual trees promote bark beetle population) depending the outcome of these complex interspecies, tropic interactions.

In this study, I investigated whether MPB outbreak affected the population of secondary beetles in post-MPB stands in Alberta. Specifically, I was interested in determining whether 1) percent lodgepole pine mortality affected the abundance of bark and woodboring beetles and their predators and (2) health conditions of residual trees affected the abundance of bark and woodboring beetles and their predators. To test these two objectives at both the tree and stand levels, I set up three types of passive (non-baited) insect traps to monitor the populations of bark and woodboring beetles, and their predators in multiple stands. I expected that the abundance of bark beetles and woodboring beetles would increase with the percent pine mortality as there would be more hosts available in these stands. I also expected that the abundance of bark and woodboring beetles would be higher on stressed or declining residual trees, compared to healthy residual trees as both bark and woodboring beetles tended to colonize stressed or declining trees. Overall, the outcome of these investigations could help to understand the complex interaction among MPB, secondary beetles and their predators after MPB outbreak in novel habitats.

Information gained through these investigations could be relevant for the restoration of lodgepole pine forests in post-MPB stands in Alberta.

4.2 Materials and Methods

4.2.1 Description of study sites, insect traps and data collection

I selected 27 sites near City of Grande Prairie in western Alberta, Canada, elevation ranging from 600 m to 1,000 m in 2017. Based on the Alberta MPB aerial survey for the last 12 years, the last trees found killed in these sites was in 2011 (Government of Alberta 2011-2017). Therefore, the sites were subjected to at least seven years of post-outbreak stand conditions before this study commenced. I established a single plot (40 m×40 m in size) in each site unless the percent lodgepole pine mortality from MPB infestation was not uniform, in which case I set up two plots in the site to represent the variability in mortality. Overall, I had three sites that contained one plot and 24 sites that contained two plots. The plot centers within a site were at least 100 m apart. In all sites, mature lodgepole pine trees were the dominant tree species (≥ 50% of tree composition) and the percent pine mortality due to MPB ranged from 2% to 83%.

To determine MPB attacked trees, I examined the external symptoms of beetle attacks on tree stems, including successful beetle entrance holes (i.e., pitch tubes), brood emergence holes, and extensive beetle galleries under tree bark (Erbilgin et al. 2017a). In each plot, I defined all live overstory mature lodgepole pine trees bigger than 15 cm in diameter at breast height (1.4 m) as residual trees and grouped them in one of the three categories based on their apparent health conditions as healthy, declining, and survived. Healthy trees showed no obvious symptoms of any pathogen or insect attacks; declining trees had dying branches, sparse crowns, yellowish needles, bark lesions or other damage caused by pathogens and/or insects. I did not determine the causal agent(s) as there were usually more than one agent that could be responsible for the declining. Survived trees showed no declining symptoms but evidence of unsuccessful MPB colonization, including failed beetle reproduction, such as presence of short maternal galleries, absence of oviposition and larval galleries, and absence of beetle emergence holes (Erbilgin et al. 2017a).

To examine the abundance of bark and woodboring beetles in post-MPB stands, I set up three types of passive insect traps: landing, emergence and flight intercept traps, in 27 sites in 2017. I set up both landing and emergence traps on individual trees while hung flight intercept traps between trees on a pipe bracket about 1 m above the ground. I constructed landing traps from a 2 L transparent plastic soda bottle by removing the bottom and then cutting the bottle in half length-wise. I attached a transparent sheet on the bottle to increase the landing surface area and inverted the bottle and stapled to the trunk approximately 1.5 m above ground (Fig. 4.1). These traps typically catch insects when they land on trees. I constructed emergence traps by wrapping a 1 m x 1.5 m fine mesh cloth (mesh size <0.5 mm) with a 0.5 m long cloth funnel around the tree trunk between 1 m and 2 m in height. Emergence traps catch insects as they emerge from tree stems. For each landing and emergence traps, I attached a 105-mL plastic cup filled with 10 mL of 50% propylene glycol solution (diluted with water) to each trap so that I could preserve the specimens between each collection (Fig. 4.2). Flight intercept traps (Advanced Pheromone Technologies, Marylhurst, OR, USA) catch insects-in-flight in stands. I set up two flight intercept traps in each plot, which were placed 5 m away from the plot center in north or south direction (Fig. 4.3). I filled the collection cups attached to the bottom of the traps with 100 ml of 50% propylene glycol solution.

I selected 24 sites to place flight intercept traps to sample across the range of percent lodgepole pine mortality. Because the other three sites had similar levels of pine mortality, I did not set up any flight intercept traps in these sites. I randomly selected one of the two plots in each of 24 sites to place two flight intercept traps. Only in one site, I set up four flight intercept traps (two per plot) because one plot had 0% host tree mortality, while the other had 45%. I set up 50 flight intercept traps in total.

I set up landing and emergence traps in all 27 sites. I accepted a general rule that if the percent pine mortality varied by more than 25% between plots in each site, I set up traps in both plots to capture the variability of host mortality within a site. Otherwise, I set up landing and emergence traps in the same plot as with the flight intercept traps. Furthermore, when selecting trees to set up traps, I wanted to include all categories of residual trees, including healthy, declining, and survived in each plot and set up one trap (landing and emergence) per category. However, since plots showed high variation in terms of number of trees in each category of residual trees, I could not always set up three traps in each plot. In cases where plots were missing one or more categories of residual trees (i.e., some plots did not have residual trees in the survived category), I set up fewer numbers of traps. In total, I selected 20 trees for landing traps and another 20 trees for emergence traps in each category of residual trees.

I set up all traps before 15 May 2017 and monitored them until the end of August. I revisited traps and collected specimens from each trap at the end of June, July, and August and transported specimens back to the laboratory for further identification. I also re-assessed the health conditions of trees on which I set up landing and emergence traps at the end of August.

Due to their close association with MPB, I primarily focused on Coleoptera, particularly in the subfamily of Scolytinae (bark beetles). I identified all beetles to family level, Scolytinae to genera level, and *Dendroctonus* to species level. I placed specimens in one of the three functional groups: bark beetles, woodboring beetles, or their coleopteran predators (Evans et al. 2007, Kenis 2007, Knížek and Beaver 2007). I separated bark beetles and woodboring beetles because of their different feeding behaviors. The woodboring beetle group included individuals from the families Buprestidae and Cerambycidae. I did not include ambrosia beetles in data analysis due to low number of specimens in my collection (*Trypodendron*, n=1). I classified several coleopteran families as predators of bark and woodboring beetles: Cleridae, Histeridae, Nitulidae, Salpingidae, Staphylinidae, Tenebrionidae, Trogrosstidae (Kenis et al. 2007, Wegensteiner et al. 2015). I did not include Carabidae as the predatory beetles, due to its highly generalist habitat requirements.

4.2.2 Data analysis

All statistical analyses were performed in R (R Core Team 2018). I standardized insect catch per day of each trap and used these standardized values in the following statistical analyses. The number of specimens caught in August was very low (Total =2,254; Coleoptera=361; Curculionidae =17), therefore, I only reported the raw data (insect abundance) of August but removed the August data from further statistical analysis to answer the research questions. The remaining analyses were based on the specimens collected in June (Total =9,175; Coleoptera=3,066; Curculionidae=781) and July (Total =5,607; Coleoptera =1,587; Curculionidae =282).

To avoid the effect of temporal variation in abundance of coleopteran species during my sampling, I first compared the abundance matrix of coleopteran families between the two sampling months for each trap type separately using Permutational Multivariate Analysis of

Variance (PERMANOVA) (Anderson 2001), with plots nested in sites as a random structure using Bray-Curtis dissimilarity for distance calculation by R package *vegan* (Oksanen et al. 2011). This method assesses the significance by permutation, and thus can process non-normal multivariable dataset. PERMANOVA results indicted significant differences between June and July samplings for each trap type. However, since PERMANOVA is sensitive to sampling dispersion, I further performed multivariate homogeneity of group dispersions (PERMDISP) with Bray-Curtis dissimilarity on the same dataset. Since the PERMDISP did not detect any differences in group dispersion between sampling months, the results of PERMANOVA were reliable, therefore, I performed statistical analysis for each month separately hereafter.

To determine whether the percent pine mortality affected the abundance of coleopteran families and species caught in each of three functional groups (bark beetles, woodboring beetles and their predators) on the stand level, I standardized the abundance per family or per each functional group to account for differences in trap numbers within a plot for each trap type. I tested the relationship between the abundance matrix of coleopteran families and the percent pine mortality by performing PERMANOVA for each trap type with plots nested in sites as a random structure after PERMDISP confirmed homogeneity of group dispersions. Both PERMANOVA and PERMDISP were performed using Bray-Curtis dissimilarity for distance calculation. To test the relationship between the abundance of each of the three functional groups and the percent pine mortality for each trap type separately, I used generalized linear mixed model (GLM) (Fournier et al. 2012) with plots nested in sites as a random effect with R package *glmmADMB* (Skaug et al. 2016). However, since insect abundance was a count data and highly skewed towards ‘0’, I first used GLM with different error distributions, including Poisson, negative binomial, and zero-inflated Poisson, to fit each response variable. I then compared the

Akaike Information Criterion (AIC) (Akaike 1973) of the three models (Poisson, negative binomial, and zero-inflated Poisson) of each response variable. Since the GLM with Poisson distribution had the lowest AIC, I reported results from this model.

To investigate whether the health conditions (healthy, declining, or survived) of residual trees affected the abundance of coleopteran families and species caught in each functional group, I only used data from emergence and landing traps because both trap types provided tree-level data. I performed PERMANOVA to test the relationship between the abundance matrix of coleopteran families and tree categories for each trap type with plots nested in sites as a random structure after PERMDISP confirmed homogeneity of group dispersions. Both PERMANOVA and PERMDISP were performed using Bray-Curtis dissimilarity for distance calculation. To compare the abundance of each functional group among three categories of residual trees for each trap type, I performed GLM with Poisson error distribution with plots nested in sites as a random effect. Furthermore, to test the probability of occurrence of each functional group in each trap type, I used mixed effects logistic regression with categories of residual trees as a fixed effect and plots nested in sites as a random effect with R package *lme4* (Bates et al. 2015).

