Variation in Anti-Insect Defenses of Three Coniferous Tree Species across Temporal and Spatial Gradients of the Colorado Front Range

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VARIATION IN ANTI-INSECT DEFENSES OF THREE CONIFEROUS TREE SPECIES ACROSS TEMPORAL AND SPATIAL GRADIENTS OF THE COLORADO FRONT RANGE

by

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A thesis submitted to the

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This dissertation entitled:
Variation in Anti-Insect Defenses of Three Coniferous Tree Species across Temporal and Spatial Gradients of the Colorado Front Range
written by Scott Ferrenberg
has been approved for the Department of Ecology and Evolutionary Biology

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The final copy of this thesis has been examined by the signatories, and we Find that both the content and the form meet acceptable presentation standards Of scholarly work in the above mentioned discipline.
Recent epidemics of bark beetles (Curculionidae: Scolytinae) have not only revealed limitations in our understanding of bark beetle-pine host interactions, but have also revealed the presence of large variations in conifer defenses across seemingly small spatial scales. This variation has clear potential to affect the dynamics of bark beetle epidemics since coniferous tree defenses play an important role in bark beetle population dynamics. At the same time, evidence suggests that bark beetle epidemics are a strong form of selection on conifer defenses as beetles selectively attack less defended trees. This selection by bark beetles on tree defenses could create a mosaic of tree resistance across the landscape where forests impacted by more frequent or recent intense bark beetle infestations would have greater anti-bark beetle defenses. To elucidate the role of tree defenses in resistance to bark beetles, I investigated the effect of bark texture of limber pines (Pinus flexilis) on beetle attack densities and characterized the vertical resin ducts of limber pine and lodgepole pine (Pinus contorta) trees that were bark beetle susceptible (killed by bark beetles) to trees that were resistant (survived attack). Following these investigations, I characterized variation in resin defenses (characterizing both resin duct and resin chemical traits) of limber, lodgepole, and ponderosa (Pinus ponderosa) pines across spatial (elevational) and ontogenetic (tree age) gradients of the Colorado Front Range. I found increasing proportional coverage by smooth bark was negatively related to bark beetle attacks/m² on limber pines. I also found resistant trees for both lodgepole and limber pine had significantly more resin ducts in
recent xylem growth rings than susceptible trees. Resin duct production was also positively related to tree radial growth rates suggesting that both tree size/age (which are strongly associated with radial growth rates) and resource availability across spatial and temporal gradients is an important influence on pine tree anti-insect defenses. Similar to other forms of anti-insect defense (i.e. secondary chemistry, leaf toughness) both stem coverage by smooth bark and resin duct production are influenced by plant ontogeny whereby younger or smaller trees have greater defenses than older or larger trees. While historical selection pressure by bark beetles may have influenced current patterns of pine defenses, past and present human activities have strongly affected tree age structure in forests across elevation in the Colorado Front Range and elsewhere. At present, higher elevation forests tend to consist of older trees on average than lower elevation forests thereby creating an elevational gradient in pine anti-insect defenses that can be best explained by the underlying gradient in tree ontogeny and allometry.
I thank Boulder County Parks and Open Space, the Indian Peaks Wilderness Alliance, the John Marr Fund, and the University of Colorado’s Department of Ecology and Evolutionary Biology for their generous support of this research. I would also like to thank Akasha Faist for assistance with field work, Jeff Kane for insight into resin duct analyses, Joe Langenhan for assisting with chemical analyses, Ken Keefover-Ring for supplying chemical standards, and Deane Bowers for comments on this work during the design phase. I am indebted to the members of my graduate committee for their input and support. I am particularly grateful for the thoughtful input and constant support of Bill Bowman in his role as Director of the University of Colorado Mountain Research Station where much of this work was completed. Finally, I would like to thank my major advisor, Jeff Mitton for his tireless support, good humor, and encyclopedic knowledge of the natural history of western North America’s conifers. While he is short in physical stature, I have had no larger champion. I hope we continue to find time to roam the forests together in the future.
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CHAPTER 1
INTRODUCTION

Insect epidemics are among the leading causes of tree mortality, with native bark beetles (Curculionidae: Scolytinae) that attack conifers as part of their reproductive cycle being the most destructive forest insects worldwide (Allen et al. 2010; Meddens et al. 2012). The mountain pine beetle (Dendroctonus ponderosae) alone has killed billions of pine trees (genus Pinus) in recent epidemics from Alaska to Northern Mexico (Meddens et al. 2013, 2014). While extensive tree mortality suggests otherwise, conifer defenses against insects can be formidable (Franceschi et al. 2005; Mumm and Hilker 2006) and enable some trees to survive bark beetle attack (Hodges et al. 1979; Sturgeon & Mitton 1986; Strom et al. 2002; Kane & Kolb 2010). In pines, oleoresin (hereafter resin) is a primary defense against bark beetles. Resin is produced and stored in a system of vertical and horizontal ducts in a tree’s phloem and xylem (Bannan 1936; Franceschi et al. 2005). Resin ducts function as a physical constitutive-defense as they exist in a tree prior to insect attack, and as an inducible defense that produces resin in response to wounding or infection (Kolosova & Bohlmann 2012; Franceschi et al 2005). Pine resin exudation can also be both a mechanical defense that repels or mires bark beetles and a chemical defense as resin is typically laden with monoterpenes and diterpene acids documented to reduce bark beetle fitness (Franceschi et al. 2005; Raffa et al. 2005; Faccoli & Schlyter 2007).

Plant secondary chemistry, such as monoterpenes in pine resin, is widely considered the most important factor in plant resistance to insects (see Carmona et al. 2011), and the role of
secondary chemistry as an anti-insect defense is well documented for many plants including coniferous trees (Mumm & Hilker 2006; Trapp & Croteau 2001; Kolosova & Bohlmann 2012). However, a recent meta-analysis suggests that physical defense mechanisms (e.g. latex, trichomes) and life history traits (e.g. growth rate, phenology) are larger influences on plant resistance to insects than plant chemistry at broad scales (Carmona et al. 2011). While much work has explored the role of tree secondary chemistry and mechanical resin flow on bark beetle host selection (Byers 1995; Raffa et al. 2005; Boone et al. 2011; Kolosova & Bohlman 2012), less work has focused on pine tree anatomical characteristics that might be associated with resistance to bark beetles such the abundance of resin ducts in xylem material. The possibility that physical traits of coniferous trees, independent of defensive chemistry, might determine resistance to insects has support from studies linking increasing amounts or size of resin ducts to resistance against several common, destructive forest-pests (King et al. 2011; O’Neill et al. 2002; Moreira et al. 2012; Kane & Kolb 2010; Gaylord et al. 2013). Collectively, these studies suggest that resin duct measures or other anatomical/physical traits of pines can be used to assess tree resistance to insects such as bark beetles.

Importantly, experimental evidence from pines and other conifers indicates that resin defense factors are heritable genetic traits and thus subject to selection from insects and pathogens (White & Nilsson 1984; Rosner & Hannrup 2004; Neale & Savolainen 2004; Sampedro et al. 2011; Westbrook et al. 2013, 2014). In the Colorado Front Range, primary bark beetles had an historical upper elevation range limit of 2740 m above sea level (Amman 1973, 1977; Amman & Schmitz 1988), while all of the common pine species of this region are found in forests both above and below 2740 m. However, after decades of increasing temperatures, particularly in the spring (Mitton & Ferrenberg 2012), the upper elevational limit of the
mountain pine beetle in Colorado has increased from roughly 2740 m to more than 3350 m. Thus, historical patterns of bark beetle attacks may have produced elevational patterns of host tree resistance. Trees found at low elevations, for example, occur within the long term historical range of bark beetles such as the mountain pine beetle (*Dendroctonus ponderosae*), and have likely been selected for greater resin defenses. Evidence of bark beetle selection on pine defenses comes from studies showing that pines possessing more-toxic resin or those that allocate more energy to resin-duct production have a lower probability of being attacked during times of local bark beetle infestation (Sturgeon & Mitton 1986; Kane & Kolb 2010). Thus, selection for resistance to bark beetle attack would make lower-elevation pines more difficult to colonize than higher-elevation pines do possibly to the number of vertical resin ducts found in the xylem rings or the chemistry of resin found in trees along these historical gradients of selection.

In addition to resin ducts and mechanical resin flow, other physical or anatomical traits may influence tree defenses against bark beetles. Smooth-textured bark on trees was historically hypothesized to be an anatomical defense against epiphytic vegetation and insect pests (Black & Harper 1979). Smooth bark was thought to make it harder for epiphytes and insects to grip a tree’s surface, reducing their ability to remain on a tree’s stem and limbs. The smooth-bark defense hypothesis was most widely considered in tropical forests where many tree species have smooth bark and plant and insect pests abound. However, the idea fell from favor when no differences in liana infestations were found between smooth and rough barked trees (Boom & Mori 1982) and alternative defenses against epiphytic vegetation were proposed (Putz 1984). Importantly, no empirical tests of bark texture effects on the interactions of trees and their phytophagous insect pests had been completed before this hypothesis was dismissed.

While bark texture has received little consideration as a potential defense against insect
pests in any tree species, a number of widespread conifers, including limber pines (*P. flexilis*) can have smooth bark (Farjon 1984; Biswas & Johri 1997; see Fig. 1). Conifers are also of particular interest in the context of potential bark texture defenses against insects because most conifers are hosts of specialist bark beetles, like the mountain pine beetle (*D. ponderosae*), that attack and kill conifers by landing upon and crawling along the tree’s stem before chewing deeper into the bark or wood (Wood 1982; Franceschi et al. 2005). Interestingly, an association of bark beetle attack locations and bark texture was indicated by a report in the secondary literature where attacks by the bark beetle *Dendroctonus pseudotsu*gae (*Douglas fir beetle*) on *Pseudotsuga menziesii* (var. *menziesii*; coastal Douglas fir) were more common on rough bark than smooth (Hedden & Gara 1972). However, the authors of this report did not consider possible mechanisms behind the observed pattern creating uncertainty over the possible defensive role of bark texture.

In the absence of direct, repeatable metrics of tree defense, forest management for reducing risk of bark beetle infestation is often based on the assumption that slow growing trees are “weaker” than fast growing trees and thus more likely to be killed by insects (e.g. Fettig et al. 2007). This assumption is not consistent with the widely documented pattern of greater overall mortality rates in faster growing trees (Loehle 1988; Wright et al. 2010), as well as plant defense theory which predicts that anti-insect defenses are costly to produce in terms of fitness and/or growth so fast growth should be accompanied by less defense (Herms & Mattson 1992; Heil & Baldwin 2002; Sampedro et al. 2011; Moreira et al. 2012). Indeed, growth-defense tradeoffs are documented for a range of defense mechanisms and plant species—including coniferous trees (Korchevia 2002; Mooney et al. 2010; Zust et al. 2011). However, studies finding no evidence of growth-defense tradeoffs in conifers are also available (Hard 1985; McDowell et al. 2007).
These conflicting reports could be due to variation in experimental designs such as the time frames over which the defenses of long-lived conifers are measured or the types of defenses (anatomical or chemical) considered, or these conflicts might stem from differing methods for measuring growth rates (i.e. tree radial growth versus total basal area increase) or complicating effects of resource limitation on plant physiology (Korchevia 2002). Resolving these conflicts will require studies of conifer defenses that utilize repeatable metrics of tree anti-insect defenses and growth rates, with additional consideration of possible spatial (landscape-level) and temporal gradients in these defenses.

In the research described here, my first goal was to investigate the importance of two anatomical traits—bark surface texture and vertical resin duct characteristics—which have been previously considered as anti-insect defense in trees, yet remain poorly studied. After verifying the importance of these anatomical traits in the defense of pines against bark beetles, my second and third goals were to determine how these defense traits varied across temporal (tree age) and spatial (elevation) gradients of the Colorado Front Range. Understanding these temporal and spatial patterns is a necessity for identifying potential mechanisms underlying variation in these defense traits at both local and regional scales. Because plant secondary chemistry has long been established as having important influences on plant-insect interactions, including interactions among conifers and bark beetles, I also characterized constitutive resin chemistry (primarily monoterpene diversity and concentrations) in a subset of the pine trees sampled for resin duct characteristics. Collectively, these research efforts have revealed many interesting interactions among tree defenses and tree age, growth, and location across elevation. Because these interactions can be complex, I have organized the presentation of my results into three chapters which respectively discuss the background and findings of three primary research hypotheses:
Chapter 2 (hypothesis 1): (a) Bark beetle-resistant pine trees (attacked and survived) have more vertical resin ducts, greater resin duct density (ducts/wood area), and/or larger resin ducts (cross-sectional area) in recent annual growth in the xylem than bark beetle-susceptible trees (attacked and killed), and (b) resistant trees will exhibit growth-defense tradeoffs whereby their larger investment in resin duct defenses results in slower measures of growth than in susceptible trees.

