

AN ABSTRACT OF THE DISSERTATION OF

Daniel S. Ott for the degree of Doctor of Philosophy in Forest Ecosystems and Society presented on March 5th, 2019.

Title: Tree Physiological, Physical, and Chemical Characteristics Related to Spruce Beetle Colonization: Engelmann versus Blue Spruce.

Abstract approved: _____

Darrell W. Ross

Blue spruce (*Picea pungens*) and Engelmann spruce (*Picea engelmannii*) experience varying levels of spruce beetle (*Dendroctonus rufipennis* (Kirby)) colonization, yet the underlying differences and mechanisms resulting in lower colonization for blue spruce are not known. Both spruce species have important roles in subalpine ecosystems where examining changes in mortality, distribution, and growth are key to understanding climate change effects at high elevation. I use a variety of disciplines and methodologies to better understand the differing levels of spruce beetle colonization between blue spruce and Engelmann spruce.

In Chapter 2, I evaluate mixed stands of blue spruce and Engelmann spruce trees at three sites to ascertain levels of water stress and water use efficiency at the time of the study and for the previous five years. This involved measuring water potential (Ψ_p) and carbon stable isotope ratios ($\delta^{13}\text{C}$) obtained from tree-ring cellulose. While water potential did not differ significantly between blue spruce and Engelmann spruce, water potential did follow predictable seasonal precipitation patterns. Carbon stable isotope ratios were corrected for atmospheric ratios ($\Delta^{13}\text{C}$) giving an estimate of discrimination, which is directly related to intrinsic water use efficiency (iWUE). Higher iWUE implies greater relative stomatal limitation of photosynthesis. Discrimination was higher in blue spruce than Engelmann spruce. This demonstrates higher iWUE in Engelmann spruce, which may affect growth and tree carbon-based defenses related to spruce beetle colonization.

Tree physical attributes associated with spruce beetle colonization were also compared. Bark and phloem thickness may conversely provide both a barrier and a habitat for spruce beetle colonization. Bark was thicker in blue spruce compared to Engelmann spruce, while phloem was thinner for blue spruce compared to Engelmann spruce. Resin flow has been shown to encapsulate pioneering beetles, slowing or halting colonization. Resin flow was higher in blue spruce compared to Engelmann spruce. These physical attributes may indicate that blue spruce has higher constitutive defenses against spruce beetle.

Other tree attributes that influence spruce beetle colonization are related to tree chemistry, in particular terpene compounds. Volatile terpenes affect spruce beetle host tree selection, and phloem-based terpenes may affect spruce beetle aggregation as precursors to pheromones. In addition, specific phloem-based terpenes negatively affect spruce beetle survival and reproduction due to toxic effects. While species-level significance was rare in volatile terpene comparisons, trends indicate overall higher rates of volatile terpenes emanating from Engelmann spruce than blue spruce. Phloem terpenes exhibited species-level differences, with blue spruce having higher concentrations of the most abundant terpenes, potentially toxic terpenes like 3-carene, and total terpenes. These results may indicate less tree attraction and higher levels of defense for blue spruce than Engelmann spruce.

To evaluate the effect of spruce beetle vision on tree colonization, I measured bark reflectance of the two species and analyzed possible spruce beetle visual cues. Bark reflectance was higher in Engelmann spruce than in blue spruce and highest for both species in the visible spectrum. Electro-Retino Gram (ERG) results indicate spruce beetle responds to multiple wavelengths of the visual spectrum including violet/purple, blue, green, and red. Spruce beetles also responded to wavelengths outside the visual spectrum in the infrared. Due to spruce beetle responding to multiple wavelengths of light and no difference between bark reflectance trends, it is unclear if the spruce beetle makes a visual selection of Engelmann spruce rather than blue spruce.

The final part of Chapter 2 compares the effects of synthetic pheromone lures on colonization at the two sites with spruce beetle present. A subset of trees from

both species were randomly selected to receive spruce beetle lures. As expected, lures significantly increased spruce beetle landing and colonization on Engelmann spruce. However, even with the presence of spruce beetle lures, fewer blue spruce trees were landed upon and colonized than Engelmann spruce.

My dissertation demonstrates a unique approach to compare spruce beetle colonization between two species, utilizing physiological, physical, chemical, and spectral components of both tree species to better understand differences in spruce beetle colonization. Change in climate may affect physiological mechanisms including carbon allocation and resin flow resulting in changes to both spruce species' resistance to spruce beetle colonization. Factors that inhibit colonization and are more species specific, such as thin phloem, thick bark, and bark reflectance, are likely unchanged under future climate predictions. This study will provide researchers and land managers vital information and establish a knowledgebase for future scientific studies relating to spruce beetle colonization in blue and Engelmann spruce.