Since both landing and flight intercept traps caught insects from ambient environment (in contrast to emergence traps catching only insects emerging from trees), I further compared the effectiveness of these two trap types. However, since the surface areas of landing and flight intercept traps were different and such differences could potentially affected the total insect catches by each trap type, I standardized insect abundance from two trap types to the same surface area (surface areas of landing and flight intercept traps were 0.06 m^2 and 0.94 m^2 respectively) (Tabacaru 2015). After these standardizations, I first performed non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity to visualize the variation on abundance of coleopteran families between landing and flight intercept traps in June and July samplings separately (McCune and Grace 2002). The stress values were assessed by scree plots using R package *goeveg* (Goral and Schellenberg 2018). If the stress value was over 0.3, then the third axis was considered to add when performing NMDS (Zuur et al. 2007). I then compared the abundance from the two trap types using PERMANOVA with plots nested in sites as a random structure after PERMDISP confirmed homogeneity of group dispersions. Again, I used Bray-Curtis dissimilarity for distance calculation to perform PERMANOVA and PERMDISP. To compare the abundance of each of the three functional groups among landing and flight intercept traps, I performed GLM with Poisson error structure with plots nested in sites as a random effect, except for bark beetle abundance in June and woodboring beetle abundance in July. For these two groups, I used GLMs with negative binomial error distribution as the models having smaller AIC, compared to other error distributions. To determine the probability of occurrence of each functional group, I used mixed effects logistic regression with the trap types as a fixed effect, with plots nested in sites as a random effect.

4.3 Results

I collected over 30,000 specimens from 170 traps over 105 trapping days. Of these, over 10,000 specimens were Collembola and Arachnida which were not analyzed or discussed further in this study. For the remaining catches, I identified 17,036 specimens from 12 insect orders (Table 4.1). Among them, coleopteran beetles consisted of 46 families (Table 4.2).

Among beetles, 985 Scolytinae specimens were caught from the middle of May to the end of August; of these, I classified 870 specimens as bark beetles in the following genera, *Dendroctonus*, *Ips*, *Hylurgops*, *Hylastes*, *Hylurgopinus*, *Pityophthorus*, *Polygraphus* and

Pseudips. Among bark beetles, *Hylurgops* spp. were the most abundant, followed by *Ips* spp. and *Hylastes* spp. *Dendroctonus* spp. did not contribute more than 2% of the total bark beetle abundance. I identified three species of *Dendroctonus*: *D. ponderosae* Hopkins, *D. murrayanae* Hopkins, and *D. valens* LeConte. Among them, *D. murrayanae* was the most abundant species (8 out of 13 specimens) and only two *D. ponderosae* were caught. There were very few specimens from *Hylurgopinus*, *Pityophthorus*, *Polygraphus*, and *Pseudips* (Table 4.3). Among trap types, landing traps caught the most bark beetles (Table 4.3).

I caught 318 woodboring beetles; of which 79% and 21% belonged to Cerambycidae and Buprestidae families, respectively. Among trap types, flight intercept traps caught the most woodboring beetles (Table 4.4). Among the predators, Staphylinidae contributed 48% of predators, followed by Cleridae (21%). About 45% of clerid beetles emerged from trees, but overall flight intercept traps had the highest number of predators (Table 4.5).

Overall, neither the percent pine mortality nor the different categories of residual trees influenced the abundance of coleopteran families or functional groups from any trap types in June or July (Figs. 4.4 & 4.5) The occurrence of each functional group did not differ among different categories of residual trees. The health conditions of any of the trees used for trapping did not change at the end of the sampling season.

Landing and flight intercept traps shared 78% of coleopteran families caught in the summer of 2017. Curculionidae was one of the most abundant families and partially drove the differences between flight intercept and landing traps (Fig. 4.6). PERMANOVA revealed differences in abundance of families between landing and flight intercept traps in both June and July ($P_{June}<0.001$, $P_{July}<0.001$). However, since PERMDISP revealed differences in multivariate dispersion of abundance of families between landing and flight intercept traps in June

($P_{June}<0.001$, $P_{July}=0.612$; Fig. 4.7), the differences in abundance of families in June could only be partially explained by the trap types. Furthermore, after standardization, the abundance of bark beetles, woodboring beetles and their predators was significantly higher in landing traps than in flight intercept traps in both June and July, except for the woodboring beetle abundance in July which showed no significance between trap types (Fig. 4.8). However, flight intercept traps had a higher probability of catching predators in June and woodboring beetles in July, while landing traps had a higher probability of catching bark beetles in June (Fig. 4.9).

4.4 Discussion

Overall the low abundance of primary and secondary beetles in this study show that seven years after the last MPB outbreak in lodgepole pine stands, populations of MPB and the other bark and woodboring beetle species are not in epidemic phase, although the abundance of secondary beetles was much higher than that of MPB. In fact, no residual trees with landing or emergence traps died or changed its health condition (e.g. from healthy to survived) by the end of August. In addition, the percent lodgepole pine mortality did not correlate with the abundance of either bark beetles or woodboring beetles across 27 sites sampled. These results illustrate that stands with higher proportions of dead pine trees did not necessarily lead to the elevated populations of bark and woodboring beetles compared to the stands with relatively lower proportions of pine mortality. Likewise, different categories of residual trees did not influence the abundance of bark and woodboring beetles. I found bark beetles more often in landing traps than in flight intercept traps, suggesting that trees may provide additional visual and chemical cues for the attraction of beetles (Hughes 1973, 1974, Pureswaran and Borden 2003). Overall these results are in disagreement with some of the earlier studies, which reported increased populations of secondary beetles following MPB outbreaks in its historical range (e.g., Amman and Safranyik 1985, Wood et al. 1987, Safranyik and Carroll 2006). As an invasive species, the population dynamics of MPB in its expanded range may be different from its historical range, resulting in cascading effects on the populations of its competitors and predators (Sakai et al. 2001, Arim et al. 2016).

Through my results, four likely explanations can be provided to describe the low population of bark and woodboring beetles in post-MPB outbreak stands. First, bark beetles may have been limited by the low abundance of suitable trees for colonization in these stands. Although bark beetles tend to attack stressed or dying trees, they, and woodboring beetles in early larval stages, require moist phloem to complete their development within the inner bark or phloem during adult and larval stages (Reid 1963, Knížek and Beaver 2007). In Alberta, although dead trees are still standing, I often noticed that these trees were without attached barks or phloem, suggesting that they were not suitable for bark beetle colonization and development (Franceschi et al. 2000).

Second, in Chapter 3, I investigated the relationship between resin duct characteristics and growth of residual lodgepole trees in post-MPB stands and found that residual trees favored anatomical defenses over growth after outbreak (relative to the pre-outbreak period). In particular, residual trees had more resin ducts and these resin ducts occupied larger proportion area in tree xylem after outbreak. As anatomical defense structures, resin ducts are critical in both anatomical and chemical defenses of conifers and are responsible for producing, storing and translocating oleoresins (Franceschi et al. 2005). The resin duct characteristics found in the residual trees suggest that these trees could quickly accumulate and transport more volume of toxic resins to the site of beetle attacks (Schopmeyer et al. 1954, Franceschi et al. 2005, Mason et al. 2019), resulting in an increased probability of preventing further beetle colonization and successful establishment (Hood and Sala 2015, Erbilgin et al. 2017a, Erbilgin 2019).

Third, since both bark and woodboring beetles share some living space during their life cycles, interspecific competition between bark and woodboring beetles may be taken place under bark, which likely limited the population growth of both beetle groups (Safranyik et al. 1999, 2010, Boone et al. 2008). Bark beetles primarily feed and reproduce in tree phloem, while woodboring beetles start feeding on the phloem but continue chewing into tree xylem to complete their development. It has been reported that woodboring beetles compete with bark beetles for the same phloem resources and occasionally prey on the immature stages of bark beetles under bark (Safranyik et al. 1999, Klutsch et al. 2016). In addition, although different bark beetle species partition along tree stems (Lindgren and Raffa 2013), the overlapped territory between species usually leads to competition for limited food and space (Rankin and Borden 1991, Boone et al. 2008, Safranyik et al. 2010).

Finally, predators likely limited the population growth of bark and woodboring beetles. I found that about 45% of clerid predators emerged from residual trees, suggesting that they likely preyed upon subcortical insects. Clerid beetles such as *Thanasimus undatulus* are considered habitat specialists in that they feed on the fauna within trees colonized by bark and woodboring beetles (Erbilgin and Raffa 2001a, Boone et al. 2008). There is increasing evidence that coleopteran predators can have important effects on the population dynamics of bark beetles (Schroeder 1996, Turchin et al. 1991, 1999, Reeve 1997, 2011).

I found that landing traps caught a higher number of species from each functional group than flight intercept traps. Furthermore, landing traps had a higher probability of catching bark beetles than flight intercept traps while flight intercept traps had a higher probability of catching woodboring beetles and predators than landing traps. These results suggest that landing traps appear to be more effective at catching bark beetles than flight intercept traps. Differences in beetle attraction between these two trap types likely reflect the types of cues provided by each trap type. Many bark beetle species use both visual (i.e., tree stems) and chemical (i.e., volatile monoterpenes) cues to locate host trees (Hughes 1973, 1974, Borden 1989, Byers 1989, Miller et al. 1997, Pureswaran and Borden 2003). Since I attached the landing traps to trees, the additional visual and volatile cues available from these trees likely improved the attraction of bark beetles. In contrast, flight intercept traps provided only a short visual cue for beetles in-flight, which likely resulted in lower bark beetle attraction. While many bark and woodboring beetles are attracted to dying trees, woodboring beetles can also colonize recently dead trees (Evans et al. 2007). Therefore, the chemical cues emitted from these dead trees differ in composition and concentration of individual chemicals from those emitted from live trees (Guevara-Razo et al. 2019), which likely reduced woodboring beetle attraction to the landing traps. Predators commonly respond to bark beetle pheromones and host tree volatiles (Erbilgin and Raffa 2001b, Lindgren and Miller 2012). However, as the predatory functional group included several predatory beetle families, the diversity of attractive cues that these predators respond to may lower the overall probability landing on residual trees. Additional studies are needed to determine how host volatiles affect the attraction of bark and woodboring beetles and their predators in novel habitat of MPB.

Insect behaviors can affect insect abundance in terms of ecological service of insect groups. In this study, I separated coleopteran beetles into three groups based on their functions. Compared to bark and woodboring beetles which require habitat under tree bark, some predators may have several habitats other than tree bark as their development. For example, the larvae of

Enoclerus sphegeus prey bark beetles under tree bark, but pupae in the duff at the tree base in fall and emerge as adults in the following spring (Reid 1957). Therefore, using emergence traps to catch *E. sphegeus* in spring and summer would cause bias of predator abundance as adult *E. sphegeus* does not emerge from tree bark. Future study may avoid such bias by identifying insects to species and analysing based on species level, instead of using functional groups.