Chapter 3 (hypothesis 2): (a) Bark surface texture influences bark beetle attack locations and density on trees, with ‘smooth’ bark reducing bark beetle attacks and ‘rough’ bark not impeding attacks, and (b) smooth bark texture defends trees by limiting the ability of bark beetles to grip the surface as compared to rough bark.

Chapter 4 (hypothesis 3): Resin defenses measured by resin duct density (and monoterpane chemistry) will vary along (a) temporal (tree age) and (b) elevational (climatic) gradient in the Colorado Front Range. Specifically, anti-insect defenses of trees from three common species of pines are predicted to decline as a function of increasing age and elevation (i.e., younger trees at lower elevations will be the most defended considering the larger landscape, while older trees at higher elevations will be the least defended).
CHAPTER 2
BARK TEXTURE INFLUENCES TREE DEFENSE AGAINST BARK BEETLES

INTRODUCTION

Smooth-textured bark on trees was historically hypothesized to be an anatomical defense against epiphytic vegetation and insect pests (Black & Harper 1979). Smooth bark was thought to make it harder for epiphytes and insects to grip a tree’s surface, reducing their ability to remain on a tree’s stem and limbs. The smooth-bark defense hypothesis was most widely considered in tropical forests where many tree species have smooth bark and plant and insect pests abound. However, the idea fell from favor when no differences in liana infestations were found between smooth and rough barked trees (Boom & Mori 1982) and alternative defenses against epiphytic vegetation were proposed (Putz 1984). However, no empirical tests of bark texture effects on the interactions of trees and their phytophagous insect pests have been published prior to this study.

Bark texture can influence the density, diversity, and location of both sessile and mobile organisms on tree bark in temperate forests (Cramer 1975; Stephenson 1989). Habitat suitability for epiphytic lichens and bryophytes increases in relation to increasing bark texture (roughness) in various forest types (Forsyth & Miyata 1984; Holien 1997; Kim et al. 1997; Friedel et al. 2006). Bark with great texture or roughness has also been shown to host a greater diversity and abundance of micro- and macro-animals, while smooth barked trees harbor far less diversity (Wardle et al. 2003). While bark texture has received little consideration as a potential defense against insect pests in any tree species, a number of widespread conifers can have smooth bark
(Farjon 1984; Biswas & Johri 1997; see Fig. 1). Conifers are also of particular interest in the context of potential bark texture defenses against insects because many conifers are hosts of specialist bark beetles that attack and kill conifers by landing upon and crawling along the tree’s stem before chewing deeper into the bark or wood (Wood 1982; Franceschi et al. 2005). Interestingly, an association of bark beetle attack locations and bark texture was indicated by a report in the secondary literature where attacks by the bark beetle *Dendroctonus pseudotsugae* (Douglas fir beetle) on *Pseudotsuga menziesii* (var. *menziesii*; coastal Douglas fir) were more common on rough bark than smooth (Hedden & Gara 1972). However, the authors of this report did not consider possible mechanisms behind the observed pattern.

As with any type of anti-insect defense, understanding when chemical and physical traits have evolved in response to herbivory versus other biotic and abiotic pressures (i.e. ‘neutral resistance’) remains a central challenge in plant defense theory (Edwards 1989; Hanley et al. 2007). Nevertheless, plant resistance to insects is often based on a collection of plant traits which can be collectively viewed as defensive regardless of their primary function (Strauss & Agrawal 1999; Agrawal & Fishbein 2006). While research into tree defenses against insects, including defenses of conifers against bark beetles, is overwhelmingly focused on secondary chemistry (e.g., Trapp & Croteau 2001) conifers also have formidable anatomical defenses that can influence tree-resistance to bark beetles (Baier 1996; Franceschi et al. 2005). Importantly, a recent meta-analysis found plant physical traits and structural defenses to be far more effective against insects than secondary chemistry on broad scales (Carmona, Lajeunesse & Johnson 2011). Thus, a direct role of bark texture in tree resistance to insects is not only possible, but potentially combines with a suite of physical and chemical traits to form a complex of anti-insect defense over a tree’s lifespan (Boege & Marquis 2005; Agrawal & Fishbein 2006).
As noted by Hanley et al. (2007), studies that identify how phytophagous insects interact with structural/anatomical traits are important for understanding not only defense trait evolution, but also how plant-insect interactions might be influenced by climate-induced changes in phenology and ranges. From a practical point of view, investigating the role of bark texture as an anatomical defense against tree-killing insects is both novel and important in light of climate-induced insect epidemics that have recently killed billions of trees across North America and Europe (Meddens, Hicke & Ferguson 2013). *Dendroctonus ponderosae* (mountain pine beetle), for example, is currently among the most destructive forest pests in the world, having killed millions of hectares of pine trees (*Pinus spp.*) across western North America (Meddens et al. 2013). In recent years, I observed stands of *Pinus flexilis* (limber pine) in the Colorado Rocky Mountains, USA, where only trees with large proportions of smooth textured bark on their stems had survived stand-level infestations of *D. ponderosae*. During these observations I qualitatively defined smooth textured bark as those areas with no visible cracks, flakes, crenulations, or other surface features that would aide an insect’s grip (Fig. 1c). Detailed inspection revealed that trees with predominantly smooth bark surfaces had often been attacked by *D. ponderosae*, but only on rough bark around limb insertions for example (Fig. 1d). These observations inspired a test of the role that bark texture may play in defense against bark beetles.

I predicted that smooth textured bark hinders the ability of bark beetles to land on or crawl upon a tree’s stem and tested two related hypotheses: 1) bark surface texture influences bark beetle attack locations and attack density on pine trees, and 2) bark texture affects bark beetles’ ability to grip a tree’s surface. I also predicted that smooth textured bark is more common in younger/smaller trees as reported for bark texture in other tree species (Whitmore 1963; Lev-Yadun & Aloni 1993; Biswas & Johri 1997; Friedel et al. 2006), and as also reported
for a number of anti-herbivore defenses in numerous plant species (Boege & Marquis 2005). Thus, I tested a third hypothesis: 3) bark texture (roughness) increases with increasing tree size (as a correlate of age). To understand the larger potential effects of bark texture on tree-insect interactions, I estimated bark texture effects on bark beetle attack density (a correlate of attack success as bark beetles must aggregate to kill trees) expected across a range of tree sizes, and illustrated this effect in comparison to expected attack densities in the absence of a smooth bark effect.

![Figure 1](image)

**Figure 1:** Bark texture of trees varies both within and between species: (a) an example of variable bark texture within lodgepole pines (*P. contorta*) of similar age and in a shared environment, (b) a limber pine (*P. flexilis*) with rough bark texture and attacked by bark beetles (red coloured resin visible from attacks by the mountain pine beetle, MPB), (c) a limber pine with a high proportion of smooth-textured bark and (d) a limber pine with MPB attacks present on rough/crenulated bark, but absent on nearby smooth bark.
METHODS

Study system

In this study, I used *P. flexilis* (limber pine) as the model tree, and *D. ponderosae* (mountain pine beetle) as the model insect. Both species are native to western North America, where they are widespread and ecologically important organisms. *Pinus flexilis* is a five-needled pine (of the subgenus *Strobus*) that can have a mixture of smooth and rough bark on its stem and is found across a large range of latitude and elevation (Biswas & Johri 1997; Schoettle & Rochelle 2000). *Dendroctonus ponderosae* attacks all true pines (*Pinus spp.*) found within its native range, and has recently caused extensive mortality in subalpine forests that were historically above its elevational range limit (Mitton & Ferrenberg 2012). During epidemic phases, *D. ponderosae* can attack and kill seemingly healthy trees, overcoming tree defenses through the use of aggregation pheromones to increase their attack densities and by vectoring a potentially lethal fungal symbiont that aides in killing host trees (Wood 1982; Raffa & Berryman 1983). Pine trees with high volume of defensive resin (Kane & Kolb 2010; Ferrenberg et al. 2014) or high concentrations of monoterpenes (Sturgeon & Mitton 1986) can escape bark beetle-induced mortality by lowering bark beetle attack densities; indicating that defenses which reduce bark beetle attacks can lead to concomitant decreases in tree mortality (Franceschi et al. 2005).

Hypothesis 1: bark texture and insect attack

I tested my first hypothesis that bark surface texture influences *D. ponderosae* (hereafter referred to as ‘bark beetles’) attack locations on tree stems using limber pines (hereafter referred to as “trees”) from four bark beetle infested stands found on U.S. National Forest lands at 40° 04' 20'' N; 105° 125 30' 36'' W (2800 m asl) and at the University of Colorado’s Mountain Research
Station (CU-MRS) at 40° 02' 09" N; 105° 32' 09" W (3021 m asl), Colorado, USA. Climate and soils common to forest of this area were described by Duhl, et al. (2013). Stands were selected using visible evidence of recent bark beetle activity. Trees used to assess bark beetle attack locations met two criteria: 1) the tree had been attacked by at least 5 bark beetles which was determined by the presence of bark beetle caused pitch tubes (Fig. 1b), and 2) the tree had a significant proportion of both smooth and rough bark surfaces on its main stem (i.e. a minimum proportion of 0.25 to a maximum of 0.75 smooth bark surfaces). I considered bark to be smooth textured when it had no visible cracks, flakes, crenulations, or other notable features aside from micro-topography—i.e. rolling or buckling can still be covered by smooth surfaces (Fig. 1c). Bark patches covered by lichens were considered to be rough textured, as the underlying surfaces were typically rough (Fig. 1d) and lichens adds obvious texture to tree surfaces.

I determined proportional coverage by smooth and rough bark on a tree’s stem by measuring the amount of smooth and rough bark found along four, vertical bark-transects, each 2 m in length running along the tree from just above the ground’s surface to a height of 2 m and oriented on the tree stem with the cardinal directions. I also measured each tree’s diameter at breast height (DBH—1.37 m above the ground surface). I examined the surface of each tree and recorded whether attacks were seen on rough bark and on smooth bark (i.e. each tree received a ‘yes’ or ‘no’ for each surface texture type); these categories were then turned into counts for a 2 × 2 contingency table. I used a Yate’s corrected $\chi^2$ test to compare the frequencies of trees in the two categories and calculated Cramer’s V—a measure of tendency toward an outcome or association with a category within a test’s variables (Agresti 1996). Cramer’s V ranges from 0 to 1, with a value close to or equal to 1 in this test indicating a strong tendency for attacks to be associated with rough bark and not smooth bark.
I tested the relationship between bark texture and insect attacks/m² using 44 limber pine trees that were mass-attacked by bark beetles between 2010 and 2012, and that had similar diameters (20 to 25 cm) at breast height (DBH) to control for potential effects of tree size on attack density. To assess bark texture on each of these 44 trees, I affixed a flexible, nylon grid (20 × 50 cm = 1000 cm²) to a limbless area of the trunk between 1 and 2 m above the ground’s surface, and used the number of cells covering rough versus smooth bark to calculate proportional coverage of each bark type. I then determined insect attack density by counting the number of bark beetle attacks per the area under the attached grid. Proportion of smooth bark (independent variable) was then related to bark beetle attack density (dependent variable) using linear regression.

**Hypothesis 2: insect ability to grip smooth vs rough bark**

I tested a second hypothesis that bark beetle ability to grip tree stems is influenced by bark texture using *P. flexilis* trees located at CU-MRS (described above). To minimize possible effects of tree chemistry on bark beetle behavior, I tested bark beetle ability to grip bark surfaces by comparing each beetle’s performance on smooth and rough bark found on the same tree. To prevent the loss of bark beetles and to eliminate lengthy falls, I attached a paper shelf below each bark patch. I randomly selected 22 individual beetles from ~50 bark beetles captured in flight traps earlier in the day (July 2011) and wearing powder free nitrile-gloves, I placed each beetle into timed trials on both smooth and rough bark (the starting bark texture type was chosen at random). Based on observation of bark beetle’s ability to grip the different bark textures during the development of my methods, I chose to run each bark beetle through one trial on rough bark, and three trials on smooth bark to create a mean time on smooth bark. The use of multiple trials
on smooth bark was intended to ensure that I was measuring a true effect of bark texture and not a fall caused by my handling of bark beetles which had trouble gripping smooth bark. Each beetle was given time to grip the bark and orient for 5 seconds before I started a timer to measure the number of seconds until fall. A maximum time of 300 seconds (5 mins.) could elapse before trials were ended if the bark beetle had not yet fallen. I statistically verified that different trees (block effect) had no effect on the time trials ($P > 0.25$) and then compared each beetle’s time on smooth bark to its time on rough bark with a paired $t$-test.