©Copyright by Daniel S. Ott
March 5, 2019
All Rights Reserved

Tree Physiological, Physical, and Chemical Characteristics Related to Spruce Beetle
Colonization: Engelmann versus Blue Spruce

by
Daniel S. Ott

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented March 5, 2019
Commencement June 2019

Doctor of Philosophy dissertation of Daniel S. Ott presented on March 5, 2019

APPROVED:

Major Professor, representing Forest Ecosystems and Society

Head of the Department of Forest Ecosystems and Society

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Daniel S Ott, Author

ACKNOWLEDGEMENTS

I am very thankful for all the help I received during my PhD research and my time in Corvallis. I would first like to thank Dr. Darrell Ross for guiding me and always being supportive throughout the PhD process. Darrell wasn't just my advisor, but also a friend. Thanks to my committee members Chris Fettig, Rick Meinzer, and Steve Munson who provided me with a knowledge base anytime I had questions and who helped me in the lab and field. Next I would like to thank everyone at Forest Health Protection in Ogden, Utah for giving me an outlet for my Forestry inclinations, helping me with field work, and encouraging me to continue with my studies.

I also thank everyone who have supported me at Oregon State University and in the College of Forest Ecosystems and Society. During my time at OSU I have made me friends that not only explored Oregon with me during outdoor recreation adventures, but also were sounding boards for my problems in and out of school.

Finally, thanks to my parents and Kat Morici. This degree has been a long time in the making and they never stopped supporting me.

TABLE OF CONTENTS

| | <u>Page</u> |
|---|-------------|
| 1. INTRODUCTION | 1 |
| 1.1 References..... | 8 |
| 2. PHYSIOLOGICAL, PHYSICAL, AND CHEMICAL CHARACTERISTICS OF BLUE AND ENGELMANN SPRUCES IN RELATION TO SPRUCE BEETLE COLONIZATION..... | 16 |
| 2.1 Abstract..... | 17 |
| 2.2 Introduction..... | 18 |
| 2.3 Materials and Methods..... | 20 |
| 2.4 Results..... | 29 |
| 2.5 Discussion..... | 32 |
| 2.6 References..... | 38 |
| 3 Conclusion | 56 |
| 3.1 References..... | 57 |

LIST OF FIGURES

| <u>Figure</u> | <u>Page</u> |
|--|-------------|
| Figure 1. Map of research sites. | 46 |
| Figure 2. Water potential in (MPa) for Engelmann and blue spruces at midday (A) and predawn (B) during the summer of 2015. Black diamonds represent the mean for each species by month. | 47 |
| Figure 3. Mean bark thickness of blue spruce and Engelmann spruce separated at three study sites in Utah and Wyoming. Error bars (SE)..... | 48 |
| Figure 4. Phloem and volatile terpenes in blue and Engelmann spruces. A. Mean phloem terpenes (bars=SE) (n=41 blue, n=39 Engelmann). B. Mean volatile terpenes (n=12). (bars=SE) | 49 |
| Figure 5. Selected ratios of phloem and volatile terpenes in blue and Engelmann spruces. A. Ratios of mean phloem terpenes (n=41 blue, n=39 Engelmann). B. Ratios of mean volatile terpenes (n=12). (bars=SE)..... | 50 |
| Figure 6. Mean bark reflectance of blue and Engelmann spruces for the visual spectrum into infrared. | 51 |

LIST OF TABLES

| <u>Table</u> | <u>Page</u> |
|--|-------------|
| Table 1. Marginal <i>F</i> test for Ψ_p (repeated in 2015) (MPa), $\Delta^{13}C$, bark thickness (mm), phloem thickness (mm), and resin amount (g) for Engelmann spruce and blue spruce at three sites in Utah and Wyoming. Statistically significant results are in bold. | 52 |
| Table 2. Marginal <i>F</i> Test for volatile and phloem based terpenes and ratios of specific terpenes by species, site, and the interaction of species by site. For phloem terpenes, same variable as volatile unless indicated. Statistically significant results are in bold. | 53 |
| Table 3. Species frequency tests (chi squared, Fisher’s exact) of binary variables for Engelmann and blue spruces. Lure 2014 (9 per species), Landing 2014, Colonization 2014 (trees with attempted colonization), Lure 2015, Landing 2015, Colonization 2015. Site specific data is also included (Lost Mill and Shingle Creek). Lures were used at both Lost Mill (Engelmann = 2 Lures, Blue = 4 Lures) and Shingle Creek (Engelmann = 7 Lures, Blue = 8 Lures) in 2015. Percentage (%) is for trees if variable A and variable B (e.g. the likelihood if a lure was placed on a tree then it was landed upon). Count (#) is data for if variable A and variable B (as described for percentage). Degrees of freedom (<i>df</i>) is 1 for all variables. No statistical test could be complete for blue at Shingle Creek in 2015 because there was no variation (represented by “.”) Statistically significant results in bold. | 55 |