In conclusion, the accumulated dead and dying standing trees resulting from MPB outbreaks did not affect the population growth of bark beetles at least seven years since the last outbreak occurred in these stands. Furthermore, I found that low abundance of suitable host trees, elevated host tree resistance, competition within and between bark and woodboring beetle species, and predation might contribute to the low populations of secondary beetles. Additionally, landing traps appear to be more effective capturing bark beetles at low population levels than flight intercept traps. Presence of different health conditions of residual trees did not affect the preference by the secondary beetles, suggesting that post-outbreak stands did not promote bark and woodboring beetle populations after MPB outbreak. However, forest managers should continue monitoring these stands as populations of these insects may be elevated due to sudden disturbances, such as windthrown or fire.

Table 4.1 Main insect orders caught in landing, emergence, and flight intercept traps set up in *Pinus contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks in Alberta, Canada from the middle of May to the end of August in 2017. Orders were ranked based on the total insect abundance of each order.

Orders	Emergence Traps	Flight Intercept Traps	Landing Traps	**Total**
Diptera	539	2,644	2,791	**5,974**
Coleoptera	579	1,893	2,542	**5,014**
Hymenoptera	1,850	1,453	1,057	**4,360**
Hemiptera	131	250	322	**703**
Lepidoptera	26	278	93	**397**
Psocodea	57	34	306	**397**
Thysanoptera	15	18	102	**135**
Neuroptera	14	10	8	**32**
Orthoptera	0	13	0	**13**
Trichoptera	3	1	1	**5**
Ephemeroptera	0	2	1	**3**
Plecoptera	1	2	0	**3**
Total	**3,215**	**6,598**	**7,223**	**17,036**

Table 4.2 Coleopteran beetle families collected from landing, emergence, and flight intercept traps set up in *Pinus contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks in Alberta, Canada from the middle of May to the end of August in 2017. Families were ranked based on the total insect abundance of each family.

Family	Emergence Traps	Flight Intercept Traps	Landing Traps	**Total***
Latridiidae	184	361	1041	**1,586**
Curculionidae	95	180	793	**1,068**
Elateridae	38	210	183	**431**
Cerambycidae	17	176	59	**252**
Melandryidae	7	133	82	**222**
Staphylinidae	25	125	61	**211**
Stenotrachelidae	1	134	2	**137**
Leiodidae	0	94	8	**102**
Cleridae	41	12	39	**92**
Nitidulidae	15	58	16	**89**
Carabidae	36	31	19	**86**
Silphidae	0	78	0	**78**
Buprestidae	2	37	27	**66**
Corylophidae	21	8	33	**62**
Anobiidae	0	31	24	**55**
Bothrideridae	41	2	9	**52**
Cryptophagidae	7	22	21	**50**
Scraptiidae	1	30	13	**44**
Scirtidae	1	20	13	**34**
Ciidae	1	14	12	**27**
Chrysomelidae	3	13	10	**26**
Dermestidae	4	9	13	**26**
Tenebrionidae	2	6	8	**16**
Trogossitidae	0	14	1	**15**
Lycidae	0	11	3	**14**
Salpingidae	0	8	6	**14**
Ptiliidae	6	4	3	**13**
Cantharidae	2	4	5	**11**
Mordellidae	1	7	2	**10**
Sphindidae	0	8	2	**10**
Histeridae	2	3	3	**8**

Cucujidae	0	4	3	**7**
Scarabaeidae	0	3	4	**7**
Monotomidae	1	3	2	**6**
Coccinellidae	2	1	2	**5**
Anthicidae	0	0	3	**3**
Brentidae	0	0	2	**2**
Erotylidae	0	2	0	**2**
Eucnemidae	0	1	1	**2**
Lampyridae	1	1	0	**2**
Noteridae	0	2	0	**2**
Bostrichidae	0	1	0	**1**
Cerylonidae	0	1	0	**1**
Eucinetidae	0	1	0	**1**
Melyridae	0	0	1	**1**
Mycetophagidae	0	0	1	**1**
Synchroidae	1	0	0	**1**
Total	**558**	**1863**	**2530**	**4951**

*The larvae of Coleoptera were not included (63 larvae in total).

Table 4.3 Bark beetles in the subfamily Scolytinae (Coleoptera: Curculionidae) collected from landing, emergence, and flight intercept traps set up in *Pinus contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks in 2017 in Alberta, Canada. Genera and species were ranked based on the total insect abundance of each genus or species.

Trap Types	Bark Beetles	June	July	August	**Total**
Landing	*Ips* spp.	206	69	2	**277**
	Hylurgops spp.	229	18	0	**247**
	Hylastes spp.	61	70	0	**131**
	Dendroctonus murrayanae	5	1	0	**6**
	Pityophthorus spp.	3	0	0	**3**
	Dendroctonus valens	3	0	0	**3**
	Polygraphus sp.	0	1	0	**1**
	Pseudips sp.	1	0	0	**1**
	Dendroctonus ponderosae	0	0	0	**0**
	Hylurgopinus sp.	0	0	0	**0**
	Total	**508**	**159**	**2**	**669**
Emergence	*Hylastes* spp.	10	21	0	**31**
	Hylurgops spp.	21	3	0	**24**
	Ips spp.	11	1	2	**14**
	Dendroctonus ponderosae	0	2	0	**2**
	Dendroctonus murrayanae	0	0	0	**0**
	Dendroctonus valens	0	0	0	**0**
	Hylurgopinus spp.	0	0	0	**0**
	Pityophthorus spp.	0	0	0	**0**
	Polygraphus spp.	0	0	0	**0**
	Pseudips spp.	0	0	0	**0**
	Total	**42**	**27**	**2**	**71**
Flight Intercept	*Hylurgops* spp.	91	1	0	**92**
	Hylastes spp.	9	21	0	**30**
	Ips spp.	4	2	0	**6**
	Dendroctonus murrayanae	1	0	1	**2**
	Hylurgopinus sp.	1	0	0	**1**
	Polygraphus sp.	1	0	0	**1**
	Dendroctonus ponderosae	0	0	0	**0**
	Dendroctonus valens	0	0	0	**0**
	Pityophthorus spp.	0	0	0	**0**
	Pseudips spp.	0	0	0	**0**
	Total	**107**	**24**	**1**	**132**

Table 4.4 Coleopteran families of woodboring beetles collected from landing, emergence, and flight intercept traps set up in *Pinus contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks in 2017 in Alberta, Canada. Families were ranked based on the total insect abundance of each family.

Insect Traps	Woodboring Beetles	June	July	August	**Total**
Landing	Cerambycidae	31	26	2	**59**
	Buprestidae	18	8	1	**27**
	Total	**49**	**34**	**3**	**86**
Emergence	Cerambycidae	5	11	1	**17**
	Buprestidae	1	1	0	**2**
	Total	**6**	**12**	**1**	**19**
Flight Intercept	Cerambycidae	40	106	30	**176**
	Buprestidae	20	14	3	**37**
	Total	**60**	**120**	**33**	**213**

Table 4.5 Coleopteran families of predators collected from landing, emergence, and flight intercept traps set up in *Pinus contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks in 2017 in Alberta, Canada. Families were ranked based on the total insect abundance of each family.

Trap Types	Families	June	July	August	**Total**
Landing	Staphylinidae	26	27	8	**61**
	Cleridae	24	14	1	**39**
	Nitidulidae	8	7	1	**16**
	Tenebrionidae	6	1	1	**8**
	Salpingidae	3	1	2	**6**
	Histeridae	3	0	0	**3**
	Trogossitidae	1	0	0	**1**
	Total	**71**	**50**	**13**	**134**
Emergence	Cleridae	29	8	4	**41**
	Staphylinidae	6	12	7	**25**
	Nitidulidae	7	5	3	**15**
	Histeridae	1	1	0	**2**
	Tenebrionidae	0	2	0	**2**
	Salpingidae	0	0	0	**0**
	Trogossitidae	0	0	0	**0**
	Total	**43**	**28**	**14**	**85**
Flight Intercept	Staphylinidae	64	44	18	**126**
	Nitidulidae	31	22	5	**58**
	Trogossitidae	14	0	0	**14**
	Cleridae	8	3	1	**12**
	Salpingidae	8	0	0	**8**
	Tenebrionidae	6	0	0	**6**
	Histeridae	3	0	0	**3**
	Total	**134**	**69**	**24**	**227**

Figure 4.1. A non-baited landing trap on a residual *Pinus contorta* var. *latifolia* tree to trap bark beetles, woodboring beetles and their coleopteran predators in a *P. contorta* var. *latifolia* forest stand after *Dendroctonus ponderosae* outbreaks.

Figure 4.2. A non-baited emergence trap on a residual *Pinus contorta* var. *latifolia* tree to trap bark beetles, woodboring beetles and their coleopteran predators in a *P. contorta* var. *latifolia* forest stand after *Dendroctonus ponderosae* outbreaks.

Figure 4.3. A non-baited flight intercept trap set up in a *Pinus contorta* var. *latifolia* forest stand after *Dendroctonus ponderosae* outbreaks to trap bark beetles, woodboring beetles and their coleopteran predators.