**Hypothesis 3: bark texture across tree size and height**

To test my third hypothesis that bark texture is related to tree size and height on a tree’s stem, I measured tree diameter at breast height (DBH—1.37 m above the ground surface) and the coverage of smooth and rough textured bark on 151 trees growing in subalpine forests described above for hypothesis one. Because mountain pine beetles rarely attack tree limbs or upper reaches of tree stems, I focused on calculating the mean coverage of rough and smooth bark texture on the lower 2 m of each trees’ trunk, again using four vertical transects of 2 m length and oriented on the tree stem with the cardinal directions as described above. I sampled trees that were ≥2 m tall and that fell within 10 m of a randomly oriented (random compass vector), 20 m long transect at each sample point. I used linear regression to relate tree size (DBH; the independent variable) to bark texture (dependent variable) selecting the best fit regression model as the one with the lowest Akaike information criterion ($AIC$) and Bayesian information criterion ($BIC$) scores which are assessments of model fit that balance overall fit with the number of terms included (Yang 2005). To test for a tree height effect on bark texture, I compared the proportional coverage of smooth bark on the lowest 1 m of each tree to the next highest 1 m on
each stem with paired t-tests.

*Bark texture effects on insect attack density*

To estimate the effect of bark texture on insect attacks, I combined measured effects of both tree size and bark texture on bark beetle attack densities. I first used linear regression to relate tree diameter (DBH) with the number of bark beetle attacks/m² of bark surface using data collected from 23 rough-barked pine trees recently killed by bark beetles at CU-MRS. I used the resultant regression equation to estimate the number of bark beetle attacks expected across tree size. My field surveys and experimental trials with mountain pine beetles described above indicated that bark beetles cannot effectively attack smooth bark surfaces. As a result the proportion of smooth bark on a tree’s stem strongly influences bark beetle attack densities. In order to combine the effect of tree size on bark beetle attack density with an effect of smooth bark on attack density, I next calculated the expected proportion of smooth bark on a tree’s lower stem (bottom 2 m) as related to tree size using the linear regression models described above for hypothesis 3. Finally, to estimate the effect of both tree size and bark texture on bark beetle attack densities, I combined my estimates of bark beetle attack density in relation to tree diameter with the equation describing the effect of smooth bark proportional coverage across tree size to create an estimate of “smooth bark effect” on total bark beetle attacks. While bark beetles are less commonly found attacking trees <10 cm DBH than larger trees, the mechanisms behind this pattern are poorly researched. Thus I included estimated attack densities for trees ranging from 1 to 40 cm DBH in my model to illustrate the point in tree size and defense when potential bark beetle attacks drop to zero.
RESULTS

Hypothesis 1: bark texture and insect attack

I found 52 trees, with a mean DBH ± 1SE of 22.2 ±1.1 cm, that fit my criteria of having ≥ 5 bark beetle attacks and at least 0.25 to 0.75 proportion smooth bark on the lower 2 m of the tree’s main stem. I did not count total attacks on each tree, but given that all trees included in my survey met a minimum threshold of 5 attacks I examined a minimum of 260 attacks collectively across the 52 trees and found only one bark beetle attack on smooth bark. Thus 51 trees were categorized as ‘0’ (no attack on smooth bark) and one tree as a ‘1’ (attack on smooth bark) resulting in a highly significant difference via Yate’s corrected $X^2 = 96.19$ ($P < 0.0001$, d.f. = 1) and a Cramer’s $V = 0.98$ (i.e. bark beetle attacks are strongly associated with rough bark and rarely associate with smooth bark). In addition to attack locations on a tree, I also found evidence that bark texture significantly influences bark beetle attack density. As the proportion of smooth bark increased, total bark beetle attacks decreased resulting in a negative relationship between proportion of smooth bark and attacks/m$^2$ ($R^2_{(1,42)} = 0.83$, $P < 0.0001$; Fig. 2).

![Graph](image.png)

**Figure 2:** Increasing proportional coverage of smooth bark on a pine tree’s lower stem reduces the density of bark beetle attacks (mountain pine beetle attacks/m$^2$). The relationship of proportional coverage of smooth bark and bark beetle densities were tested via linear regression on data measured from a 50 × 20 cm grid placed on 43 limber pines between 20 and 25 cm in diameter at breast height in the Colorado Rocky Mountains, USA.
Hypothesis 2: insect ability to grip smooth vs rough bark

In experimental trials, bark beetles were less capable of gripping smooth bark than rough bark (paired \( t_{21} = -17.5, P < 0.0001; \) Fig. 3). When placed onto rough bark surfaces, 21 of the 22 bark beetles tested (95%) remained on the bark until the 300 second (5 min) trial cut-off time, giving the beetles a mean time of 293.9 seconds before falling from the tree. All but one of the 22 bark beetle placed onto smooth bark fell from the tree’s stem in less than 60 seconds (95%), with a mean time of 43.3 seconds before falling from the smooth bark (Fig.3).

**Figure 3:** Time (seconds) until mountain pine beetles (MPB) fell from the surface of rough versus smooth textured bark in paired trials. Trials were stopped at a maximum time of 300 seconds (5 minutes) if MPB had not yet fallen. Smooth and rough bark surfaces were located on the same tree to reduce variation in chemical cues that might influence MPB behavior. Means (grey bars) and one standard error of the mean are shown; \( P \) value is from a paired \( t \)-test.

Hypothesis 3: bark texture across tree size and height

I found that smaller trees have a greater proportion of smooth bark on their main stem than larger trees. Specifically, I found a significant, negative relationship between tree size (DBH) and the proportion of smooth bark on a tree’s stem \( (R^2_{(2,149)} = 0.76; P < 0.0001; \) Fig. 4) with the best fit regression model found when DBH was considered as a 2\(^\text{nd}\) order polynomial term. Regardless of tree size, the proportion of smooth bark texture was significantly greater with
increasing height on the tree stem—i.e. the lower meter of bark closest to the ground surface had less smooth surfaced bark coverage (mean proportional coverage of 0.44) than the upper measured meter (mean proportional coverage of 0.57) (paired \( t_{151} = 9.5, P < 0.0001 \)).

Figure 4: Proportion of total bark surface that is smooth textured on the lower 2 m of limber pine trees as related to tree size (diameter at 1.37 m above the ground surface). Data are from 151 trees sampled in the Colorado Rocky Mountains, USA. The solid line and values of \( P \) and \( R^2 \) are from polynomial regression.

Bark texture effects on insect attack density

Mean attack density measured from 23 pines killed by bark beetles in recent years was 99.1 (SE = 6.6) attacks/m\(^2\), with a minimum of 37.1 and a maximum of 145.0 attacks/m\(^2\) found among all trees. However, bark beetle attacks/m\(^2\) were positively related to tree size whereby larger trees have greater attack densities than smaller trees (\( R^2_{(1,20)} = 0.22; P = 0.028; y = 52.4 + 1.49 \times \text{DBH} \)). Combining estimates of bark beetle attack densities across tree size with estimates of bark beetle attack density in relation to the proportion of smooth bark across tree size revealed the largest total reduction in bark beetle attacks/m\(^2\) due to the smooth bark effect was found for intermediate tree sizes (Fig. 5). The largest effect of smooth bark on bark beetle attacks for any sized tree was found for trees 9 cm in diameter which see a reduction of 65.5 attacks/m\(^2\). All
trees measuring ≤ 23 cm in diameter have an estimate reduction of bark beetle attacks ≥ 20.0 attacks/m², while all trees ≤ 28 cm in diameter have an estimated reduction of ≥ 10.0 attacks/m². Reduction in bark beetle attacks for larger trees range from 9.0 attacks/m² in trees 28 cm in diameter to 2.1 attacks/m² in trees 40 cm in diameter.

**Figure 5:** The interacting effects of smooth bark texture and tree size on the number of mountain pine beetle (MPB) attacks/m² of bark on limber pine trees (lower two meters of a tree’s stem considered here as MPB rarely attack tree tops or limbs). The solid line shows the effect of tree size on MPB attack density; the dashed line shows the possible number of MPB attacks/m² of bark based on the effect of bark texture and tree size. The shaded area illustrates the difference in total MPB attacks due to an effect of bark texture. While bark beetles rarely attack very small trees (trees < 10 cm in diameter), the mechanisms behind this pattern are poorly described. Thus, the effect of bark texture and tree size across trees of 1 to 40 cm is shown here to demonstrate the point when bark beetle attacks reach zero.

**DISCUSSION**

Tree bark is a first line of defense against insects and pathogens. Bark can contain not only chemical means to combat insect attacks (Krabel & Petercord 2000; Petrakis et al. 2011), but also anatomical structures (Franceschi et al. 2005). While smooth bark texture was historically hypothesized to be an anti-insect defense that operates by reducing the ability of
insects to grip tree stems, no empirical tests of this hypothesis are available in the literature. I found that bark beetle attacks on *P. flexilis* (limber pines) were overwhelmingly located on rough bark and virtually absent on smooth bark (Fig. 1b,d), and that increasing proportions of smooth bark on tree stems led to decreases in bark beetle attack densities (Fig. 2). I also found in experimental trials that bark beetles were less able to grip smooth bark and quickly fell from the smooth bark surfaces, but not from rough bark surfaces of a tree (Fig. 3). This result supports the supposition that smooth bark defends trees by mechanically ‘shedding’ attacking insects, and collectively, my results support my first two hypotheses that smooth bark on pines influences the location of insect attacks by reducing an insect’s ability to grip the bark. Importantly, in my study, smooth bark on pine trees acted as an anatomical defense against one of the world’s most destructive forest pest, the mountain pine beetle (*D. ponderosae*), which are insects specialized to attack trees by boring through the bark.

I found that even for trees with mixtures of rough and smooth bark, smooth surfaces reduce total bark beetle attacks by $\geq 20$ attacks per m$^2$ of bark surface in trees $\leq 23$ cm in diameter (DBH). This is a notable reduction given that my model estimated average starting attack densities (i.e., densities expected in the absence of smooth bark effects) of 89.9 attacks/m$^2$ across trees 10 to 40 cm in diameter. Importantly, the estimated attack densities used in my predictive model are within the range of attacks/m$^2$ reported for other mountain pine beetle infested forests (Duhl et al. 2012; Clark et al. 2012), and are similar to the threshold densities reported to bring about pine mortality (Raffa & Berryman 1983). Also, while it is commonly stated or assumed that bark beetles primarily attack only the largest trees in mature forests stands, recent mountain pine beetle epidemics in the U.S. Rocky Mountains have been marked by substantial mortality in small trees. For example, trees $<23$ cm in diameter account for
roughly 50% of bark beetle-induced mortality in numerous stands of lodgepole pine (*Pinus contorta*), a tree that rarely has smooth bark surfaces (Progar et al. 2013). The large numbers of attacks on smaller size classes of trees combined with the need for bark beetle aggregation on individual tree stems in order to overcome tree defenses suggests that smooth bark could realistically reduce bark beetle attack density and related tree mortality across the many coniferous tree species with smooth bark traits during or throughout their lifespans (Biswas & Johri 1997). It should be noted that the number of insect attacks necessary for overcoming plant defenses, in this and other study systems, is influenced by plant physiological status and other abiotic and biotic factors over time (Huberty & Denno 2006; Gaylord et al. 2013). Nevertheless, defense mechanisms that reduce total bark beetle attacks have been shown to reduce tree mortality in several coniferous species (Wood 1982; Raffa & Berryman 1983). Also, while defensive resin flow and tree secondary chemistry can enable conifers to withstand bark beetle attacks (Sturgeon & Mitton 1986; Kane & Kolb 2010), these defenses are mainly encountered after beetles have already damaged the tree (Franceschi et al. 2005). Smooth bark, however, inhibits bark beetle attacks before damage is incurred which presumably leaves overall tree vigor unaffected and makes this bark defense highly effective for predominantly smooth trees.

Tree bark texture has long been considered to be of adaptive significance (Esau 1967; Roth 1981). While I found that smooth bark defends against insect attack, bark texture could enhance tree fitness in other ways. For example, rough textured bark can increase resistance to fire and drought (Glitzenstein & Harcombe 1979; Pinard & Huffman 1997), and improve tree heat balance (Derby & Gates 1966). Meanwhile, smooth bark can increase the volume and nutrient content of water flowing along tree stems after precipitation (Van Stan & Levia 2010). Taken together, these reports indicate that smooth bark could be an adaptation to stresses other
than herbivory or insect attack which would make its contribution to defenses a form of “neutral resistance” (Edwards 1989). Nevertheless, neutral resistance traits (traits that offer defense but are evolved for other purposes) still influence overall plant anti-insect defense. As noted by Strauss and Agrawal (1999), a trait that confers anti-insect defense to a plant must be considered in study of defenses regardless of its adaptive function. It is also important to note that plant surface texture has been previously shown to influence plant-insect interactions in ways that could promote coevolved relationships. For example, some bees can identify flowers by touch (Kevan & Lane 1985; Erber et al. 1998; Scheiner et al. 2005; Yoshioka et al. 2007) and increased surface texture of flower petals increases pollinator visits (Comba et al. 2000). Experiments also indicate that bees avoid smooth, steeply angled flowers because of an inability to grip their surfaces (Whitney et al. 2009a & 2009b). Taken collectively, these studies and my results indicate a need for further study of the roles that plant surface textures might play in plant-insect interactions and evolutionary biology.