Spruce Tree Characteristics Influencing Spruce Beetle Colonization

1. INTRODUCTION

Climate change predictions indicate an overall warming trend for higher elevations (Chmura et al. 2011, Intergovernmental Panel on Climate Change 2014). It is important to understand the potential effects this warming may have on bark beetle and tree interactions so that land managers can meet future resource objectives in high elevation forests. Over recent decades, natural disturbances (e.g. insect outbreaks, wildfires, heatwaves, drought) have been increasing in frequency and/or severity in western North America (Allen et al. 2010, Bentz et al. 2010, Allen et al. 2015, Kolb et al. 2016). Increases in average monthly temperatures and extended periods of drought result in tree physiological stress and enhance bark beetle development (Bentz et al. 2010). This, in combination with land management practices that have increased tree density and diameter, and altered forest species composition over large areas, has resulted in widespread, historically unprecedented bark beetle outbreaks with millions of hectares of tree mortality killed by native bark beetles (Bentz et al. 2009, Bentz et al. 2010). These changes are having more extreme impacts at high elevations and/or latitudes where few tree species can survive, such as spruce forests in the Rocky Mountains of the United States (DeRose and Long 2007, Hebertson and Jenkins 2008). There is limited research on regeneration of spruce trees and forests at high elevations above 2000 meters, but studies indicate that greater than average high temperatures along with a decrease in precipitation, result in range contraction and loss of suitable habitat (DeRose and Long 2007, Hebertson and Jenkins 2008, Bentz et al. 2010, Ryan 2011, DeRose and Long 2012, Stocker et al. 2013, Kolb et al. 2016).

Engelmann spruce (*Picea engelmannii*) is one of a few tree species found at high elevations in the Rocky Mountains and, consequently, is a critical species for many ecological processes and provides important ecological services to humans (Alexander 1987, Negron et al. 2008). Blue spruce (*Picea pungens*) is found at lower elevations and on more mesic sites than Engelmann spruce (Schmid and Frye 1977, Fechner 1990). High elevation conifer forests hold snow pack through much of the

growing season, providing an important source of water in much of the arid western United States. Most of the spruce forests in the West occur on public land and provide critical habitat for many animal species, including hiding cover for ungulates, nesting for birds, and hold water for aquatic species. A variety of eco-tourism activities in these forest types can also serve as crucial revenue sources for small mountain communities (Grilli et al. 2014), which in turn provide financial support for education and land conservation.

While Engelmann spruce has been experiencing spruce beetle (*Dendroctonus rufipennis* (Kirby)) outbreaks over the last 25 years in the Intermountain west and Alaska, blue spruce is rarely colonized by spruce beetle in most forest stands, including mixed stands with Engelmann spruce (Massey and Wygant 1954, Schmid and Frye 1977). With the exception of blue spruce and black spruce (Hansen et al. 2016), most spruce species found within the geographic range of the spruce beetle are highly susceptible to colonization. The characteristics of blue spruce that contribute to low spruce beetle colonization are unknown.

Several studies propose that bark beetle outbreaks will be more frequent and severe under a warmer, drier climate and that some bark beetle species will expand their range with the predicted changes in climate (Carroll et al. 2003, Bentz et al. 2010). Therefore, climate change will likely alter the interactions between spruce and spruce beetles, making it imperative to investigate the mechanisms underlying low colonization in blue spruce to better predict how colonization patterns might change in the future. Comparing mechanisms underlying colonization in a species that is highly colonized and one that is rarely colonized will help us better understand and predict colonization patterns under future climates.

A warming climate may increase spruce beetles' rate of population growth, rate of expansion, and colonization success. In fact, research has shown warming temperatures can decrease a spruce beetle's average lifecycle from two years to one (Bentz et al. 2010). The overall effects of climate change on spruce beetle and spruce forests are difficult to predict, but blue spruce habitats will likely become warmer and drier (Chmura et al. 2011), increasing the water stress of blue spruce trees and possibly increasing susceptibility to spruce beetle colonization (McDowell et al.

2008, Anderegg et al. 2015). Already, drought has been linked to spruce beetle colonization success in Engelmann spruce (Berryman 1972, Hebertson and Jenkins 2008, Hart et al. 2013, Jenkins et al. 2014).

Water is an integral resource for trees and drives a number of important biological and physiological processes, including photosynthesis. Water is taken up by roots from the soil, then travels through the stems in xylem tissue to the leaves. At the leaf level, water is lost to the atmosphere in exchange for carbon dioxide (CO₂) through stomata, small pores on leaves. During photosynthesis, plants use this CO₂ to produce photosynthates (sugars), which are then used for growth, respiration, and other metabolic processes, such as the production of defensive compounds. When stomata are open, CO₂ is taken up for photosynthesis while water is lost to the atmosphere through evaporation or transpiration. This creates a negative tension gradient that moves water through the hydraulic system along the soil-tree-atmosphere continuum. This negative tension is measured as water potential (Ψ_p) (Lambers et al. 2008). Water potential is an important variable that assists in estimating tree water stress, as the tension gets progressively negative with water limitations. This tension gradient from the soil to the top of the tree is greatest during peak daylight hours because the combination of sunlight, warmer temperatures, and open stomata results in increased loss of water from the foliage. Although water loss occurs daily, it can also have a cumulative effect over time as multiple days of water stress deplete soil moisture and may cause higher water stress to occur earlier each day (Lambers et al. 2008, Woodruff et al. 2016). As a plant becomes water stressed, it begins to close its stomata to reduce water vapor loss. However, stomatal closure also depresses rates of photosynthesis and decreases sugar production via photosynthesis (Lambers et al. 2008).