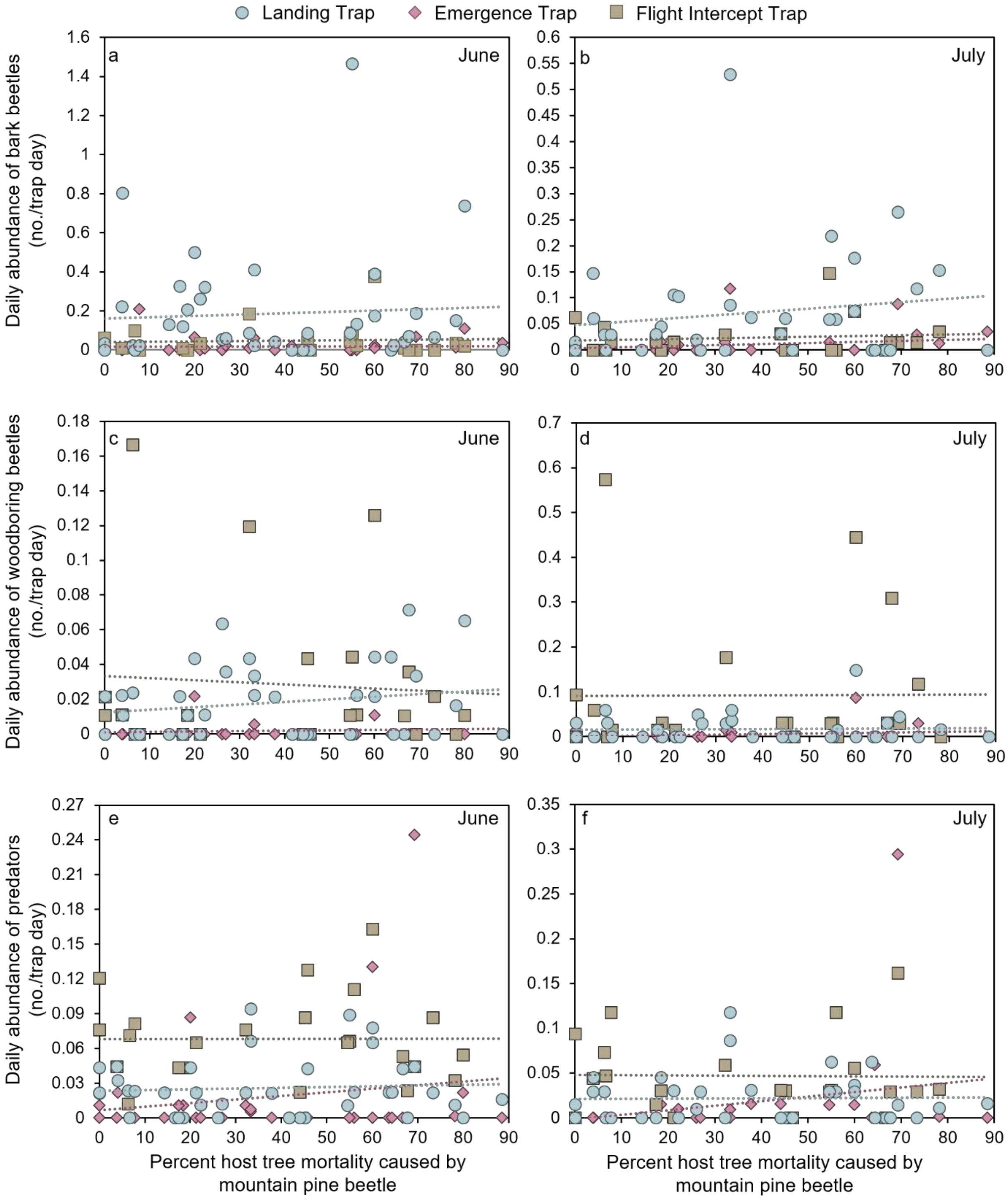


Figure 4.4 The relationship between the percent host tree (*Pinus contorta* var. *latifolia*) mortality due to *Dendroctonus ponderosae* outbreak and daily abundance of bark beetles in June (a) and July (b), woodboring beetles in June (c) and July (d), and predators in June (e) and July (f) in

each of the three types of insect traps. Blue circles represent beetle abundance from landing traps, pink diamonds represent beetle abundance from emergence traps, and brown squares represent beetle abundance from flight intercept traps. A breaking line indicates the tendency of the insignificant relationship between beetle abundance per trap per plot and percent host tree mortality. The color coding of breaking lines is the same as scattered dots.

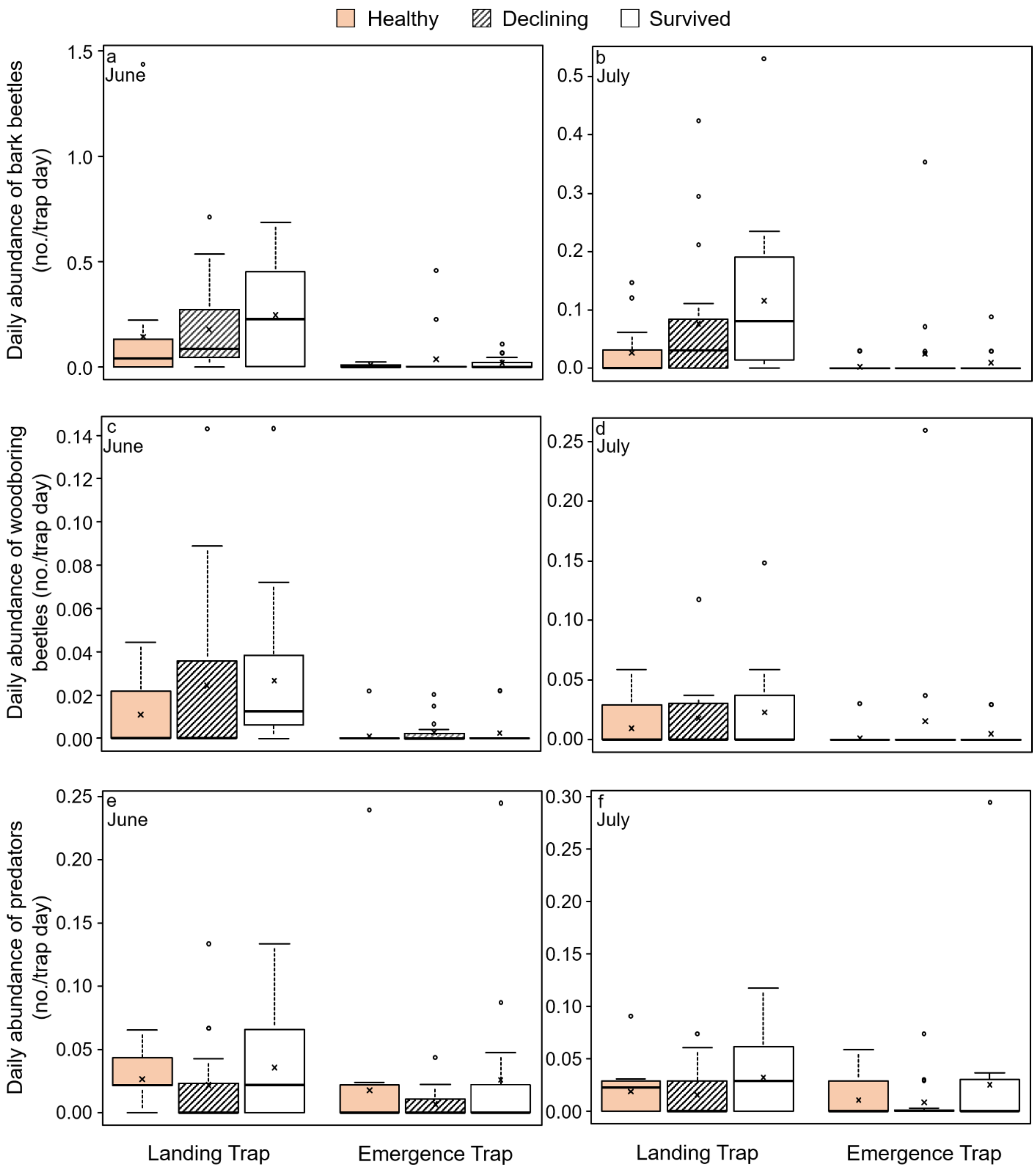


Figure 4.5 Daily abundance of bark beetles in June (a) and July (b), woodboring beetles in June (c) and July (d), and predators in June (e) and July (f) on healthy, declining and survived *Pinus contorta* var. *latifolia* trees from landing and emergence traps in *P. contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks. Bars with shadow represent insect abundance

caught from landing traps, while bars without shadow represent insect abundance caught from emergence traps. In each box plot, the heavy line is the median, the × mark indicates the mean, the box represents the 1st and 3rd quartiles, whiskers are Tukey's 1.5 interquartile range, and the open circles indicate outliers.