I found support for my third hypothesis that bark texture is influenced by tree ontogeny, whereby increasing tree size leads to decreasing coverage by smooth bark (Fig. 4); and that surface area of smooth bark tends to increase with increasing height on tree stems. These results suggest an effect of age-related or growth-related factors on bark texture in *P. flexilis*. Similar changes in bark texture with stages of ontogeny have been described for other deciduous (Lev-Yadun & Aloni 1993; Pinard & Huffman 1997; Friedel et al. 2006) and coniferous species (Biswas & Johri 1997; Malone & Liang 2009). I found the defense offered by smooth bark against bark beetles is greatest for smaller trees, but is retained into trees that are respectively large in size (~ 30 cm in diameter; Fig. 5) in my study system—a subalpine forest with short growing seasons and slow tree growth (Duhl et al. 2013). The greater defense offered by smooth
bark on smaller trees is important since younger trees located near mature con specifics or congeners can have higher probabilities of being attacked by shared pests (Janzen 1970; Peters 2003; Terborgh 2012). Also, coniferous trees like those studied here often grow in dense, low diversity stands which can promote the movement of pests and reduce survival of younger/smaller trees (Schupp et al. 2002). Thus, smooth bark surfaces might benefit pines more in early ontogenetic stages—when proportional coverage of stems is greatest—by helping them to escape shared insect pests. However, increased resistance against insect attack offered by even small proportions of smooth bark in larger trees (Fig. 5) could have long term consequences for pine survival as warming temperatures promote insect range expansion (Mitton & Ferrenberg 2012), and as insect-induced mortality continues to exceed drought-induced mortality in high elevation forests (Das et al. 2013).
CHAPTER 3
RESIN DUCT CHARACTERISTICS ASSOCIATED WITH
TREE-RESISTANCE TO BARK BEETLES

INTRODUCTION

Native bark beetles (Curculionidae: Scolytinae) that attack conifers as part of their reproductive cycle are a leading cause of recent, extensive tree mortality across western North America (Allen et al. 2010; Meddens et al. 2012). While this extensive tree mortality suggests otherwise, conifer defenses against insects can be formidable (Franceschi et al. 2005), and some trees repel bark beetle attacks even during epidemics (Hodges et al. 1979; Strom et al. 2002). The possibility that anatomical structures linked to physical defenses in coniferous trees, independent of defensive chemistry, might determine resistance to insects has support from studies linking increasing numbers or size of resin ducts to resistance against several destructive insects (O’Neill et al. 2002; Kane & Kolb 2010; King et al. 2011; Moreira et al. 2012; Gaylord et al. 2013). Collectively, these studies suggest that resin duct measures can be used to assess tree resistance to insects such as bark beetles. However, studies comparing resin duct characteristics of trees that were attacked and killed by bark beetles to trees that directly resisted bark beetles are not readily available in the literature. To investigate the importance of resin ducts for tree resistance to bark beetles, I compared bark beetle-resistant and susceptible trees attacked over a two year period in a shared environment.

In pines, oleoresin (hereafter resin) is a primary defense against bark beetles. Resin is produced and stored in a system of vertical and horizontal ducts in a tree’s phloem and xylem (Bannan 1936; Franceschi et al. 2005). Resin ducts function as a physical constitutive-defense as
they exist in a tree prior to insect attack, and as an inducible defense that produces resin in
response to wounding or infection (Franceschi et al 2005; Kolosova & Bohlmann 2012). Pine
resin exudation can also be both a mechanical (physical) defense that repels or mires bark beetles
and a chemical defense as resin is typically laden with monoterpenes and diterpene acids
documented to reduce bark beetle fitness (Raffa et al. 2005; Facolli & Schlyter 2007).
Importantly, experimental evidence from pines and other conifers indicates that resin defense
characteristics such as flow rates and chemistry are heritable genetic traits and thus subject to
selection from insects, pathogens, and environmental factors (Rosner & Hannrup 2004;
Sampedro et al. 2011; Westbrook et al. 2013).

Forest management to reduce the risk of bark beetle infestation is often based on the
assertion that slow growing trees are “weaker” than fast growing trees and thus more likely to be
killed by insects (e.g. Fettig et al. 2007). This assertion conflicts with documented patterns of
greater herbivory and mortality rates in faster growing trees (Loehle 1988; Ruel & Whitham
2002; Wright et al. 2010). In addition, plant defense theory predicts that anti-insect defenses are
costly to produce in terms of fitness, so fast growth should be accompanied by reduced allocation
to defense (Herms & Mattson 1992; Heil & Baldwin 2002). Growth-defense tradeoffs have been
documented for a range of defense mechanisms and plant species including coniferous trees
(Korchevia 2002; Mooney et al. 2010; Zust et al. 2011). However, studies finding no evidence of
growth-defense tradeoffs in conifers are also available raising questions about how growth
influences defense in coniferous trees (Hard 1985; McDowell et al. 2007; Gaylord et al. 2007).
These conflicting reports could be due to variation in experimental designs, differing measures of
growth rates (i.e. tree radial growth vs. basal area growth), or complicating effects of resource
limitation and/or climate on plant physiology and defense (Korchevia 2002). Regardless, vertical
resin ducts offer are a long-term record of tree defense in relation to growth rates estimated from tree rings, and may offer a way to assess tree resistance to insects prior to management efforts.

Using two widespread tree species, lodgepole pine (Pinus contorta, subgenus Pinus) and limber pine (Pinus flexilis, subgenus Strobus), I compared bark beetle-resistant trees (survived mass attack) to bark beetle-susceptible trees (killed by mass attack) to assess the roles of vertical resin duct number, density, and size, along with tree growth rates in resistance to the mountain pine beetle (D. ponderosae). Both lodgepole and limber pine have suffered extensive mortality from the mountain pine beetle and often co-occur in high elevation forests. By comparing bark beetle resistant to susceptible trees in a common environment and using species representing long-diverged subgenera (~90 million years ago; He et al. 2012) my study is the first, to my knowledge, to quantify resin duct defenses in pines known to be bark beetle-resistant, and also the first study of resin duct defenses and bark beetle attack for the pine subgenus Strobus (five-needle pines). I hypothesized (1) that bark beetle-resistant trees of both species would have more vertical resin ducts, greater resin duct density (ducts/wood area), or larger resin ducts (cross-sectional area) in recent annual growth in the xylem, and (2) that resistant trees would exhibit growth-defense tradeoffs whereby their larger investment in resin duct defenses would result in slower growth. Ultimately, my goal was to determine if resin duct and/or growth rate characteristics could be used to differentiate among “resistant” and “susceptible” trees—my results supported this goal as models based primarily on resin duct counts accurately assessed tree-resistance both within and among the pine species studied.

METHODS

Study site and tree sampling
I compared bark beetle-resistant to bark beetle-susceptible trees at the University of Colorado Mountain Research Station, 10 km east of the Continental Divide, Colorado, USA (N 40° 1' 49", W 105° 31' 56"; 2900 m asl). The climate and soils of this site were described by Duhl, et al. (2013); monthly air temperatures from 1970 to 2008 were described by Mitton and Ferrenberg (2012). To minimize environmental and climatic differences across my study, I studied 102 trees (replicates) attacked by bark beetles; 25 resistant and 25 susceptible lodgepole pines and 26 resistant and 26 susceptible limber pines selected from a common 2.5 ha plot.

Lodgepole pines in my study had a mean age (± 1 SE) of 117 (± 6) years and a mean diameter at breast height (DBH, 1.37 m above ground level) of 22.0 (± 0.7) cm. Limber pines had a mean age of 253 (± 10) years and a mean DBH of 23.7 (± 1.0) cm. All trees were mass-attacked by the mountain pine beetle (≥ 20 individual attacks on the lower 2 m of the trunk) between 2008 and 2010. Trees categorized as bark beetle-resistant survived for ≥ 2 years after attack; trees categorized as susceptible died within 1 year of attack. I extracted a 12 mm diameter increment-core at DBH from each tree and mounted cores on wooden blocks, sanded each with a progression of coarse to fine sandpaper, and created a flat cross-section for analysis.

Resin duct measures and tree growth

Using a stereo-microscope I counted the number of vertical resin ducts in each of the most recent 20 growth rings (# yr\(^{-1}\)). Mean resin duct cross-sectional areas (mm\(^2\)) and variation in area (standard error of mean duct area) of each tree were calculated from all resin ducts in the most recent five years of growth rings using high resolution scans (9600 dpi) and analysis tools in Photoshop version CS2. I measured annual radial growth (mm·yr\(^{-1}\)) of the most recent 20 years using scans in CooRecorder 7.6 (Cybis Elektronik & Data AB, 2012). To simplify analyses
and to understand what intervals of tree growth are most important for insect resistance, I binned resin duct totals and densities, as well as growth measures for each tree into four overlapping time intervals that included the most recent 5, 10, 15, and 20 year totals, and used the growth rate and resin duct totals of the entire interval to calculate resin duct density (resin ducts·mm\(^{-2}\) of wood). I also used radial growth to calculate each tree’s basal area increment (BAI) for the 5 through 20 year intervals of each tree. BAI in my study estimates tree growth as the percentage of a tree’s total cross-sectional area (mm\(^2\)) comprised by a specific interval of growth.

Data analysis

For each species, I compared resin duct numbers, density, and size; and radial growth and BAI of resistant and susceptible trees for each time interval (5, 10, 15, and 20 years) via t-tests on log-transformed data or Wilcoxon tests when assumptions of normality were not met by transformations. I examined relationships between tree growth and defenses within species via linear regression to relate radial growth or BAI for the 20 year interval (independent variables) to vertical resin duct counts and density (dependent variables) over the 20 year interval in each tree. Similarly, I used linear regression to examine the relationship between growth (radial and BAI) over the most recent five years to mean vertical resin duct size for each tree (dependent variable).

Using discriminant analysis (DA; a multivariate method that tests the ability of continuous variables to identify pre-assigned categorical groups), I attempted to correctly categorize bark beetle-resistant and susceptible trees of each species based on the 5 through 20 year interval measures of resin duct characteristics (counts, density, size, and variation) to 5 through 20 year tree growth (radial and BAI). I created three DA models for lodgepole and limber pine, and three models for the species combined that considered resistant and susceptible
trees independent of species identity. For the first model (individual species or grouped), I used principle coordinates analysis (PCoA) to reduce the data from all resin duct and growth measures of the overlapping time intervals into axis scores—a method that avoids violating assumptions regarding multiple collinearity in DA. The PCoA axis scores were then used to create a model that used the fewest axes to achieve the highest level of correct categorization of trees. The second DA models represented a best fit model constrained to only one time interval measure for each factor (i.e. each factors such as resin duct density could only be represented by one interval from the 5 through 20 years) to correctly categorize the largest % of trees. The third and final DA models were based only on the five year interval of each factor and used the fewest factors necessary to correctly categorize the largest % of trees.

RESULTS

Resin ducts and bark beetle-resistance

Bark beetle-resistant trees for both lodgepole pine and limber pine had significantly more resin ducts in the most recent 5 and 10 years of growth measured in 12 mm wide increment cores ($P < 0.05$; Fig. 6), than did susceptible (bark beetle-killed) trees. Specifically, bark beetle-resistant lodgepole pines had 23% more resin ducts and resistant limber pines had 37% more resin ducts in their most recent 5 years of radial growth (5 years of rings) on average than susceptible trees. Extended to the most recent 10 years of growth, resistant lodgepole pines had 21% more, and resistant limber pines had 18% more resin ducts than bark beetle-susceptible trees. Over the 15 and 20 year growth interval resistant trees of both species consistently had more resin ducts, but with no significant difference between groups ($P > 0.05$; Fig. 6).
**Figure 6:** Vertical resin duct counts in the most recent 5, 10, 15 and 20 years of growth rings from lodgepole (*P. contorta*) and limber (*P. flexilis*) pines that either resisted bark beetle attack or were killed (susceptible) by mass attack of mountain pine beetles. It is important to note that the values shown here are counts from 12 mm wide increment cores; scaling these measures up to account for the whole tree stem would result in differences of many thousands of resin ducts between resistant and susceptible trees. Significant differences are indicated by an asterisk; boxes show medians and 1st and 3rd quartiles, and whiskers indicate 1.5 IQR (inter-quartile range) or ~97% of the variation in untransformed data.