Carbon has two stable isotopes, carbon-12 and carbon-13. As CO₂ is taken into the leaf, Rubisco in the chloroplasts discriminates against carbon-13 while photosynthesizing. Partial stomatal closure causes the leaf interior to be more isolated from the external CO₂ pool, so there is less opportunity to discriminate. This results in a higher ratio of carbon-13 to carbon-12 fixed through photosynthesis (Silva et al. 2015). The ratio of these two isotopes ($\delta^{13}\text{C}$) is in the tissue of each plant,

including tree rings (Stuiver et al. 1984, Farquhar et al. 1988, Meinzer et al. 1990, Meinzer et al. 1994, McCarroll and Loader 2004, Cernusak et al. 2013).

Discrimination against carbon-13 or stable carbon isotope discrimination ($\Delta^{13}\text{C}$) is quantified by changes in atmospheric CO_2 ($\delta^{13}\text{C}$), the relative abundance of carbon-13 decreases due to human activity increasing carbon-12. Drought responses and intrinsic water use efficiency can be estimated from $\Delta^{13}\text{C}$. Intrinsic water use efficiency can be defined as a ratio of carbon assimilation to stomatal conductance (Lambers et al. 2008). This aids in understanding environmental effects on estimates of photosynthesis, stomatal conductance, and resulting intrinsic water use efficiency (McCarroll and Loader 2004).

The environment and microenvironment of a tree are linked to tree physiological stress and may also be linked to a tree's defensive capacity (Raffa and Berryman 1982, Lorio Jr. 1993). Water availability and photosynthetic performance affect the production of carbon-based defenses, such as preformed resin that flows from wounds and defensive secondary metabolites such as terpenes, furanocoumarins, and polyphenolics (Berenbaum et al. 1986, Rocchini et al. 2000, Franceschi et al. 2005). The growth differentiation balance hypothesis GDBH as reviewed in Herms and Mattson (1992), discusses tradeoffs between growth and plant defense. A plant has three major allocations of carbon, the first two being for growth through cell division and cell enlargement and the final, cell differentiation, is related to defense (Lorio Jr 1986). Cell differentiation can be chemical or morphological changes that to cell maturation and specialization (Herms and Mattson 1992). GDBH predicts drought to have non-linear impacts on carbon-based plant defenses that require carbohydrates to support metabolic costs of synthesis. During mild to moderate drought stress, predictions indicate an increase in carbon-based defense due to excess carbohydrates available as plant growth suffers. However, prolonged periods of intense water stress causes plants to close stomata reducing photosynthesis and carbohydrates supply, affecting all plant processes including defense.

Further influencing carbon allocation for plant defense is natural selection from environmental pressures of herbivory over time and competition from other plants for growing space. Defensive differences likely vary more by species than due

to environmental conditions due to the coevolved nature of many of these defensive traits. Many conifer species have been shown to produce traumatic resin flow in response to wounding by herbivorous predators (Lombardero et al. 2000, Faldt et al. 2003, Klepzig et al. 2005, Lombardero et al. 2006). This resin flow can push a pioneering beetle out of an entrance tunnel and encapsulate it in sticky resin, often leading to death of the beetle (Roberds et al. 2003). These resins also contain defensive compounds, such as terpenes, that are toxic to the beetles, interfering with metabolism if the beetles survive (Trapp and Croteau 2001).

Terpenes are present in the phloem tissue of trees where adult beetles construct egg galleries and where larvae feed and spend the majority of their lifecycle (Ott et al. 2011). Some terpenes have been shown to be toxic to bark beetles at high concentrations (Harris et al. 1983, Huber and Borden 2001, Alfaro et al. 2002). However, beetles can use a combination of terpenes to produce pheromones for communication with conspecifics (Byers 1995, Pureswaran et al. 2008). These combinations result in aggregation and may change to antiaggregation once a tree is colonized (Gillette and Munson 2009). In addition, volatile tree compounds including terpenes affect bark beetle aggregation (Gray et al. 2015, Giunta et al. 2016). For particular species of bark beetle, pheromones are not used and volatile terpenes are the beetles only chemical cue for host finding (Byers 1995). Therefore, the differences in quantity and ratio of terpenes a tree produces can have a considerable impact on bark beetle establishment and reproductive success (Smith 1967, Harris et al. 1983, Smith 2001, Huber et al. 2004, Huber and Bohlmann 2006). While some studies have shown this to be important, other studies suggest random landing (Hynum and Berryman 1980, Byers 1996) and the physical traits of a tree that pioneering beetles encounter during host tree selection may be just as important (Herms and Mattson 1992, Franceschi et al. 2005). These traits include growth rate (Hard 1985), bark and phloem thickness (Graf et al. 2012), and resin flow (Phillips and Croteau 1999, Rocchini et al. 2000, Trapp and Croteau 2001, Roberds et al. 2003) among others.