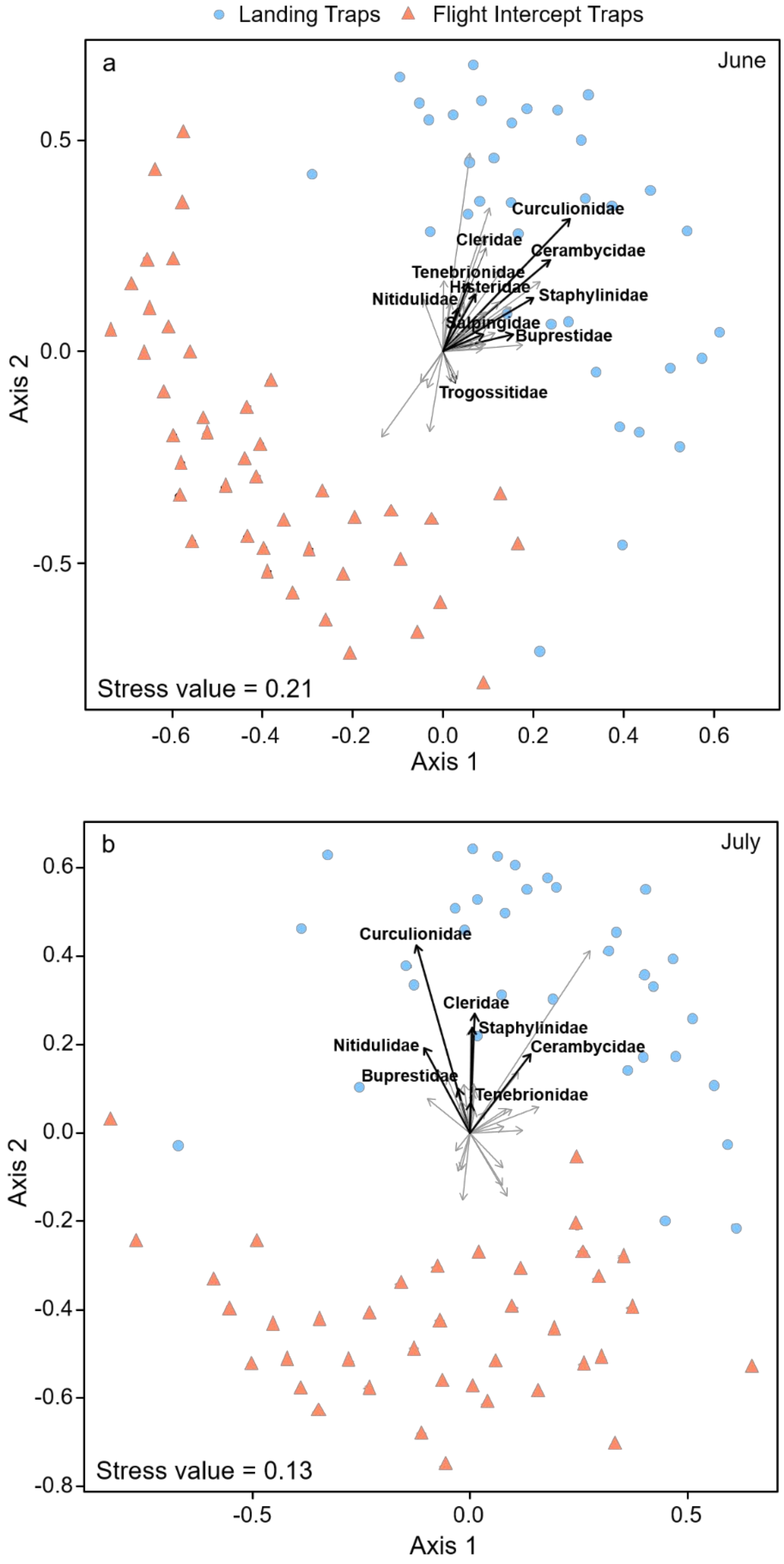
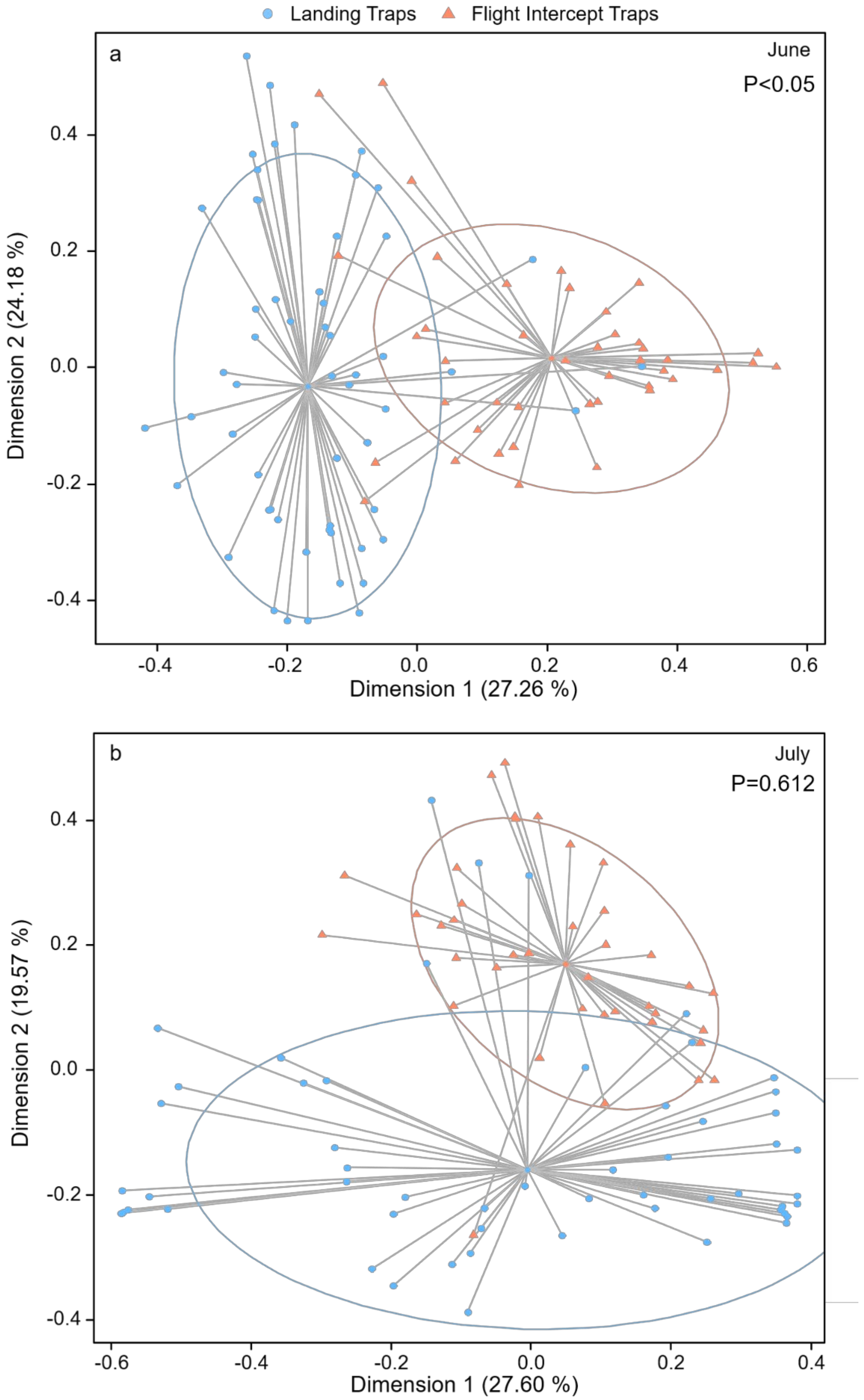

Landing Traps
Flight Intercept Traps
a
June
Curculionidae
Cleridae
Cerambycidae
Tenebrionidae
Histeridae
Nitidulidae
Staphylinidae
Salpingidae
Buprestidae
Trogossitidae
Stress value = 0.21
Axis 2
Axis 1
0.5
0.0
-0.5
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
b
July
Curculionidae
Cleridae
Staphylinidae
Nitidulidae
Cerambycidae
Buprestidae
Tenebrionidae
Stress value = 0.13
Axis 2
Axis 1
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-0.5
0.0
0.5

Figure 4.6 Results of an unconstrained ordination (nonmetric multidimensional scaling, NMDS) of coleopteran family abundance in June (a) and July (b) between landing and flight intercept traps in *Pinus contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks. Insect abundance was standardized to the same trap surface area. Relevant beetle families included in functional groups were labeled by their names and represented by black arrows. Bark beetles: Curculionidae. Woodboring beetles: Buprestidae and Cerambycidae. Predators: Cleridae, Histeridae, Nitidulidae, Salpingidae, Staphylinidae, Tenebrionidae, Trogosstidae. Gray arrows represent other coleopteran families which were not included in functional groups. Blue circle represents beetle abundance in landing traps, orange triangle represents beetle abundance in flight intercept traps.



● Landing Traps ▲ Flight Intercept Traps
a
June
P<0.05
Dimension 2 (24.18 %)
0.4
0.2
0.0
-0.2
-0.4
-0.4
-0.2
0.0
0.2
0.4
0.6
Dimension 1 (27.26 %)
b
July
P=0.612
Dimension 2 (19.57 %)
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
Dimension 1 (27.60 %)

Figure 4.7 Results of principal coordinate analysis (PCoA) of dispersions of coleopteran family abundance between landing and flight intercept traps in June (a) and July (b) in *Pinus contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks. Insect abundance was standardized to the same trap surface area. Blue circle represents beetle catches from landing traps, orange triangle represents beetle catches from flight intercept traps. P-values indicate significant differences of dispersions of beetle catches from traps. $P < 0.05$ indicates significantly different dispersions. Ellipses' area was calculated based on standard error which indicated 68% of data falling into the circled area.

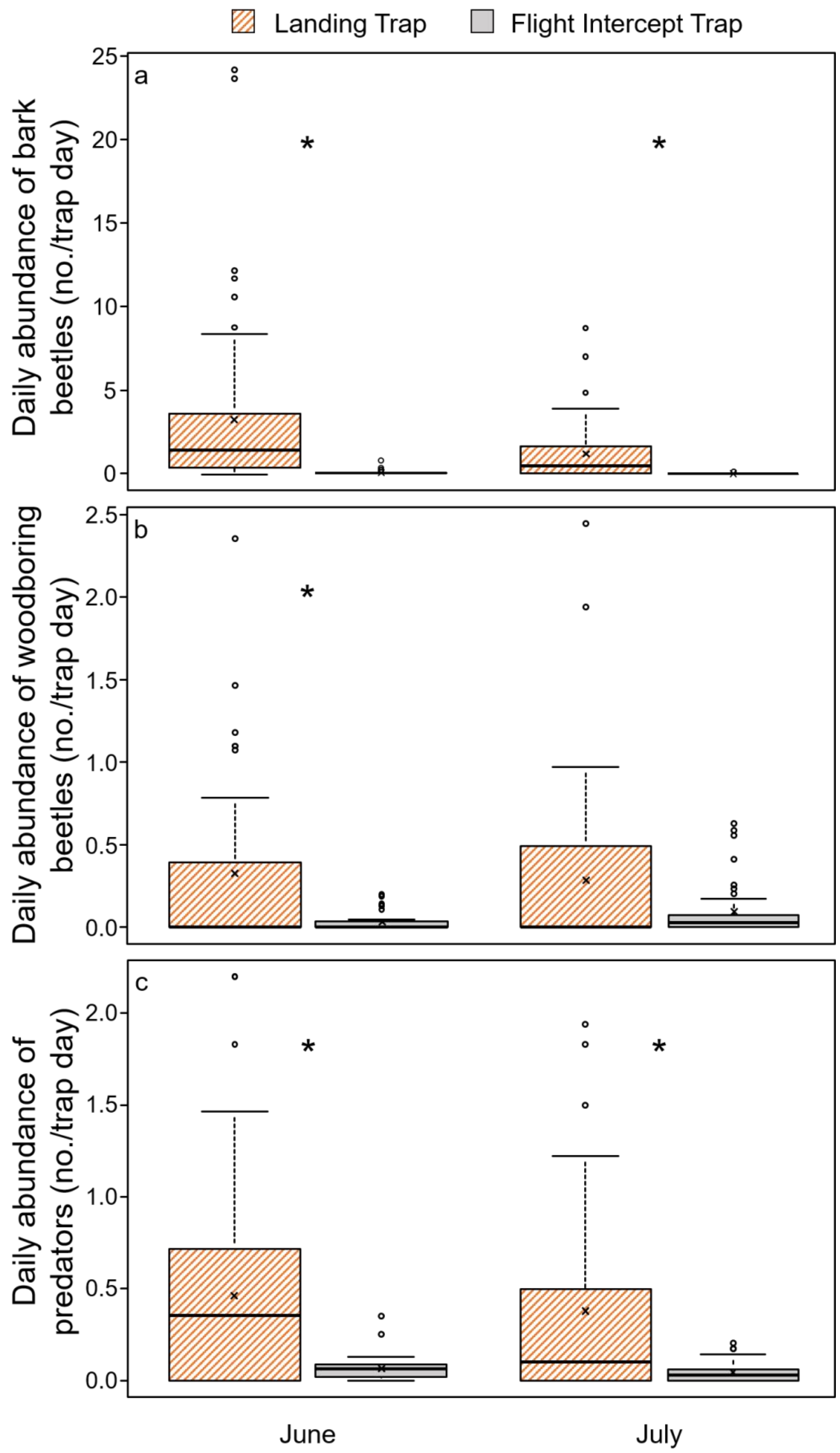

Landing Trap
Flight Intercept Trap
a
Daily abundance of bark beetles (no./trap day)
25
20
15
10
5
0
*
*
b
Daily abundance of woodboring beetles (no./trap day)
2.5
2.0
1.5
1.0
0.5
0.0
*
c
Daily abundance of predators (no./trap day)
2.0
1.5
1.0
0.5
0.0
*
*
June
July

Figure 4.8 Daily abundance of bark beetles (a), woodboring beetles (b), and their coleopteran predators (c) caught in June and July from landing and flight intercept traps in *Pinus contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks. Insect abundance was standardized to the same trap surface area. Bars with shades represent beetle abundance in landing traps, while gray bars represent beetle abundance in flight intercept traps. In each box plot, the thick line is the median, × mark indicates the mean, the box represents the 1st and 3rd quartiles, whiskers are Tukey's 1.5 interquartile range, and the open circles indicate outliers. Asterisk indicates significant differences between landing and flight intercept traps in catching each of the three functional beetle groups as determined from generalized linear mixed models ($\alpha = 0.05$).