While mean resin duct density (vertical resin ducts/mm$^2$ of wood growth) of bark beetle-resistant lodgepole pine trees was roughly 8% greater than that of bark beetle-susceptible trees, resin duct density did not significantly differ between the groups for any interval of lodgepole annual growth rings ($P > 0.05$; Fig. 7). However, resin duct density in bark beetle-resistant limber pine trees was significantly greater than in bark beetle-susceptible trees over all of the time intervals from 5 to 20 years ($P < 0.05$; Fig. 7). Resistant limber pines had 39%, 37%, 32%,
and 28% greater resin duct densities than susceptible trees over the 5, 10, 15, and 20 year growth intervals respectively.

**Figure 7:** Vertical resin duct density (ducts/mm$^2$ of wood) in the most recent 5, 10, 15, and 20 years of growth rings from lodgepole (*P. contorta*) and limber (*P. flexilis*) pines that either resisted bark beetle attack or were killed (susceptible) by beetles. Significant differences are indicated by an asterisk; boxes show medians and 1st and 3rd quartiles, and whiskers indicate 1.5 IQR (inter-quartile range) or ~ 97% of the variation in untransformed data.

Resin duct size (mm$^2$ of cross-sectional area) did not differ significantly between bark beetle-resistant (0.018 ± 0.001) and susceptible (0.020 ± 0.001) lodgepole pine trees ($P > 0.05$), but was significantly different ($P < 0.05$) in limber pine with resistant trees having smaller resin ducts (0.014 ± 0.001) that susceptible trees (0.017 ± 0.001). Similar to resin duct size, variation in resin duct size (standard error of resin duct areas within a tree) did not significantly differ between bark beetle-resistant (0.0155 ± 0.0002) and susceptible (0.00143 ± 0.0001) lodgepole
pine trees ($P > 0.05$), but did differ for limber pine with resistant trees having less variation in duct size ($0.0008 \pm 0.00007$) than susceptible trees ($0.0011 \pm 0.00009$) ($P < 0.05$).

Three growth and bark beetle-resistance

Tree radial growth (mm time interval$^{-1}$) in bark beetle-resistant lodgepole pines exceeded radial growth of susceptible trees by up to 12%, but was not significantly greater for any of the 5 through 20 year growth intervals ($P > 0.05$; Table 1). An opposite trend was present in limber pines, where bark beetle-resistant trees had less total radial growth over the 5 and 10 year growth intervals and significantly less radial growth over the 15 and 20 year intervals ($P < 0.05$; Table 1). Specifically, resistant limber pines had 38% and 32% less radial growth than susceptible limber pines over the most recent 15 and 20 years of growth respectively.

<table>
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<th>Growth type</th>
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<th>Killed</th>
<th>Resist</th>
<th>Killed</th>
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<td>8.06 (0.54)</td>
<td>6.89 (0.56)$^b$</td>
<td>9.10 (0.90)$^a$</td>
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<td>14.15 (1.13)</td>
<td>12.17 (1.08)</td>
<td>14.29 (2.87)</td>
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</table>

Note: Tree radial growth is shown in mm, while basal area increment (BAI) is a measure of the % of total stem area comprised by the specified interval. Trees that resisted bark beetles were compared to those killed within a species via t-tests on log transformed values; untransformed means with 1 SE in parentheses are shown here; means ± SE followed by different letters are significantly different ($P < 0.05$).
Tree growth considered as the basal area increment (BAI) also indicated that bark beetle-resistant lodgepole pine trees tended to have greater growth rates than bark beetle-susceptible trees, while resistant limber pine trees had lower growth rates than susceptible trees (Table 1). BAI of resistant lodgepole pines was significantly greater (20%) than in susceptible trees over the most recent 5 years of tree growth ($P < 0.05$; Table 1), but for all other time intervals (10, 15, and 20 years) difference were non-significant ($P > 0.05$) despite resistant tree have roughly a 15% greater BAI over time. While bark beetle-resistant limber pine trees had a BAI roughly 50% less than bark beetle-susceptible trees over the 20 years of growth rings, the lower BAI in resistant trees was not significantly different from susceptible trees ($P > 0.05$; Table 1).

Tree growth had a moderate, significantly positive relationship with resin duct defenses in both lodgepole pine and limber pine trees. In both species, tree radial growth over the most recent 20 years was positively related to resin duct counts ($R^2 = 0.21$ and 0.22 in lodgepole and limber pine respectively; $P < 0.05$ for both) (Fig. 8). BAI was also significantly related to resin duct counts in lodgepole pine trees ($R^2 = 0.17$, $P < 0.05$; Fig. 8) but the relationship was weaker than that of radial growth and resin duct counts. BAI was not related to resin duct counts in limber pine trees ($R^2 = 0.04$, $P > 0.05$; Fig. 8). Tree radial growth had a stronger positive relationship than BAI with resin duct size for both tree species ($R^2 = 0.36$ and 0.15 in lodgepole and limber pine, respectively; $P < 0.05$) (Fig. 9). BAI was also significantly related to resin duct size in both lodgepole ($R^2 = 0.20$, $P < 0.05$) and limber pine trees ($R^2 = 0.13$, $P < 0.05$; Fig. 9).
Figure 8: Relationship between tree radial growth (mm·yr$^{-20}$) or basal area increment (BAI; % of total tree basal area comprised by the last 20 years of growth) and resin duct counts over the last 20 years in lodgepole ($P. contorta$) limber ($P. flexilis$) pines.

Figure 9: Relationship between tree radial growth (mm·yr$^{-20}$) or basal area increment (BAI; % of total tree basal area comprised by the last 20 years of growth) and resin duct size (mm$^2$) in lodgepole ($P. contorta$) and limber ($P. flexilis$) pines.
Predicting tree resistance and susceptibility

Discriminant analysis (DA) was consistently able to better categorize limber pine trees as resistant or susceptible than lodgepole pines. While the best fit DA models (explained the greatest amount of variation with the fewest variables) for both pine species included resin duct counts and density, as well as radial growth and BAI measures, the two species differed in the importance of resin duct size for categorizing trees—i.e., the best fit model for limber pine did not include measures of resin duct size or variation in resin duct size, while resin duct size was included in the best fit model for lodgepole pine. Overall models based on multivariate combinations of resin duct measures and tree growth reduced into PCoA axis scores were able to correctly categorize 84.0% of lodgepole pines and 92.3% of limber pines as resistant (repelled bark beetle attacks) or susceptible (killed by bark beetle attacks) (Table 2). Both DA models resulted in an area under the receiver operating characteristic (ROC) curve ≥ 0.93 (a value of 1.0 indicates a perfect test, a value of 0.5 indicates a worthless test) indicating that both models are strong tests for discriminating between resistant and susceptible tree groups. Simplifying the DA models to best fit using one time interval (either 5, 10, 15, or 20 year values) for resin duct total, density, size, or duct size variation, and radial growth and BAI resulted in the correct categorization of 80.0% of lodgepole (area under ROC = 0.78) and 88.5% of limber pine (area under ROC = 0.92) trees as resistant or susceptible (Table 2). Finally, best fit DA models using only measures from the most recent 5 years of annual growth rings correctly categorized 72.0% (area under ROC = 0.72) and 80.8% (area under ROC = 0.88) of lodgepole and limber pine trees respectively (Table 2).
Combining the two species into one best fit DA model, independent of species identity, correctly categorized 82.3% of trees as resistant or susceptible (area under ROC = 0.89) (Table 2, Fig. 10). A best fit simple DA model, using only one time interval (either 5, 10, 15, or 20 year values) for resin duct total and density, and radial growth and BAI resulted in the correct categorization of 76.5% of trees (area under ROC = 0.83), while a final DA model based only on the most recent 5 year resin duct totals from each tree correctly categorized 72.6% of trees as resistant or susceptible (area under ROC = 0.75) (Table 2).
**DISCUSSION**

*Resin ducts and bark beetle-resistance*

Bark beetle epidemics have renewed interest in forest pest dynamics, and much work has explored the role of tree secondary chemistry and mechanical resin flow on bark beetle host selection (Byers 1995; Raffa et al. 2005; Boone et al. 2011; Kolosova & Bohlman 2012). Less work has focused on pine tree anatomical characteristics that might be associated with resistance to bark beetles such as bark traits or the expression of resin ducts, yet available studies suggest strong influences of anatomical characters on bark beetle attack densities and tree mortality.
Importantly, a recent meta-analysis indicates that plant life history and physical traits are often stronger influences on plant resistance than chemical traits (Carmona et al. 2011). While I did not measure tree secondary chemistry, my results offer additional support for a strong role of a physical trait—i.e. vertical resin duct production—in determining pine tree resistance to bark beetles. I found support for my first hypothesis that resistant trees would have greater numbers of resin ducts, as both lodgepole and limber pines that resisted bark beetles had significantly more vertical resin ducts over the last decade of growth than trees killed by bark beetle attacks (Fig. 6). More resin ducts should translate into greater resin production, storage, and flow during insect attack, likely explaining the differences in resin duct numbers between resistant and susceptible trees. Through de novo formation of traumatic resin ducts, conifers can increase the number of vertical resin ducts when attacked by insects or infected by fungal pathogens. However, these traumatic resin ducts form in the developing secondary xylem (the woody portion of a developing growth ring) and not in the previously developed xylem of earlier growth rings (Martin et al. 2002; Hudgins & Franceschi 2004). The formation of additional resin ducts in a portion of only one growth ring in trees used in my study is possible, but cannot be the driver of the results I present here given that the pattern of greater resin duct numbers and density in resistant trees than in susceptible trees is present over the full two decade period (Figs. 6 and 7). Also, significant differences in resin duct counts between resistant and susceptible trees are found over a decade of growth rings in both tree species (Fig. 6)—a result that is retained (but not shown to avoid redundancy) when the growth ring with the greatest number of resin ducts are excluded from each tree’s total count and data are reanalyzed.

Also, using discriminant analysis I was able to correctly categorize 84% of lodgepole and 92.3% of limber pines as bark beetle-resistant or susceptible based on combinations of resin duct
and growth rate measures (Table 2). Considering both species together, I was still able to correctly categorize 82.3% of trees using combinations of factors and 72.6% of trees using just the resin duct counts from the last five years of growth (Table 2).

My models had reasonably high success categorizing trees as resistant and susceptible without measures of secondary chemistry for these two pine species (from long-diverged subgenera) that vastly differ in their respective constitutive and inducible secondary chemistry (Smith 2000). While my study is the first to compare vertical resin ducts in the xylem of pines that resisted bark beetle attacks to pines that died from attacks, the role of resin ducts in tree resistance to insects is not without some precedent. For example, greater vertical resin duct density was shown to increase resistance of sitka spruce (*Picea sitchensis*) to white pine weevils (*Pissodes strobe*) (O’Neill et al. 2002; King et al. 2011; Moreira et al. 2012), while smaller resin duct size was linked to higher densities of the piñon *Ips* beetle (*Ips confuses*) on piñon pines (*P. edulis*) (Gaylord et al. 2013). Also, a combination of resin duct density and size was highly successful at separating trees selected and killed by bark beetles from neighboring trees that were not attacked by bark beetles in logistic models following a bark beetle infestation in ponderosa pines (*P. ponderosa var. scopulorum*) (Kane & Kolb 2010).

Tree growth and bark beetle-resistance

I found evidence that tree growth rates may influence anti-insect defense in conflicting ways for lodgepole and limber pine, leading to mixed support for my second hypothesis, derived from the growth-differentiation balance hypothesis (Lerdau et al. 1994), that greater resin duct defenses would result in less growth. Results from lodgepole pines countered this hypothesis as susceptible trees had smaller basal area increment (BAI) growth over the last five years of their
lives (as well as fewer resin ducts) than resistant lodgepole pines with no significant difference in radial growth between groups. Meanwhile, results for limber pines supported my hypothesis as limber pines killed by bark beetles had greater radial growth over the past 15 to 20 years of their lives (along with fewer resin ducts suggesting a growth-defense trade-off) than resistant limber pines, but with no significant difference in BAI between groups. Interestingly, I found a positive relationship between both growth measures and total resin ducts for lodgepole pine, and between radial growth and total resin ducts in limber pine (Fig. 8); as well as positive relationships of growth measures and resin duct size for both species (Fig. 9). These concomitant increases in growth and defense seem to run counter to plant defense theory as well as reports of growth-defense trade-offs in other plant species (Herms & Mattson 1992; Heil & Baldwin 2002; Mooney et al. 2010; Zust et al. 2011; Sampedro et al. 2011; Moreira et al. 2012). However, an absence of growth-defense trade-offs has been noted for systems characterized by resource limitation and plants with terpenoid based defenses including other coniferous forest types and trees (Hard 1985; Christiansen et al. 1987; Koricheva 2002; McDowell et al. 2007), and neither radial growth or BAI are complete measures of tree growth since they consider only changes in stem diameter or area. Nevertheless, if all trees had equal energy budgets to be apportioned between growth and defense, then tradeoffs would likely become apparent (Koricheva 2002). However, conifers are among the most genetically variable groups of organisms known, with substantial variability in photosynthetic capacity, respiration and growth rates, water potential, and resin duct density within and among species and many of these traits are heritable, or at least influenced by genetics (Linhart & Mitton 1985; Cornelius 1994; Neale & Savolainen 2004; Chhatre et al. 2013; Benkman & Parchman 2013). Therefore, the energy budgets of trees in a
stand can vary; and some trees, by virtue of greater ability to fix carbon can allocate to both
growth and defense with no immediate trade-off.