Like other insect species, bark beetles preferentially select tree hosts that provide an adaptive advantage. It is thought that beetles use a combination of

olfaction, vision, gustation, and random landing to sense, locate, and evaluate the suitability of host trees. Emerald ash borer (*Agrilus planipennis*) have been shown to respond to specific wavelengths of light emitted/reflected by the host tree (Crook et al. 2009). Some butterflies have evolved blue-absorbing pigments in their vision to find host flowers (Wakakuwa et al. 2010). Many studies have indicated insects have photoreceptors for the UV, violet, blue, green, and red regions of the spectrum (Crook et al. 2009, Belušič et al. 2010, Pirih et al. 2010, Stavenga 2010). Most studies on insect vision have been conducted on pollinators and long-studied species such as fruit flies (*Drosophila melanogaster*). Work has begun on coleopterans, specifically with the red palm weevil (*Rhynchophorus ferrugineus*), but bark beetle vision has yet to be fully explored (Ilić et al. 2016). Older studies indicate beetles have relatively simple vision with fewer ommatidia than many other insects and with possibly only two color receptors in the eyes (Byers 1995). Studies have indicated that bark beetles are first attracted to dark silhouettes, then land on the bark of a tree for host selection through gustation and smell (Shepherd 1966, Byers 1995, Strom et al. 2001, Safranyik and Carroll 2006).

Bark is the first line of protection and defense for trees during bark beetle host selection. Beetles need rough bark areas or crevices for creating head pressure to begin boring through the bark during initial colonization attempts (Shepherd 1965). Once the beetle begins entry, other physical structures of the tree serve as additional barriers to colonization. This includes lignin (Wainhouse et al. 1990), resin stores in both axial and radial ducts (Gaylord et al. 2015), antifeedant structures such as stone cells (Franceschi et al. 2005), and the overall thickness of bark as a beetle uses energy reserves to masticate through bark tissue (Graf et al. 2012, Raffa et al. 2015). In addition, phloem thickness and quality play a role in bark beetle success. Phloem contains possible toxins in the form of terpenes. However, phloem with bark beetle associated fungi (Davis et al. 2019), is the primary food source and habitat for bark beetle larvae and the habitat where bark beetles spend the majority of their lives. Therefore, phloem thickness, nutritional quality, and propensity to propagate bark beetle associated fungi play a role in beetle colonization success (Ayres et al. 2000, Davis et al. 2018).

The difference between Engelmann spruce and blue spruce in spruce beetle colonization is also related to beetle and tree life history and evolution. Many studies indicate selective pressures on trees cause defenses to arise, so as plants evolve more defenses, insects subsequently adapt, resulting in coevolution in a genetic arms race for survival (Ehrlich and Raven 1964, Raffa and Berryman 1983, Franceschi et al. 2005). Alternatively, some studies have proposed non-reciprocal relationships, where certain taxa of plants have adapted defenses and some have not, also known as sequential evolution (Jermy 1976). Within this latter theory, insects colonize some taxa of plants, but not others, allowing the uncolonized plants to propagate and for insects to ultimately adapt to these new taxa and become specialists. Thompson (2005) developed a modern theory indicating coevolution and sequential evolution are occurring in a geographic mosaic of hot spots of coevolution and cold spots of sequential evolution. This has been observed in parsnip webworm (*Depressaria radiella*) populations (Berenbaum et al. 1986) and in geographically separated half-sibling families of lodgepole pine (*Pinus contorta*) (Ott et al. 2011).

The study described in Chapter 2 will 1) compare the physiological, physical, and chemical defenses of Engelmann spruce and blue spruce, and 2) improve our understanding of bark beetle host selection and tree characteristics related to bark beetle colonization success under future climate change including warmer and drier climates. Previous studies have identified tree physical and chemical characteristics that affect the success of subcortical phloem-feeding herbivores like bark beetles. Others have compared tree species-level variation in water stress and water use efficiency (WUE). However, very few studies have linked the physiological, physical, and chemical characteristics of tree species in relation to insect herbivory. This study uses a unique combination of techniques and approaches to predict spruce beetle transference and success in a novel host, blue spruce. Comparing the mechanisms that affect spruce beetle colonization in Engelmann spruce versus blue spruce may help researchers and land managers prevent or reduce tree mortality during future spruce beetle outbreaks under a changing climate and predict future bark beetle colonization in other regions under future climates.