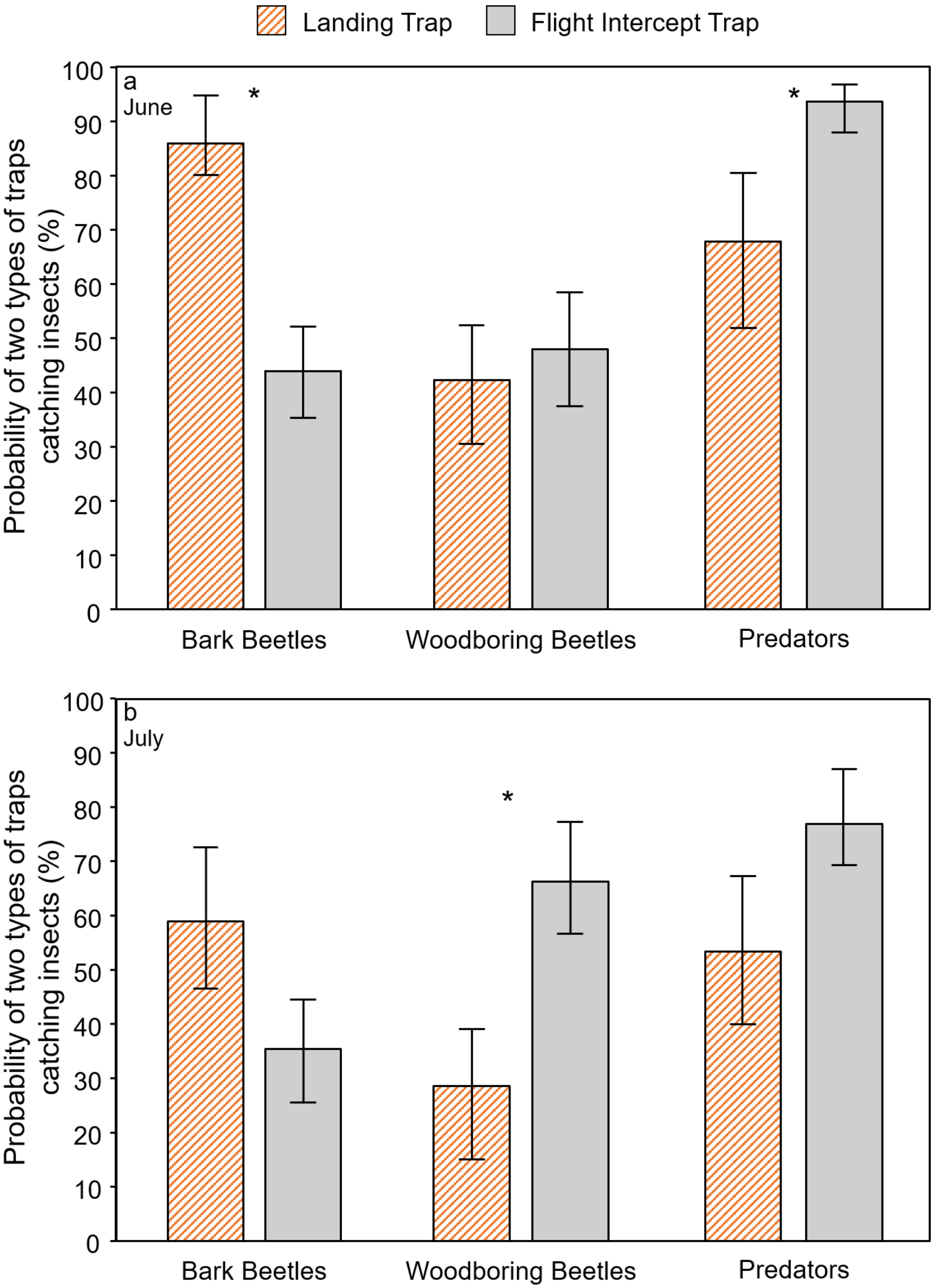

Landing Trap
Flight Intercept Trap
a
June
b
July
Probability of two types of traps catching insects (%)
0
10
20
30
40
50
60
70
80
90
100
*
Bark Beetles
Woodboring Beetles
Predators

Figure 4.9 Probability (±SE) of landing and flight intercept traps caught bark beetles, woodboring beetles and their coleopteran predators in June (a) and July (b) in *Pinus contorta* var. *latifolia* forest stands after *Dendroctonus ponderosae* outbreaks. Insect abundance was standardized to the same trap surface area. Bars with shades represent the probability of landing trap catching beetles, while gray bars represent the probability of flight intercept trap catching beetles. Asterisk indicates significant differences between landing and flight intercept traps in probability of catching each of three functional insect groups as determined from mixed effects logistic regression ($\alpha = 0.05$).

Chapter 5. Discussion

In this doctoral thesis, I used residual lodgepole pine trees in post-mountain pine beetle (MPB) stands to study growth and resin-duct based anatomical defenses and to determine the potential threats that these trees may face from growing populations of bark and woodboring beetles in post-MPB stands. This thesis yielded several important outcomes that would contribute to the current knowledge on the short- and long-term impacts of MPB outbreak on lodgepole pine forests in western Canada.

5.1 Resin duct-based anatomical defenses appear to contribute to the survival of lodgepole pine trees during MPB outbreak

I found that residual trees had fewer but larger resin ducts, compared to the MPB-killed trees, which had more but smaller resin ducts annually before MPB outbreak started. Furthermore, both healthy and survived trees had relatively larger resin ducts than declining trees. Larger resin ducts likely result in storage and biosynthesis of a higher volume of constitutive resin, thereby increasing resin accumulation within a tree (Schopmeyer et al. 1954, Hood and Sala 2015). Trees with larger resin ducts are more likely to hinder and cease on-going MPB colonization and thus can significantly improve the probability of their survival (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015). In contrast, higher resin duct production and total resin duct area measured in MPB-killed trees do not appear to be effective defense characteristics against MPB attacks because trees with such resin duct characteristics, ultimately killed by beetles.

Although I did not investigate the profiles of defense chemicals, mainly toxic terpenes, in residual trees, a study by Erbilgin et al. (2017a) reported that the lodgepole pine trees categorized as survived MPB attacks had lower concentration of MPB-attractive volatile terpenes, but higher concentration of toxic terpenes than those healthy trees. Balogh et al. (2018) also suggested that seedlings grown from cones of residual trees had higher concentration of terpenes compared to seedlings grown from cones of MPB-killed trees. These results suggest that MPB-killed trees might have higher concentrations of beetle-benefiting compounds and lower concentrations of toxic compounds (Erbilgin et al. 2017a, Balogh et al. 2018, Erbilgin 2019). Furthermore, although my results showed that resin duct size changed after MPB outbreak, these changes were within an expected (historical average) range of resin duct size in residual trees, suggesting that the size of resin ducts may be under strong genetic control in pines. These results are in agreement with a recent study which found that the genotypes of lodgepole pine trees that survived during high density beetle attacks could be separated from those lodgepole pine trees in stands that have never experienced MPB outbreaks (Six et al. 2018). Future studies should focus on the relationship between tree genetics and resin duct characteristics in lodgepole pine trees to understand the role of genetics in anatomical defense of pines. Nevertheless, the survival of trees with larger resin ducts suggests that bark beetle outbreaks likely drive selection for better-defended lodgepole pine phenotypes, at least in the naïve lodgepole pine forests.

5.2 Changes in stand conditions altered the growth and defense relationship of residual lodgepole pine trees

Growth and defense, as two major carbon sinks in trees, are expected to interact due to their reliance on the same photosynthetic products, carbohydrates (Herms and Mattson 1992, Stamp 2003). My results indicated that residual trees tended to prioritize resin duct-based anatomical defenses over growth after MPB outbreak. Specifically, I found that residual trees had higher resin duct density and larger relative resin duct area after MPB outbreak compared to the same characteristics before outbreak. In contrast, I did not observe any significant changes in radial growth rate after MPB outbreak, suggesting that the post-MPB stand conditions were unfavorable for tree growth so that residual trees likely favored defense over growth, which is consistent with the growth-differentiation balance hypothesis (Herms and Mattson 1992, Stamp 2003). Furthermore, resin duct density in healthy residual trees increased with the percent lodgepole pine mortality, but I failed to observe a similar relationship in the declining and survived trees. Actually, these results are expected because trees in the declining and survived categories have experienced continuous attacks from a number of biotic or abiotic agents, which might affect their ability to produce resin ducts.

In addition, residual trees favored defense over growth as the stand density (density of both pine and non-pine trees) increased before MPB outbreak, indicating possible effects of overstory competition on pine defenses (Slack et al. 2017). Interestingly, the resin duct density and relative resin duct area in residual trees were associated with stand density before outbreak. Nevertheless, the higher resin duct density and larger relative resin duct area of residual trees after outbreak, compared to before outbreak, suggests that residual trees in post-MPB stands are highly important for the continuing success of lodgepole pine trees in novel habitats because they appear to be more resistant to the future MPB outbreaks.