The increase in resin duct size with larger growth rates I found here, regardless of
underlying cause, could explain why I did not find resin duct density to be significantly different
for bark beetle-susceptible and resistant lodgepole pines. Resistant lodgepoles had a greater BAI
and resin duct counts than trees killed by bark beetles. The associated increase of resin duct size
with increasing growth could lead to greater defense without increased duct density thereby
minimizing the effect of total wood area on defense (Fig. 7). Yet, I found that resistant and
susceptible lodgepole pines did not differ with respect to resin duct sizes, and that resistant
limber pine trees had smaller resin ducts than susceptible trees calling into question the
importance of resin duct size in defense against insects for the pine species studied here.

Predicting tree resistance and susceptibility

Anti-insect defenses of plants are influenced by genotype, phenology, physiology, and
location on the plant itself (e.g. roots vs leaves) among other factors (Barton & Koricheva 2010;
Muola et al. 2010; Erbilgin & Colgan 2012) which can lead to difficulty in characterizing plant
defenses under natural conditions. Additionally, plant defenses can be plastic in response to
biotic and abiotic factors that can vary over time (Huberty & Denno 2006; Heil 2010; Hart et al.
2013). As a result, characterizing anti-insect defenses of long-lived trees based on secondary
chemistry may accurately assess current status, but fail to capture trends in tree defense. My
results indicate that relatively short-term trends in tree defenses (5-10 years) are sufficient to
characterize defense against bark beetles. Nevertheless, vertical resin ducts of lodgepole pine can
remain active for 25 years (Reid et al. 1967) and are responsive to inter-annual climate factors in
Norway spruce (Wimmer & Grabner 1997), suggesting that tree defense structures might operate on, or be influenced by conditions over long temporal and spatial scales. However, insect life histories could influence the importance of older structures for tree resistance. For example, primary bark beetles, (e.g. the mountain pine beetles) are active beneath the tree bark near newer growth, while wood-boring beetles (Coleoptera: Buprestidae and Cerambycidae) burrow deeper into a tree’s xylem possibly contacting older resin ducts.

I found the inclusion of measures such as resin duct size (mm$^2$), variation in resin duct size (standard error of resin duct areas), and tree growth rates in discriminant analysis can lead to a greater percentage of trees being correctly classified as “resistant” or “susceptible”. However, using just resin duct counts from five years of tree growth led to the correct categorization of > 72% of all trees across species. Thus, reasonable levels of accuracy for characterizing pine tree resistance to bark beetles can be achieved without time consuming measures. Also, while I and others have pointed out the possibility that physical defenses outweigh chemical defenses of pines in their effect on insect-resistance (e.g. Strom et al. 2002), a role of tree secondary chemistry in defense against bark beetles has been shown in numerous studies (e.g. Christiansien et al. 1987; Mumm & Hilker 2006; Raffa et al. 2013). My results indicate that resin duct assessments are more accurate for categorizing limber pine trees as opposed to lodgepole pine—a result that is possibly due to the “stronger” anti-insect defensive chemistry of lodgepole pine compared to white pine species (subgenus Strobus) such as limber pine (Smith 2000; Raffa et al. 2013). Thus, the effort placed on characterization of pine defenses should be guided by pine evolutionary history and experimental design.

My results suggest that vertical resin ducts in coniferous tree xylem can be used to assess insect-resistance for ecological and evolutionary biology studies and also for forest management.
While measuring resin duct characteristics in forest stands that have not undergone recent bark beetle infestations complicates the identification of characteristics associated with trees predisposed to bark beetle-mortality, vertical resin ducts in recent xylem could still be used to characterize “mean resistance” of trees. This assessment of variation in resin duct characters might then enable "resistance-based thinning" where harvesting efforts target trees with lower resin duct counts until the desired tree density is attained. While tree survival if attacked by bark beetles or other insects is not ensured by this method, overall levels of resistance against insects should increase across the managed forest as per capita levels of resin defense increase.
CHAPTER 4

INFLUENCES OF ONTOGENY AND CLIMATIC GRADIENTS ON RESIN DUCT CHARACTERISTICS AND RESIN CHEMISTRY

INTRODUCTION

Ecologists have long been interested in the causes and consequences of biogeographical variation in plant defenses against herbivores and pathogens (Fraenkel 1959, McKey 1979). This interest has led to the rise of many major hypotheses regarding potential influences on plant defense evolution and expression (Stamp 2003). Despite the many frameworks for considering plant defenses, the various hypotheses collectively stem from a central thesis that plant defenses increase plant fitness in the face of phytophagous insects and pathogens (Marquis 1984, Belsky 1986, Kane and Kolb 2010, Ferrenberg et al. 2014), and that defenses come with costs given resource availability and the need for other developmental and physiological processes that maintain fitness (Simms 1992, Koricheva 2002, Strauss et al. 2002). Given their purported role in plant fitness and investment costs, plant defense allocation within and across plant species should be influenced by selection pressure imposed by a mixture of abiotic and biotic factors (Stamp 2003, Agrawal and Fishbein 2006, Agrawal 2007). At the same time, abiotic conditions and the intensity of antagonistic interactions between plants and their natural enemies vary across the landscape (Dobhansky 1950, Levin 1976, Coley and Aide 1991, Ågren et al. 2008, Pennings et al. 2009, Schemske et al. 2009, Vanhonenacker 2013) which results in spatially variable selection pressures on plant defense phenotypes (Thompson 2005, Muola et al. 2010).

In light of the putative role of biotic selection on plant defense evolution and expression,
two types of non-mutually exclusive hypotheses have risen to the top in consideration as possible
drivers of landscape-level variation in plant anti-insect/pathogen defenses: 1) process-defense
tradeoffs that interact with resource availability gradients and abiotic stresses (McKey 1974,
Rutledge 1996, Ohnmeiss and Baldwin 2000), and 2) climatic suitability gradients for insects
and pathogens which influence the intensity of antagonistic interactions such that plants from
lower latitudes or elevations should be better defended than plants from higher latitudes or
Support for the former group of hypotheses is relatively strong as indicated by the meta-analyses
of Koricheva (2002) and Endara and Coley (2011), while the existence of hypothesized clines in
herbivory and plant defenses along climatic gradients are generally accepted to exists, yet are
largely unsupported by the literature. While some studies find clear evidence of latitudinal
climatic-defense gradients (e.g. Levin 1976, Coley and Aide 1991, Pennings et al. 2009,
Rasmann and Agrawal 2011, Pearse and Hipp 2012), two recent meta-analyses indicate the
majority of studies measuring difference in plant defenses across latitudes refute the existence of
climatic-defense gradients (Moles et al. 2011a, 2011b). How plant defenses are partitioned along
elevation has received far less study than latitude making generalization difficult, but there is
some support for decreasing defenses with increasing elevation (e.g. Pellissier et al. 2014,

A majority of available studies of climatic-defense gradients focus on plant secondary
chemistry in vegetative tissue (often in germinant, seedling, and juvenile stages) as the primary
marker of defense allocation (Moles et al. 2011a, 2011b) and the assumption that these chemicals
arose and are maintained in response to selection pressure from insect herbivores. Indeed, the
overwhelming majority of available studies, either over latitude, or elevation are focused on plant secondary metabolites and other chemical defenses, but a recent meta-analysis found no overall association between plant secondary chemistry and plant susceptibility to herbivory (Carmona et al. 2011). Physical and structural plant defenses, however, were found to be good predictors of plant susceptibility to natural enemies, thus drawing into question whether the traits commonly considered in studies of climatic-defense gradients are truly anti-insect/pathogen defenses that improve plant fitness (Johnson and Rasmann 2011). At the same time, defensive traits vary over a plant’s lifespan with differences in the trajectories of chemical versus physical defenses within plants both across ontogeny and plant functional groups (Barton and Koricheva 2010). However, there are relatively few studies of changes in plant physical defenses with increasing age, and an overall lack of studies in the mature stages of long-lived plants such as shrubs and trees (Barton and Koricheva 2010, Hanley et al. 2007). Thus, plant age and defense type must become key consideration in the assumptions and design of any study of plant defense clines, but particularly across large climatic gradients which can influence plant life history strategies in relation to age (Franks and Weis 2008, Futuyma 1998).

Interest in understanding how plant physical and chemical defenses change with age and elevation in long-lived trees inspired me to study resin defenses in three species of pines (Pinaceae: Pinus) with Neartic ranges covering large swaths of western North America: Pinus flexilis (subgenus Strobus), and Pinus ponderosa and Pinus contorta (subgenus Pinus) (Richardson 1998). While studies of plant defenses are often performed in short-lived, herbaceous species for obvious logistical reasons, these three pine species are long-lived, have large intraspecific elevational ranges, and can be found com mingled in naturally occurring forest stands. Also, pines and other conifer species have coevolved with several genera of aggressive
bark beetles (Curculionidae: Scolytinae) that attack mature, healthy pines *en masse* (for the purpose of reproduction and feeding) during intense epidemics (Wood 1982). As a result of these coevolutionary relationships, pines and related conifers possess potent anti-insect/pathogen defenses, with the physical flow of oleoresin (hereafter resin) produced from a system of vertical and horizontal duct structures being a primary defense against stem- and wood-boring insects (Franceschi et al. 2005, Mumm and Hilker 2006). Recent field studies revealed that trees of all three species studied here (as well as additional pine species) with particular vertical resin duct characteristics (higher duct density, larger duct sizes) can either escape bark beetle attack to begin with (Kane and Kolb 2010), or can resist bark beetle-induced mortality after attack (Ferrenberg et al. 2014); these studies verify an important role of resin ducts in pine defenses against bark beetles, while also indicating that bark beetles continue to exert strong selection pressure on pine defense structures and characters through their choices of host trees and subsequent mortality probabilities linked to resin duct characteristics (Ferrenberg et al. 2014, Macalady and Burgmann 2014, Gaylord et al. 2013, Kane and Kolb 2010). Pine resin consists of a suite of secondary chemicals, primarily sesquiterpenes, and monoterpenes dissolved in diterpene acids. While individual terpenes have been correlated to bark beetle host selection in past studies, the use of chemical markers to assess host tree resistance to attack has proven more difficult than the use of resin duct characteristics which can distinguish ‘susceptible’ from ‘resistant’ trees of all three species studied here with > 85% accuracy across studies (Ferrenberg et al. 2014, Kane and Kolb 2010).

I hypothesized that resin defenses measured by resin duct density (and monoterpene chemistry) would vary with tree age and the elevational (climatic) gradient used for sampling in the Colorado Front Range. Specifically, anti-insect defenses of trees from three common species
of pines were predicted to decline as a function of increasing age and elevation (i.e., younger trees at lower elevations would be the most defended considering the larger landscape, while older trees at higher elevations would be the least defended). Given the potential tradeoffs among tree defenses and growth (or other physiological processes), I also hypothesized that resin defenses, regardless of elevation, would be influenced by tree growth rates over time; with my prediction being that higher growth rates would lead to decreased production of resin defenses.

METHODS

Sampling design

I tested the hypothesis that tree defenses are adapted to historical herbivore pressure associated with climatic gradients by sampling tree defenses across an elevational transect covering a total range of 1440 meters in elevation and a difference of approximately 11.5°C in annual mean temperature from the lowest to highest sampling sites which were separated by 15.5 km of planar distance between the highest sampling site (3330 m asl, -0.3°C annual mean air temperature; 40.0375, -105.5739) and the lowest sampling site (1890 m asl, 11.1°C annual air temperature; 40.0061, -105.3518). The three pine species examined along this transect had variable, yet overlapping elevational ranges (all three species can be found growing together) with *P. ponderosa* sampled in this study from 1895 m to 2854 m above sea level (asl), *P. contorta* sampled from 2347 m to 3330 m asl, and *P. flexilis* sampled from 2579 m to 3330 m asl. Trees of each species were sampled between 2011 and 2014 from 5 or more locations along their elevational range, with a diverse range of tree sizes selected from along randomly oriented transects within each sampling site. The goal was to collect data on defenses from across a range of tree ages and growth rates while also effectively assessing elevational/climatic influences.
Characterization of resin ducts and resin chemistry

Defenses were assessed for each tree through characterizations of vertical resin ducts in annual xylem rings from increment cores, and in a subset of trees via comparisons of constitutive monoterpene chemistry. Increment cores were removed with a 12 mm diameter borer, dried for 2 days, and then mounted cores on wooden blocks before being sanded with a progression of coarse to fine sandpaper to create a flat cross-section for analyses. Vertical resin ducts in each of the most recent 20 growth rings (# yr⁻¹) were counted under a stereo-microscope, while annual radial growth (mm·yr⁻¹) of the most recent 20 years was measured from high resolution scans (9600 dpi) in CooRecorder 7.6 (Cybis Elektronik & Data AB, 2012). To simplify analyses, resin duct measures and growth rates were binned into four time intervals that included resin duct totals and growth rates over 5, 10, 15, and 20 year totals. Radial growth rates were then used to calculate each tree’s basal area increment (BAI) for the 5 through 20 year intervals of each tree, with BAI in this study considered as the percentage of a tree’s total cross-sectional area (mm²) comprised by a specific interval of growth. This approach to measuring BAI allows trees of different sizes to be compared on their proportional growth. Tree growth and age at 1.4 m above the ground surface were also measured from increment cores.