1.1 References

- Alexander, R. R. 1987. Ecology, silviculture, and management of the Engelmann spruce-subalpine fir type in the central and southern Rocky Mountains. Page 144 *in* R. M. Burns and B. H. Honkala, editors. Agriculture Handbook No. 659. USDA Forest Service, Washington D.C.
- Alfaro, R. I., J. H. Borden, J. N. King, E. S. Tomlin, R. L. McIntosh, and J. Bohlmann. 2002. Mechanisms of resistance in conifers against shoot infesting insects. Pages 105-130 *Mechanisms and deployment of resistance in trees to insects*. Springer.
- Allen, C. D., D. D. Breshears, and N. G. McDowell. 2015. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere* **6**:1-55.
- Allen, C. D., A. K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D. D. Breshears, and E. T. Hogg. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**:660-684.
- Anderegg, W. R., J. A. Hicke, R. A. Fisher, C. D. Allen, J. Aukema, B. Bentz, S. Hood, J. W. Lichstein, A. K. Macalady, and N. McDowell. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* **208**:674-683.
- Ayres, M. P., R. T. Wilkens, J. J. Ruel, M. J. Lombardero, and E. Vallery. 2000. Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi. *Ecology* **81**:2198-2210.
- Belušič, G., P. Pirih, and D. G. Stavenga. 2010. Photoreceptor responses of fruitflies with normal and reduced arrestin content studied by simultaneous measurements of visual pigment fluorescence and ERG. *Journal of Comparative Physiology A* **196**:23.
- Bentz, B., J. Logan, J. MacMahon, C. D. Allen, M. Ayres, E. Berg, A. Carroll, M. Hansen, J. Hicke, and L. Joyce. 2009. Bark beetle outbreaks in western North America: Causes and consequences. Page 42 *in* *Bark Beetle Symposium*. University of Utah Press, Salt Lake City, UT.
- Bentz, B. J., J. Regniere, C. J. Fettig, E. M. Hansen, J. L. Hayes, J. A. Hicke, R. G. Kelsey, J. F. Negron, and S. J. Seybold. 2010. Climate Change and Bark Beetles of the Western United States and Canada: Direct and Indirect Effects. *BioScience* **60**:602-613.

- Berenbaum, M. R., A. Zangerl, and J. Nitao. 1986. Constraints on chemical coevolution: wild parsnips and the parsnip webworm. *Evolution* **40**:1215-1228.
- Berryman, A. A. 1972. Resistance of conifers to invasion by bark beetle-fungus associations. *BioScience* **22**:598-602.
- Byers, J. A. 1995. Host-tree chemistry affecting colonization in bark beetles. *Chemical Ecology of Insects* **2**:154-213.
- Byers, J. A. 1996. An encounter rate model of bark beetle populations searching at random for susceptible host trees. *Ecological Modelling* **91**:57-66.
- Carroll, A. L., S. W. Taylor, J. Regniere, and L. Safranyik. 2003. Effects of climate change on range expansion by the mountain pine beetle in British Columbia. Pages 223-232 in *Mountain Pine Beetle Symposium: Challenges and Solutions*. Natural Resources Canada, Information Report BC-X-399, Victoria, Kelowna, BC.
- Cernusak, L. A., N. Ubierna, K. Winter, J. A. Holtum, J. D. Marshall, and G. D. Farquhar. 2013. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist* **200**:950-965.
- Chmura, D. J., P. D. Anderson, G. T. Howe, C. A. Harrington, J. E. Halofsky, D. L. Peterson, D. C. Shaw, and J. B. S. Clair. 2011. Forest responses to climate change in the northwestern United States: ecophysiological foundations for adaptive management. *Forest Ecology and Management* **261**:1121-1142.
- Crook, D. J., J. A. Francese, K. E. Zylstra, I. Fraser, A. J. Sawyer, D. W. Bartels, D. R. Lance, and V. C. Mastro. 2009. Laboratory and field response of the emerald ash borer (Coleoptera: Buprestidae), to selected regions of the electromagnetic spectrum. *Journal of Economic Entomology* **102**:2160-2169.
- Davis, T. S., F. B. Horne, J. C. Yetter, and J. E. Stewart. 2018. Engelmann Spruce Chemotypes in Colorado and their Effects on Symbiotic Fungi Associated with the North American Spruce Beetle. *Journal of Chemical Ecology* **44**:601-610.
- Davis, T. S., J. E. Stewart, A. Mann, C. Bradley, and R. W. Hofstetter. 2019. Evidence for multiple ecological roles of *Leptographium abietinum*, a symbiotic fungus associated with the North American spruce beetle. *Fungal Ecology* **38**:62-70.
- DeRose, R. J., and J. N. Long. 2007. Disturbance, structure, and composition: Spruce beetle and Engelmann spruce forests on the Markagunt Plateau, Utah. *Forest Ecology and Management* **244**:16-23.