5.3 Secondary bark and woodboring beetles do not pose any threat to residual lodgepole pine trees in post-MPB stands

My results demonstrated that MPB population were below the outbreak population levels. Likewise, populations of secondary bark and woodboring beetles were not likely threatening the residual pine trees at least seven years after the last outbreak in these stands. In addition, populations of bark and woodboring beetles and their coleopteran predators were not associated with the level of lodgepole pine mortality caused by MPB. Furthermore, the health conditions of residual trees (healthy, declining, survived) did not affect host colonization preference by bark and woodboring beetles or the predators in post-MPB stands. These results indicate that residual pine trees have not become ‘source’ (stands/individual trees promote bark beetle population growth) in post-MPB stands. It is likely that the low abundance of suitable host trees (those that are stressed or have weakened defenses), competition within and between bark and woodboring beetle species, predation from natural enemies and elevated host tree resistance may have contributed to the low populations of bark and woodboring beetles in these stands (Schmid 1979, Safranyik et al. 1999, Boone et al. 2008, Six et al. 2018, Zhao et al. 2019). Overall, the low populations of MPB and secondary bark and woodboring beetles are not likely to be a potential threat to the residual trees in post-MPB stands.

5.4 Management implications

Through these studies, I found that residual lodgepole pine trees might be resistant to future MPB attacks and that the populations of MPB and secondary beetles seem to be below the population threshold required to kill live residual lodgepole pine trees (Safranyik and Carroll

2006). Therefore, leaving residual trees as potential seed sources, instead of salvage harvesting, would be critical for the establishment of lodgepole pine forests that are less suitable to MPB in western Alberta. However, caution must still be used due to the possibility of other disturbances such as drought, windthrow, or wildfire that may weaken residual trees for future bark beetle attacks. Perhaps collecting cones of residual trees especially from those in the survived and healthy categories to populate a progeny trial and screening program may provide a new direction for the provincial tree breeding programs to prevent or minimize the impact of MPB outbreaks in Alberta. I also suggest continually monitoring the health conditions of residual trees and setting up non-baited insect traps in these stands. These management actions may be important to prevent residual trees from becoming ‘sources’, for future bark and woodboring beetle populations.

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Appendix

App. Table 1 Percent mortality of *Pinus contorta* var. *latifolia* trees caused by *Dendroctonus ponderosae* in selected sites in western Alberta, Canada.

Site No.	Density of beetle-killed pine trees (ha)	Mortality of pine trees (%)	Diameter of Trees at Breast Height (cm) Mean (SE)			
			Beetle-killed	**Healthy**	**Declining**	**Survived**
1	600	82.76	28.74 (0.70)	NA	22.90 (1.92)	26.33 (1.03)
2	287.5	76.67	30.27 (0.89)	29.70 (0.00)	27.40 (1.46)	23.80 (0.07)
3	162.5	68.42	24.30 (2.26)	38.10 (2.76)	NA	23.05 (1.32)
4	162.5	68.42	33.40 (2.33)	43.60 (0.00)	NA	30.22 (2.60)
5	212.5	65.38	22.45 (0.89)	23.68 (1.54)	NA	NA
6	312.5	60.98	31.96 (1.02)	21.70 (0.00)	23.78 (1.25)	27.59 (1.86)
7	312.5	60.98	23.55 (0.55)	19.62 (1.19)	20.70 (1.72)	20.00 (0.00)
8	312.5	59.52	21.87 (0.74)	20.26 (0.44)	19.46 (0.84)	17.90 (1.91)
9	162.5	54.17	26.60 (1.33)	NA	28.40 (0.00)	26.60 (1.21)
10	362.5	50.88	22.48 (0.69)	19.45 (1.16)	19.24 (0.63)	18.75 (0.91)
11	300	50.00	19.20 (0.63)	18.70 (0.00)	17.50 (0.76)	17.91 (0.76)
12	87.5	46.67	24.63 (2.62)	22.30 (0.00)	NA	24.93 (1.59)
13	87.5	46.67	26.71 (1.97)	NA	NA	25.83 (1.23)
14	125	45.45	36.18 (1.82)	26.15 (0.81)	23.41 (1.47)	35.93 (3.40)
15	400	43.24	20.66 (0.73)	20.02 (0.67)	18.97 (0.50)	NA
16	237.5	43.18	20.19 (0.88)	18.31 (0.89)	17.65 (0.43)	19.15 (1.52)
17	250	41.67	20.52 (0.74)	16.25 (0.81)	19.15 (0.89)	18.65 (0.50)
18	150	41.38	30.54 (1.53)	32.00 (0.00)	26.80 (4.74)	25.94 (1.29)
19	200	39.02	17.79 (0.52)	18.27 (0.84)	17.20 (0.46)	15.90 (0.00)
20	175	25.93	22.85 (1.23)	21.49 (0.52)	21.68 (1.12)	22.41 (1.32)
21	75	24.00	34.93 (2.62)	20.30 (1.91)	32.44 (3.08)	25.07 (1.08)
22	100	19.05	19.81 (0.90)	19.21 (0.64)	19.37 (0.62)	20.50 (1.56)
23	137.5	16.67	23.62 (1.78)	22.55 (0.66)	20.04 (0.53)	25.61 (1.52)
24	100	16.33	25.51 (1.00)	23.30 (1.35)	21.19 (0.53)	NA
25	87.5	14.29	27.11 (1.80)	23.69 (0.92)	23.32 (0.88)	25.50 (1.56)
26	25	14.29	40.90 (2.26)	34.60 (0.00)	18.00 (0.00)	27.20 (4.31)
27	100	13.79	22.33 (1.10)	21.26 (1.77)	20.11 (0.73)	21.67 (0.69)
28	25	12.50	32.05 (6.54)	34.25 (4.12)	42.20 (0.00)	36.38 (1.94)
29	50	11.76	30.38 (1.73)	28.07 (2.76)	24.42 (0.87)	26.27 (1.91)
30	12.5	6.67	16.50 (0.00)	20.03 (1.49)	18.05 (1.26)	24.65 (1.59)
31	12.5	2.22	16.40 (0.00)	17.20 (0.43)	17.78 (0.57)	18.43 (1.44)

Density of *D. ponderosae*-killed trees (ha) was standardized by the density of killed trees in each site. Percent mortality of trees was calculated based on the density of *D. ponderosae*-killed trees and the density of all *P. contorta* var. *latifolia* trees in each site. Healthy trees are *P. contorta* var. *latifolia* trees showing no external symptoms of any pathogen or insect attacks; declining trees are *P. contorta* var. *latifolia* trees showing presence of insect, other than *D. ponderosae*, and pathogen attacks, showing dying branches, bark lesions, sparse crown, yellow-red needles; survived trees are *P. contorta* var. *latifolia* trees showed symptoms of unsuccessful *D. ponderosae* attacks but appeared healthy.

App. Table 2 Stand age, density and percent basal area of each species of mature overstory trees presented in selected sites in western Alberta, Canada.

Site No.	Stand Age	***Pinus contorta* var. *latifolia***		***Picea glauca***		***Populus tremuloides***		***Abies balsamea***		***Betula papyrifera***		***Picea mariana***		***Populus balsamifera***	
		Density	**Basal area (%)**	**Density**	**Basal area (%)**	**Density**	**Basal area (%)**	**Density**	**Basal area (%)**	**Density**	**Basal area (%)**	**Density**	**Basal area (%)**	**Density**	**Basal area (%)**
1	103	65	99.10%							1	0.90				
2	113	43	78.91%	7	14.47	2	4.17	3	2.44						
3	102	19	86.01%	5	13.99										
4	101	19	64.03%	11	14.84	4	21.13								
5	75	29	79.80%	9	13.47	3	6.73								
6	80	42	75.45%			7	21.56			3	2.99				
7	74	43	68.71%	9	10.23	17	18.44	3	2.62						
8	49	43	64.24%	2	1.98	25	32.58	1	1.21						
9	101	24	79.59%	6	20.41										
10	59	62	100.00%												
11	64	53	98.53%	1	1.47										
12	40	15	62.46%	14	36.04	1	1.49								
13	110	15	97.94%	1	2.06										
14	88	22	61.26%	25	38.07	1	0.66								
15	57	74	93.21%	4	4.99	1	1.04					1	0.77		
16	66	45	67.44%	5	5.64	11	21.97	1	1.18	1	1.36			1	2.41
17	54	50	93.92%			1	6.08								
18	97	30	55.20%	11	24.87	5	18.94								
19	60	43	93.93%	3	6.07										
20	125	66	68.16%	30	22.18	9	9.66								
21	110	29	63.31%	2	3.25	19	33.44								
22	62	42	98.61%							1	1.39				

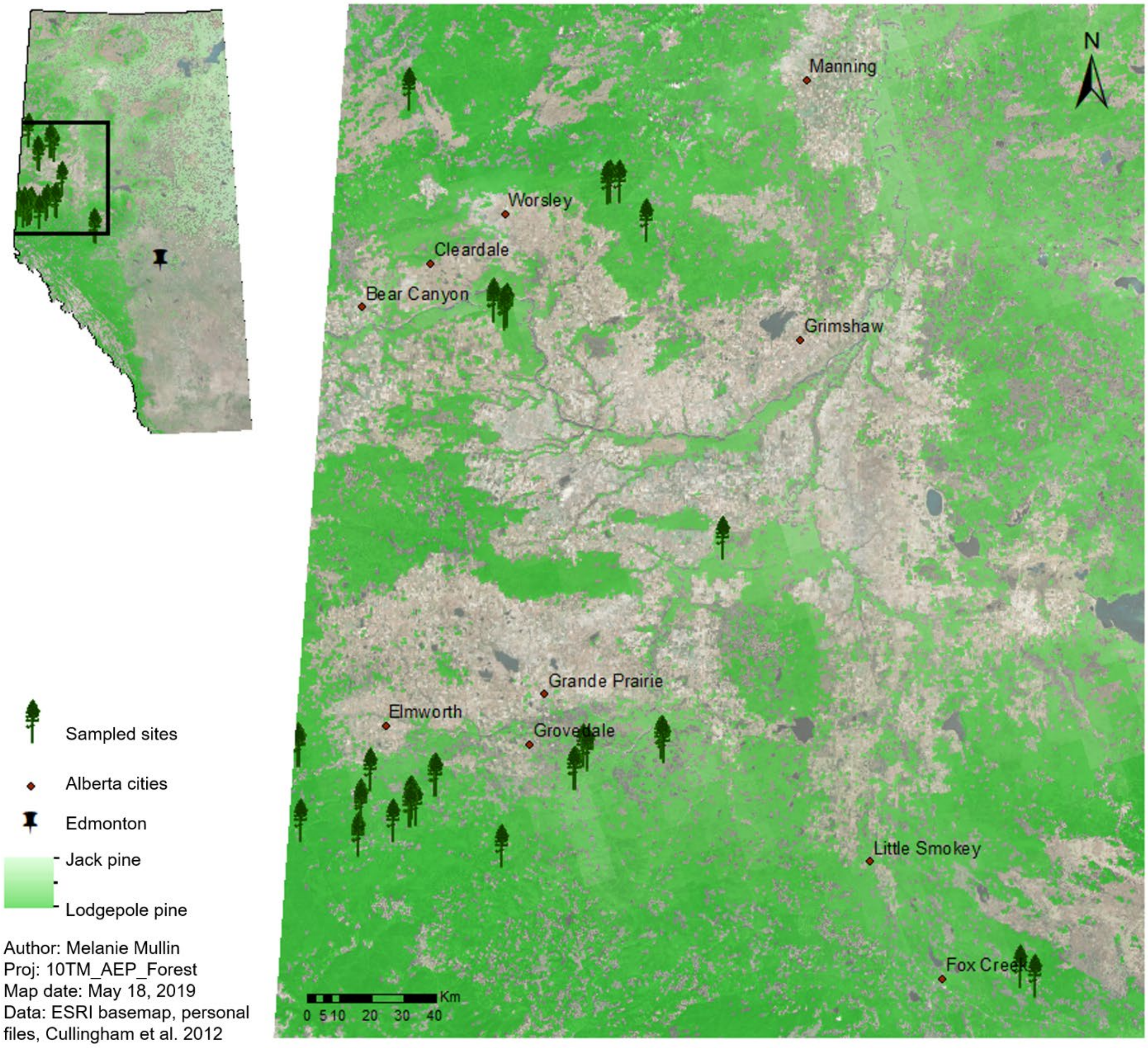
23	130	76	91.58%	7	7.64	1	0.78				
24	64	51	76.88%	5	5.59	8	15.36			2	1.50
25	109	56	86.76%	11	13.24						
26	45	14	70.03%	13	29.97						
27	123	61	68.79%	31	25.33	6	5.88				
28	100	16	55.32%	6	25.32	7	19.37				
29	100	35	66.29%	33	33.71						
30	NA	15	84.86%	4	15.14						
31	57	45	93.34%	3	6.66						