Constitutive monoterpene chemistry was measured from resin collected in glass scintillation vials placed into a 65 mm diameter bore hole drilled approximately 50 mm into each tree’s xylem. Vials were tightly inserted to reduce contact between resin and the atmosphere, left in place for 48 hours, and then capped and stored at -30°C until analysis. On the day of laboratory analysis, resin samples were allowed to warm to room temperature before an aliquot of each sample was transferred to a 3 dram glass vial which was promptly capped and weighed.
Each sample was then dissolved into methyl tert-butyl ether (MTBE, C₅H₁₂O) containing either 5 mM isobutylbenzene (C₁₀H₁₄) or cyclohexylbenzene (C₁₂H₁₆) as an internal standard. A Hewlett-Packard HP6890 Series GC/MS equipped with a Restek Rtx-5Sil MS column was used to quantify monoterpene amounts. After split injection (2 µL, 34:1 ratio, 220 °C), the oven temperature increased from an initial value of 40 °C at 10 °C/min until 200 °C was reached (5 min hold). During this time, helium flow rate was maintained at 1.6 mL/min. Identification of peaks was accomplished by comparing retention times to those of authentic standards.

Calibration curves for quantification were generated using authentic α-pinene (Fluka), β-pinene (Fluka), α-phellandrene (TCI), β-phellandrene (supplied by Dr. Keefover-Ring), Δ-3-carene (Aldrich), limonene (Fluka), myrcene (Acros), sabinene (Indofine), and terpinolene (TCI).

Statistical analyses

For each species, stepwise regression models were used to examine the association of resin duct numbers in the most recent 20 years of xylem rings and radial growth (RG) for the same 20 year interval, tree size (DBH), the interaction of RG × DBH, and elevation. Terms included were first tested to ensure no significant collinearity. Best fit models were determined by comparison of Bayesian information criterion (BIC). Following stepwise modeling, individual linear regressions were used to examine the relationships of: 1) RG/BAI and total resin ducts, 2) tree age and RG, 3) tree age and total resin ducts, and 4) tree age and tree diameter (DBH). Linear regression was also used to test for correlations among DBH and total resin ducts, and DBH and RG. The associations of RG, tree age, and total resin ducts for all three species were visualized with filled contour plots.

Monoterpene (MT) forms and abundances of constitutive resin in each tree of all three
species were treated as ‘communities’ for analyses. MT communities of each tree species were visualized with non-metric multidimensional scaling (NMDS) and compared via multi-response permutation procedure (MRPP, a non-parametric method for comparing the hypothesis of no difference among groups/communities). MRPP was also used to compare MT communities within each species among different elevational sampling sites. Mean total MT concentration, MT diversity within species (α-diversity) calculated as Simpson’s diversity index (Simpson’s D), and mean pair-wise dissimilarity in MT diversity (β-diversity) calculated as the Bray-Curtis dissimilarity index were all compared among species via Kruskal-Wallis nonparametric tests followed by Steel-Dwass comparisons (a nonparametric multiple means comparison with sequential correction).

RESULTS

I sampled a total of 692 trees: 199 P. flexilis with a mean age of 143.5 years and a basal area of 410.8 cm², 303 P. ponderosa with a mean age of 116.2 years and a basal area of 473.2 cm², and 190 P. contorta with a mean age of 89.8 years and a basal area of 375.5 cm². Stepwise regression models revealed a number of factors influenced total resin duct production in the most recent 20 years of xylem across species, with radial growth rates over the same 20 year period having the greatest influence on resin duct totals for all three species (P < 0.0001 for all; Table 3). Elevation was not a significant influence on resin duct totals for P. contorta, but elevation was a significant, yet relatively minor factor (P < 0.05) leading to a slight decrease in resin duct totals of both P. flexilis and P. ponderosa as elevation increased (Table 3). In addition to the individual factors of radial growth and elevation, the interaction term of radial growth × elevation and tree size (xylem diameter measured from increment cores collected at 1.4 m above
the ground surface) were also important factors in resin duct totals of *P. ponderosa* (*P* < 0.05 and *P* < 0.0001, respectively) but not in the other species (Table 3).

**Table 3**: Influence of radial growth rates and elevation on total resin duct production in within and across three species of pines.

<table>
<thead>
<tr>
<th>Species</th>
<th>$R^2$</th>
<th>Factor</th>
<th>$F$-ratio</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. flexilis</em></td>
<td>0.70</td>
<td>Radial growth</td>
<td>445.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tree diameter</td>
<td>—</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elevation</td>
<td>13.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RG × Elevation</td>
<td>—</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>P. ponderosa</em></td>
<td>0.76</td>
<td>Radial growth</td>
<td>793.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tree diameter</td>
<td>15.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elevation</td>
<td>7.4</td>
<td>&lt; 0.01</td>
</tr>
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<td></td>
<td></td>
<td>RG × Elevation</td>
<td>5.5</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td><em>P. contorta</em></td>
<td>0.70</td>
<td>Radial growth</td>
<td>428.8</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tree diameter</td>
<td>—</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elevation</td>
<td>—</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RG × Elevation</td>
<td>—</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Models shown were determined as the best fit for the three individual factors and the interaction term based on comparisons of BIC values in stepwise regressions. Factors included were controlled for collinearity to avoid over-fitting models.

Considered in individual linear regressions, the relationship of radial growth rates and resin duct production were strongly positive and significant for all three species with $R^2 = 0.68$ for *P. flexilis*, $R^2 = 0.74$ for *P. ponderosa*, and $R^2 = 0.70$ for *P. contorta* (*P* < 0.0001, Figure 11). A similar result was found for the relationship of resin duct production and basal area increment (BAI, or the percentage of each tree’s cross-sectional area comprised by the most recent 20 years of xylem rings); $R^2 = 0.49$ for *P. flexilis*, $R^2 = 0.62$ for *P. ponderosa*, and $R^2 = 0.47$ for *P. contorta* (*P* < 0.0001 for all).
Figure 11: The relationship of tree radial growth (mm) from the most recent 20 years of xylem rings and total resin ducts from the same time period in limber pine (*P. flexilis*, blue circles and dark blue line), ponderosa pine (*P. ponderosa*, gray squares and black line) lodgepole pine (*P. contorta*, pink triangles and red line). All relationships are significant at \( P < 0.0001 \).

Radial growth rates and tree age were significantly and negatively related across all species \( (P < 0.0001) \), with *P. flexilis, P. ponderosa, and P. contorta* having \( R^2 = 0.37, 0.50, \) and 0.39, respectively (Figure 12). Also, given the significant relationship of radial growth with both resin duct totals and tree age, increasing tree age was correlated with a significant decrease in total resin ducts with *P. flexilis, P. ponderosa, and P. contorta* having \( R^2 = 0.41, 0.50, \) and 0.28, respectively \( (P < 0.0001 \) for all, Figure 13 and 14). Importantly, while tree age and tree diameter were significantly related in all three species, DBH was not a useful predictor of tree growth rate or total resin ducts \( (P > 0.05, R^2 < 0.01 \) for all).
Figure 12: The relationship of tree age (years measured as total xylem rings) and total radial growth over the most recent 20 years of xylem rings in limber pine (*P. flexilis*, blue circles and dark blue line), ponderosa pine (*P. ponderosa*, gray squares and black line) lodgepole pine (*P. contorta*, pink triangles and red line). All relationships are significant at $P < 0.0001$.

Figure 13: The relationship of tree age (years measured as total xylem rings) and total resin ducts from the most recent 20 years of xylem rings in limber pine (*P. flexilis*, blue circles and dark blue line), ponderosa pine (*P. ponderosa*, gray squares and black line) lodgepole pine (*P. contorta*, pink triangles and red line). All relationships are significant at $P < 0.0001$. 
Figure 14: Contour plots showing the interaction of total resin ducts in the most recent 20 years of xylem rings with tree radial growth (mm) over the same time period and tree age. Darker greens indicate greater numbers of resin ducts.

Resin Chemistry

I sampled constitutive resin (the resin already present in trees prior to insect attack or pathogen infection) across elevation in 106 trees of *P. flexilis*, 105 of *P. ponderosa*, and 60 of *P. contorta*. Monoterpene communities of constitutive resin significantly differed among tree species (MRPP *P* < 0.0001, Figure 15 and 16) as well as among sample sites within each species (MRPP *P* < 0.01 for all three). The diversity of resin monoterpenes (calculated as Simpson’s D, a measure of within sample diversity or α-diversity) significantly differed among all three species.
(Kruskal-Wallis followed by Steel-Dwass comparisons, \( P < 0.0001 \)), with the highest diversity found in \( P. \text{flexilis} \) (0.73 ± 0.01), followed by \( P. \text{ponderosa} \) (0.64 ± 0.01) and \( P. \text{contorta} \) (0.54 ± 0.02). Mean pair-wise dissimilarity in monoterpane diversity (\( \beta \)-diversity, measured as Bray-Curtis dissimilarity index) was also significantly different among species (Kruskal-Wallis followed by Steel-Dwass comparisons, \( P < 0.0001 \)); \( P. \text{contorta} \) had the largest pair-wise dissimilarity (0.67 ± 0.01) followed by \( P. \text{flexilis} \) (0.54 ± 0.01) and \( P. \text{ponderosa} \) (0.50 ± 0.01).

Mean total monoterpene concentration (± 1 SE) in the constitutive resin differed among the three species (Kruskal-Wallis \( P < 0.0001 \)) with \( P. \text{flexilis} \) having a significantly lower concentration (0.14 ± 0.004) than both \( P. \text{ponderosa} \) (0.19 ± 0.005) and \( P. \text{contorta} \) (0.21 ± 0.016). Despite differences in monoterpene composition, concentration, and diversity of the three pine species there was no clear relationship between monoterpene community structure and elevation.

**Figure 15:** Non-metric multidimensional scaling plot (NMDS) showing ‘communities’ of monoterpene in the constitutive resin of limber pines (\( P. \text{flexilis} \), gray circles), ponderosa pines (\( P. \text{ponderosa} \), blue circles), and lodgepole pines (\( P. \text{contorta} \), black circles) sampled across tree ages/sizes along an elevational gradient. Monoterpene communities of each species are significantly different in permutation based comparisons of variation (MRPP, \( P < 0.0001 \)).
Figure 16: Mean relative abundance of monoterpenes in the constitutive resin of limber pines (P. flexilis), ponderosa pines (P. ponderosa), and lodgepole pines (P. contorta) sampled across an elevational gradient. Monoterpene relative abundances significantly differ among all three species ($\chi^2 = 203.7, P < 0.0001$).

DISCUSSION

Variations in plant defense phenotypes within and among species have been of longstanding interest in evolution and ecology. A number of prominent hypotheses have been presented as explanatory frameworks for variation in plant defenses (Stamp 2003). These frameworks deal with adaptive responses in defenses to: selection pressures from abiotic and biotic pressures across climatic gradients (e.g. MacArthur 1972, Levin 1976, Coley and Aide 1991), responses to resources availability (e.g. Coley et al. 1985, Fine et al. 2006), adaptive changes in resources allocation across plant ontogeny (Bryant et al. 1992, Herms and Mattson 1992, Spiegel and Price 1996, Ohnmeiss and Baldwin 2000, Barton and Koricheva 2010), and trade-offs with other physiological processes to optimize defenses (McKey 1974, Feeny 1976, Rhoades and Cates 1976, Coley et al. 1985, Herms and Mattson 1992, Zangerl and Rutledge 1996, Ohnmeiss and Baldwin 2000). Yet despite this longstanding interest in understanding
variation in plant defenses there are several prominent gaps in the coverage of existing studies; data are limited on physical defense traits in mature plants, particularly for woody plants with long life spans such as conifers. At the same time, a recent meta-analysis indicates that plant life history and physical traits are stronger influences on plant resistance to insects than chemical traits (Carmona et al. 2011). Clearly, studies of plant defenses should consider traits with a verified role in plant resistance to natural enemies. Recent studies have revealed a strong influence of anatomical characteristics of resin ducts on insect attack densities and tree mortality in conifer-bark beetle systems (Ferrenberg et al. 2014, Kane and Kolb 2010). While resin ducts are relatively unexplored physical defense traits, they offer a novel approach to plant defense studies because they are conserved over time in conifer xylem rings which allows them to be related to tree age, ontogeny, and growth rates.