- DeRose, R. J., and J. N. Long. 2012. Factors Influencing the Spatial and Temporal Dynamics of Engelmann Spruce Mortality during a Spruce Beetle Outbreak on the Markagunt Plateau, Utah. *Forest Science* **58**:1-14.
- Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18**:586-608.
- Faldt, J., D. Martin, B. Miller, S. Rawat, and J. Bohlmann. 2003. Traumatic resin defense in Norway spruce (*Picea abies*): Methyl Jasmonate-induced terpene synthase gene expression, and cDNA cloning and functional characterization of (+)-3-carene synthase. *Plant Molecular Biology* **51**:119-133.
- Farquhar, G., K. Hubick, A. Condon, and R. Richards. 1988. Carbon isotope fractionation and plant water-use efficiency. Pages 21-40 *Stable Isotopes in Ecological Research*. Springer-Verlag, New York.
- Fechner, G. H. 1990. *Picea pungens* Engelm. blue spruce. Pages 238-249 in R. M. Burns and B. H. Honkala, editors. *Silvics of North America*. USDA Forest Service, Washington D.C.
- Franceschi, V. R., P. Krokene, E. Christiansen, and T. Krekling. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist* **167**:353-376.
- Gaylord, M. L., T. E. Kolb, and N. G. McDowell. 2015. Mechanisms of piñon pine mortality after severe drought: a retrospective study of mature trees. *Tree Physiology* **35**:806-816.
- Gillette, N. E., and A. S. Munson. 2009. Semiochemical sabotage: behavioral chemicals for protection of western conifers from bark beetles. Pages 85-109 in *The Western Bark Beetle Research Group: A Unique Collaboration with Forest Health Protection*. USDA Forest Service, Portland, Oregon.
- Giunta, A. D., J. B. Runyon, M. J. Jenkins, and M. Teich. 2016. Volatile and Within-Needle Terpene Changes to Douglas-fir Trees Associated With Douglas-fir Beetle (Coleoptera: Curculionidae) Attack. *Environmental Entomology* **45**:920-929.
- Graf, M., M. Reid, B. Aukema, and B. Lindgren. 2012. Association of tree diameter with body size and lipid content of mountain pine beetles. *The Canadian Entomologist* **144**:467-477.
- Gray, C. A., J. B. Runyon, M. J. Jenkins, and A. D. Giunta. 2015. Mountain Pine Beetles Use Volatile Cues to Locate Host Limber Pine and Avoid Non-Host Great Basin Bristlecone Pine. *PLoS ONE* **10**:e0135752.
- Grilli, G., A. Paletto, and I. De Meo. 2014. Economic valuation of forest recreation in an Alpine valley. *Baltic Forestry* **20**:167-175.

- Hansen, W. D., F. S. Chapin III, H. T. Naughton, T. S. Rupp, and D. Verbyla. 2016. Forest-landscape structure mediates effects of a spruce bark beetle (*Dendroctonus rufipennis*) outbreak on subsequent likelihood of burning in Alaskan boreal forest. *Forest Ecology and Management* **369**:38-46.
- Hard, J. S. 1985. Spruce beetles attack slowly growing spruce. *Forest Science* **31**:839-850.
- Harris, L., J. Borden, H. Pierce Jr, and A. Oehlschlager. 1983. Cortical resin monoterpenes in Sitka spruce and resistance to the white pine weevil, *Pissodes strobi* (Coleoptera: Curculionidae). *Canadian Journal of Forest Research* **13**:350-352.
- Hart, S. J., T. T. Veblen, K. S. Eisenhart, D. Jarvis, and D. Kulakowski. 2013. Drought induces spruce beetle (*Dendroctonus rufipennis*) outbreaks across northwestern Colorado. *Ecology* **95**:930-939.
- Hebertson, E. G., and M. J. Jenkins. 2008. Climate factors associated with historic spruce beetle (Coleoptera: Curculionidae) outbreaks in Utah and Colorado. *Environmental Entomology* **37**:281-292.
- Hermes, D. A., and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. The quarterly review of biology **67**:283-335.
- Huber, D. P. W., and J. Bohlmann. 2006. The role of terpene synthasas in the direct and indirect defense of conifers against insect herbivory and fungal pathogens. Pages 296-308 in S. Tuzun and E. Bent, editors. *Multigenic and induced systemic resistance in plants*. Springer Science, New York City.
- Huber, D. P. W., and J. H. Borden. 2001. Protection of lodgepole pines from mass attack by mountain pine beetle, *Dendroctonus ponderosae*, with nonhost angiosperm volatiles and verbenone. *Entomologia Experimentalis et Applicata* **91**:131-141.
- Huber, D. P. W., S. Ralph, and J. Bohlmann. 2004. Genomic hardwiring and phenotypic plasticity of terpenoid-based defenses in conifers. *Journal of Chemical Ecology* **30**:2399-2418.
- Hynum, B. G., and A. A. Berryman. 1980. *Dendroctonus ponderosae* (Coleoptera: Scolytidae): pre-aggregation landing and gallery initiation on lodgepole pine. *The Canadian Entomologist* **112**:185-191.
- Ilić, M., P. Pirih, and G. Belušič. 2016. Four photoreceptor classes in the open rhabdom eye of the red palm weevil, *Rynchophorus ferrugineus* Olivier. *Journal of Comparative Physiology A* **202**:203-213.
- Intergovernmental Panel on Climate Change. 2014. IPCC Fifth Assessment Synthesis Report. IPCC 5th Assessment Synthesis Report.