Stand age was determined by increment cores taken from *Pinus contorta* var. *latifolia* trees. Stand age of site No.30 was missing because increment cores taken in site No.30 were either damaged or not sampled to tree pith. Density of each species of trees includes only mature overstory trees having diameter at breast height over 15cm. Basal area (%) was calculated based on the basal area of each species of trees having diameter at breast height over 15cm and the total basal area of all trees having diameter at breast height over 15cm.

App. Table 3 Comparison of mean resin duct characteristics between residual *Pinus contorta var. latifolia* trees in the total post-*Dendroctonus ponderosae* outbreak growth period (from outbreak started year to sampled year [2015]) and the *D. ponderosae*-killed trees in the 10-year before tree death in post-*D. ponderosae* outbreak stands in Alberta.

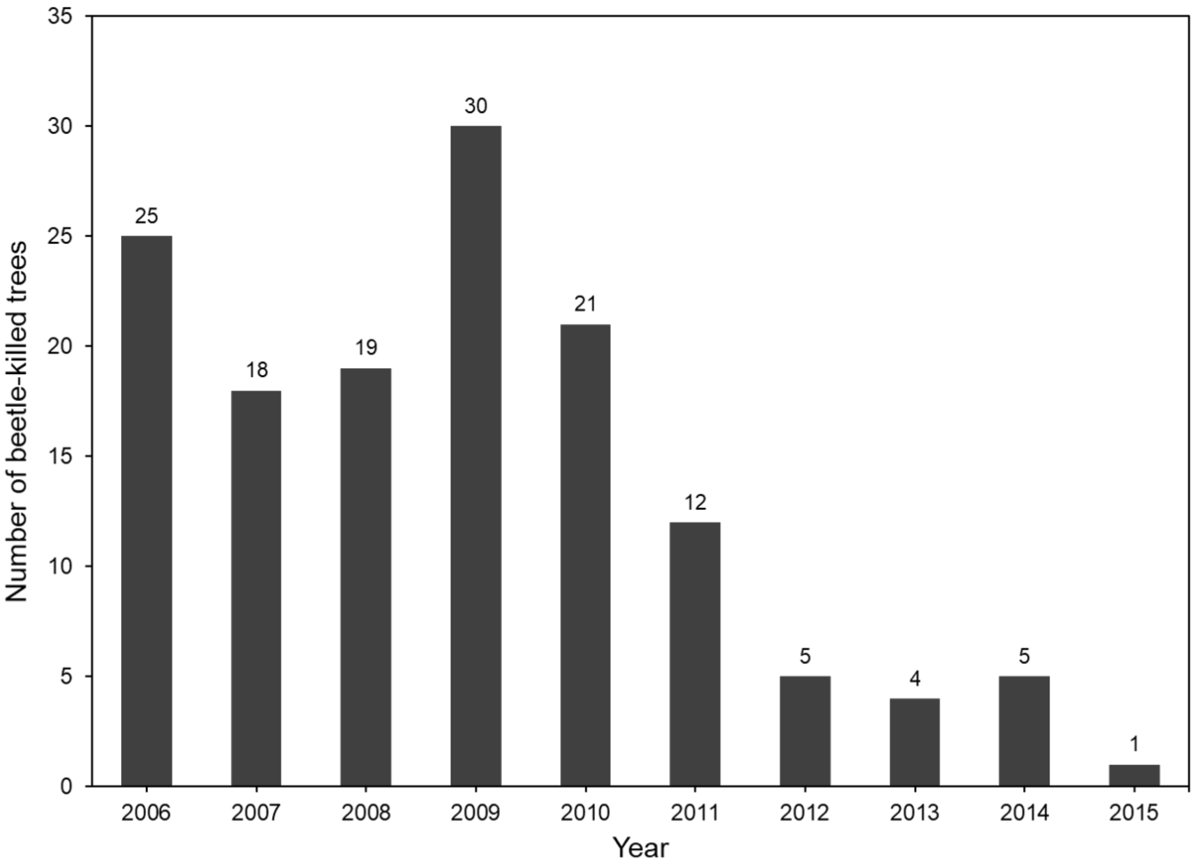
Variables	Median (25%, 75% quartile)		F-value	P
	Residual trees	Killed trees		
Resin duct production (no. yr^{-1})	2.000 (1.300, 2.900)	3.850 (2.875, 5.250)	125.58	<0.001
Resin duct density (no. mm^{-2} yr^{-1})	0.588 (0.275, 0.943)	0.865 (0.577, 1.330)	45.148	<0.001
Relative resin duct area (% yr^{-1})	0.878 (0.446, 1.383)	1.020 (0.756, 1.469)	17.454	<0.001
Total resin duct area (mm^2 yr^{-1})	0.035 (0.020, 0.054)	0.047 (0.033, 0.067)	14.331	<0.001
Resin duct size (mm^2 yr^{-1})	0.014 (0.011, 0.018)	0.012 (0.011, 0.013)	45.123	<0.001

Resin duct characteristics were log-transformed to meet the assumptions of the normality and homogeneity of variance of model residuals.

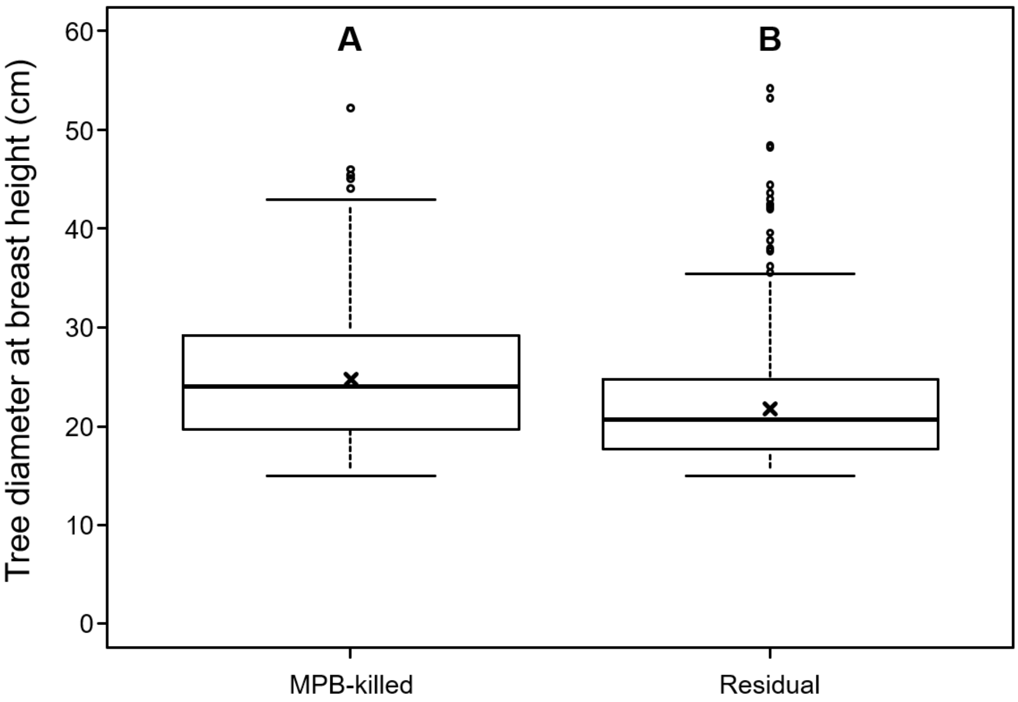


Author: Melanie Mullin
Proj: 10TM_AEP_Forest
Map date: May 18, 2019
Data: ESRI basemap, personal files, Cullingham et al. 2012

App. Figure 1 Geographic distribution of sites sampled for increment cores and wedges taken from *Pinus contorta* var. *latifolia* trees. Some sites were clustered together due to short distance between them; thus, the number of sites shown on the map does not reflect the actual number of sites sampled. Resource of base map was from personal files in Cullingham et al. 2012.



App. Figure 2 Annual number of sampled *Pinus contorta* var. *latifolia* trees killed by *Dendroctonus ponderosae* in selected study sites from 2006 to 2016. The total number of trees killed was 140. The death year of each sampled tree was determined by visual crossdating. The majority of trees (89%) were killed between 2006 and 2011.



App. Figure 3 Tree diameter at breast height of mountain pine beetle (MPB, *Dendroctonus ponderosae*)-killed and residual *Pinus contorta* var. *latifolia* trees with bark attached in post-MPB outbreak stands. Significant differences among the two categories of trees were indicated by different letters based on the results of linear mixed-effects model (α=0.05). In each box plot, the heavy line is the median, the × mark indicates the mean, the box represents the 1st and 3rd quartiles, whiskers are Tukey's 1.5 interquartile range, and the circles indicate outliers.