I measured physical characteristics of the vertical resin duct system and secondary chemical traits of constitutive resin across tree age and an elevational (climatic) gradient. I found strong support for my hypothesis that resin defenses vary with tree age and growth rate, but only weak support that resin defense vary across the climatic gradient associated with elevation in the Colorado Front Range. Specifically, resin duct totals in the most recent 20 years of xylem rings in all three pine species were positively related to radial growth rates and basal area increment growth (BAI) (Figure 11), while resin duct numbers declined with increasing age when sampled across trees ranging from 25 to 400 years old (Figure 13). Resin duct numbers declined very slightly in relation to elevation after controlling for tree growth-age factors (Table 3, Figures 12, 13, and 14), with tree elevation accounting for ≤ 3% of the variation in resin duct totals in P. flexilis and P. ponderosa but was not a significant factor for P. contorta (Table 3).

Resin chemistry, characterized here by monoterpene diversity and abundance, showed no
clear patterns in relation to elevation, but resin chemistry was consistently different among the three species and frequently differed within species across sampling sites separated by very small distances. Previous work has unveiled substantial variation in resin monoterpane chemistry both among and within conifer species (Clark et al. 2010, Pureswaran et al. 2004, Lindström et al. 1989, Pollack and Dancik 1985, Smith 1983, Forrest 1980), but this work typically considered variation within-species variation over larger spatial scales than my study. Overall, my results suggest even greater variation in pine secondary chemistry across the landscape than previously appreciated.

Importantly, experimental design is a necessary consideration when comparing plant defenses and for interpreting evidence for and against climatic-defense gradients (Schemske et al. 2009). While a majority of studies refute the existence of climatic defense gradients, primary support for these gradients comes from studies completed across latitude in coastal salt marshes of eastern North America. These studies differ from many others (which focus on interspecific comparisons across latitude) by considering the intraspecific variation in plant defenses and herbivore performance across an ecosystem where plant-insect communities remain relatively consistent across a large geographical range (i.e. Ho and Pennings 2013, Pennings et al 2009, 2007, 2001, Saldago and Pennings 2005). This approach helps to reduce the documented effects of differing life history traits and deep evolutionary history on plant defenses (Carmona et al. 2011, Koricheva 2002, Mitter et al. 1991) and allows for consideration of adaptations to more recent biotic and abiotic factors along extant climatic gradients. In short, this approach avoids a longstanding issue with a priori assumptions that similar traits found among species must have arisen and been maintained for equivalent and adaptive reasons (Gould and Lewontin 1979).

While I employed a study design that compared resin defenses within species across
elevation, I found that climatic influences associated with this large elevational gradient appear to be far less important than growth and ontogeny for physical production of resin ducts in pine species (Table 3, Figure 11). In addition to the role of plant age in pine defenses found here, evidence that plant ontogeny and life history strategies strongly influence anti-insect and pathogen defenses comes from across a wide range of plant species (Barton and Koricheva 2010, Hanley et al. 2007). Nevertheless, few previous studies have found a link between age and leaf/needle defenses in woody plants (Barton and Koricheva 2010), suggesting that the type (e.g. chemical vs. physical) and location (e.g. stem versus leaves) of defense being studied is an important consideration. Also, many available studies of conifer defenses utilized measure from seedling and juvenile stages of these extremely long-lived plants (Wainhouse and Ashburner 1996, Moreira et al. 2014). Meanwhile, studies comparing defenses among mature conifer species under field conditions fail to not only consider influences of evolutionary history described above (e.g. Raffa et al. 2013), but also fail to consider continued non-linear variation across late stages of ontogeny which has been documented in woody plants (see Barton and Koricheva 2010). Importantly, the significant decline in resin duct defenses I found with increasing tree age was roughly equivalent across all three species used in my study. Given that *P. flexilis* is from a pine subgenus (*Strobus*) which diverged ~90 million years ago from the subgenus (*Pinus*) containing *P. ponderosa* and *P. contorta* (He et al. 2012), the relationship of resin ducts to growth and tree age appear to be reasonably conserved.

My results appear to challenge support offered for climatic-defense gradients from greenhouse studies comparing pine defense across species (Moreira et al. 2014) and the assertion of Raffa et al. (2013) that pine elevational range has strongly influenced resin defenses. However, in the case of Moreira et al. (2014) defenses across 17 species of pines, two of which I
studied here (*P. ponderosa* and *P. contorta*) were measured by a mixture of constitutive and induced secondary chemistry in the needles of tree seedlings and not by physical and chemical defenses in the woody stems of mature pines under field conditions. Differences in the monoterpene chemistry of tree stems versus needles within individuals have been verified for *P. contorta* and other conifers (Pureswan et al. 2004). Thus comparisons of my results to Moreira et al. (2014) are difficult given the likely large differences in defense allocation and syndromes between tree stems and needles across conifers.

Finally, conclusions regarding influences of climatic/elevational gradients on pine defenses presented by Raffa et al. (2013) are clearly unfounded given their use of an experimental design that did not include climate/elevation as a variable. Instead, the authors compared chemical defenses between two co-occurring pine species—from different subgeneras—with results that confirmed previously reported difference in resin chemistry in these species (see Mirov 1961, Smith 2000). While Raffa and colleagues argue that these differences in resin chemistry indicate difference in susceptibility to bark beetles among the species, there was no statistically supported difference in either bark beetle preference for each species, or differences in mortality rates of the two species in their study. Also, minimal evidence from live trees, sampled under field conditions, is available to substantiate which monoterpene mixtures and concentrations confer more or less resistance to primary bark beetles at the landscape scale (Clark et al. 2014, Thoss and Byers 2006, Sturgeon and Mitton 1986, Sturgeon 1979). In fact, monoterpene profiles of various coniferous species have been associated with a range of interactions with mammalian herbivores and seed dispersers, insect herbivores and pollinators, and resistance to widespread fungal pathogens suggesting a diverse set selective pressures on pine resin chemistry beyond interactions with bark beetles (Litvak and Monson
1998, Latta and Linhart 1997, Langeheim 1994, Snyder 1993). As noted by Gould and Lewontin (1979), who cautioned against viewing extant traits as resulting from improvements through selection: “when ‘multiple adaptive peaks’ are occupied, we usually have no basis for asserting that one solution is better than another. The solution followed in any spot is a result of history; the first steps went in one direction, though others would have led to adequate prosperity as well.”

In conclusion, I found little to no support that pine resin defenses in mature tree stems have been influenced by climatic controls on the intensity of interactions with natural enemies along an elevational gradient. This topic has been of recent interest as an unprecedented epidemic of the mountain pine beetles, *Dendroctonus ponderosae*, has killed billions of true pines (genus *Pinus*) across western North America (Meddens et al. 2012). During this epidemic, the mountain pine beetle has expanded its range into high elevation forests that were historically beyond its suitable climatic range, possibly putting the beetle into contact with less defended trees as predicted in Mitton and Ferrenberg (2012). Despite finding only weak support for a decrease in pine defenses with increasing elevation, I did find that pine resin duct production declines significantly as trees age (Figure 13). While forest age structure was not a focus of my research, historical disturbance patterns from human activities have led to higher densities of older trees over many areas of the western United States (Pan et al. 2011). Natural declines in defense as pine trees age have therefore likely combined with warming temperature effects on bark beetle life history (Mitton and Ferrenberg 2012, 2014) to spur recent epidemics. Continued research into additional conifer groups and across latitude should enable detailed conifer defense models based primarily on age-growth-resin duct relationships.
CHAPTER 5
CONCLUSIONS

Tree mortality from bark beetles has renewed interest in predicting the spatial and temporal dynamics of these forest insect populations. However, consideration for tree anti-insect defenses in bark beetle risk models remains rare (e.g. Fuentealba et al. 2013) hampering our ability to identify plant-specific factors that might promote insect population growth or tree resistance. Through the work presented above, I have demonstrated that smooth bark can limit attacks by insects on trees—even for insect species specialized to attack tree stems. The reduction of bark beetle attacks on trees with large proportions of smooth bark appeared to decrease rates of bark beetle-induced mortality—an observation that initially inspired my study but that requires long term investigation. However, I did not attempt to measure bark texture impacts on rates of mortality because I selected my study sites by locating dead or dying trees which could lead to an over-sampling of trees susceptible to insect attack, and reduced my ability to determine if trees had been predisposed to insect attack due to prior stresses such as native root fungi and introduced pathogens that abound in my study region (Kinloch 2003). My results will hopefully inspire future studies of ways in which bark texture might influence the demography and ecology of trees and forest insects. While the effects of bark texture have been widely considered for lichens (Cramer 1975; Holien 1997), bark effects are overlooked in studies of forest insects. Even when differences in bark texture between trees are clear and variation in secondary chemistry is minimal, differences in the diversity and density of tree-bark associated insects are still often attributed to plant-secondary chemistry (e.g. Petrakis et al. 2011). Nevertheless, my results not only indicate that smooth bark defends a widespread and
ecologically important pine species from one of the world’s most destructive forest-insects, but also calls for a resurrection of the ‘smooth-bark as defense’ hypothesis.

I found bark beetle-resistant trees had more resin ducts than susceptible trees and that resin duct characteristics from recent growth can be used with reasonably high accuracy to differentiate among resistant and susceptible trees both within and across pine species. Combined with work by Kane and Kolb (2010) who showed that bark beetles preferentially attacked pine trees with fewer resin ducts and avoided trees with greater numbers of resin ducts, my results suggest that selection during insect epidemics favors trees with larger number of vertical resin ducts. Because resin defense traits (e.g. resin production, flow, chemical content, duct production) are heritable genetic traits in pines and other conifers (Rosner and Hannrup 2004; Sampedro et al. 2011; Chhatre et al. 2013; Westbrook et al. 2013, 2014) managed could use selective removal of “susceptible” trees as part of a strategy to select for long-term, tree resistance to bark beetles.

Identifying how phytophagous insects interact with structural/anatomical traits of their host plants is important for understanding not only defense trait evolution, but also how plant-insect interactions might be influenced by climate-induced changes in phenology and ranges. My goal was to determine if bark texture and resin duct and/or growth rate characteristics can be used to differentiate among “resistant” and “susceptible” pine trees. Because these anatomical characters are visible on the surface of the tree and in the xylem rings of pines, they represent both easily accessed and (in the case of resin ducts) long term metrics of tree defenses against bark beetles—thus they can be used to investigate and compare tree defenses across temporal and spatial scales. Collectively, these easily quantified measures of defense also offer novel ways to test overlapping, yet competing theories of plant defenses such as the growth-differentiation
balance hypothesis (Herms & Mattson 1992), the plant optimal defense theory (Rhoades & Cates 1976), and the plant age hypothesis (Bryant et al. 1992). Taken together, these hypotheses predict that plant defenses are linked to growth rate (either genetically or environmentally determined depending on the theory in question), resource availability, and plant ontogeny (immature vs. reproductive). Simply stated, the essence of these various plant defense theories is the prediction that allocation to anti-herbivore defenses results in a cost on plants’ growth and/or reproduction (Koricheva 2002). Yet to date, evidence of such trade-offs is conflicting, suggesting that fitness costs of defense are either context dependent or their detection is limited by experimental design and analysis methods (Mole 1994; Koricheva 1999, 2002); alternative experimental designs, particularly those that incorporate long-lived plants and conserved metrics of plant defense might therefore improve my ability to test plant defense theory. Finally, from a practical point of view, investigating the role of both resin ducts and bark texture as possible metrics of anatomical defense against tree-killing insects can enhance forest management—particularly forest thinning—by helping practitioners to determine tree resistance prior to harvesting.

At a larger scale, I found pine defenses among species were strongly influenced by tree growth rates but with no evidence of growth-defense tradeoffs or strong influences of climatic gradients. Yet while resin duct production was tightly and positively linked to tree growth, tree growth is a function of tree age and size such that older/larger trees tend to have smaller annual growth than younger/smaller trees under natural field conditions. The decline of growth rates with increasing tree age suggests that scaling individual tree defenses to stand-level measures of resistance to insect infestation must be done with consideration of ontogeny-defense interactions. Tree resistance is clearly dynamic, and harvesting practices and management activities from
previous generations could be a strong influence on current stand-level resistance to insects.

Future risk models for stem and bark boring insects might be dramatically improved and become more useful for predicting the spatial and temporal spread of epidemics if informed by measures of tree age and growth.
BIBLIOGRAPHY


Boom BM, Mori SA (1982) Falsification of two hypotheses on liana exclusion from tropical


Ferrenberg S, Kane JM, Mitton JB (2014) Resin duct characteristics associated with tree