- Jenkins, M., E. Hebertson, and A. Munson. 2014. Spruce beetle biology, ecology and management in the Rocky Mountains: an addendum to spruce beetle in the Rockies. *Forests* **5**:21-71.
- Jermey, T. 1976. Insect—Host-plant Relationship—Co-evolution or Sequential Evolution? Pages 109-113 *in* T. Jermey, editor. *The host-plant in relation to insect behaviour and reproduction*. Springer, Boston, MA.
- Klepzig, K. D., D. J. Robison, G. Fowler, P. R. Minchin, F. P. Hain, and H. L. Allen. 2005. Effects of mass inoculation on induced oleoresin response in intensively managed loblolly pine. *Tree Physiology* **25**:681-688.
- Kolb, T. E., C. J. Fettig, M. P. Ayres, B. J. Bentz, J. A. Hicke, R. Mathiasen, J. E. Stewart, and A. S. Weed. 2016. Observed and anticipated impacts of drought on forest insects and diseases in the United States. *Forest Ecology and Management* **380**:321-334.
- Lambers, H., F. S. Chapin, and T. L. Pons. 2008. Plant water relations. Pages 163-223 *in* *Plant physiological ecology*, editor. Springer, New York, NY.
- Lombardero, M. J., M. P. Ayres, and B. D. Ayres. 2006. Effects of fire and mechanical wounding on *Pinus resinosa* resin defenses, beetle attacks, and pathogens. *Forest Ecology and Management* **225**:349-358.
- Lombardero, M. J., M. P. Ayres, P. L. Lorio Jr., and J. J. Ruel. 2000. Environmental effects on constitutive and inducible resin defenses of *Pinus taeda*. *Ecology Letters* **3**:329-339.
- Lorio Jr, P. L. 1986. Growth-differentiation balance: a basis for understanding southern pine beetle-tree interactions. *Forest Ecology and Management* **14**:259-273.
- Lorio Jr., P. L. 1993. Environmental stress and whole tree physiology. Pages 81-100 *in* T. D. Schowalter and G. M. Filip, editors. *Beetle-Pathogen Interactions in Conifer Forests*. Academic Press Inc., San Diego.
- Massey, C., and N. Wygant. 1954. *Biology and control of the Engelmann spruce beetle in Colorado*. US Department of Agriculture, Washington.
- McCarroll, D., and N. J. Loader. 2004. Stable isotopes in tree rings. *Quaternary Science Reviews* **23**:771-801.
- McDowell, N., W. T. Pockman, C. D. Allen, D. D. Breshears, N. Cobb, T. Kolb, J. Plaut, J. Sperry, A. West, and D. G. Williams. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**:719-739.

- Meinzer, F. C., G. Goldstein, and D. A. Grantz. 1990. Carbon Isotope Discrimination in Coffee Genotypes Grown under Limited Water Supply. *Plant Physiology* **92**:130-135.
- Meinzer, F. C., Z. Plaut, and N. Z. Saliendra. 1994. Carbon Isotope Discrimination, Gas Exchange, and Growth of Sugarcane Cultivars under Salinity. *Plant Physiology* **104**:521-526.
- Negron, J. F., B. J. Bentz, C. J. Fettig, N. Gillette, E. M. Hansen, J. L. Hays, R. G. Kelsey, J. E. Lundquist, A. M. Lynch, R. A. Progar, and S. J. Seybold. 2008. US Forest Service bark beetle research in the western United States: Looking toward the future. *Journal of Forestry* **106**:325-331.
- Ott, D. S., A. D. Yanchuk, D. P. Huber, and K. F. Wallin. 2011. Genetic variation of lodgepole pine, *Pinus contorta* var. *latifolia*, chemical and physical defenses that affect mountain pine beetle, *Dendroctonus ponderosae*, attack and tree mortality. *Journal of Chemical Ecology* **37**:1002-1012.
- Phillips, M. A., and R. B. Croteau. 1999. Resin-based defenses in conifers. *Trends in plant science* **4**:184-190.
- Pirih, P., K. Arikawa, and D. G. Stavenga. 2010. An expanded set of photoreceptors in the Eastern Pale Clouded Yellow butterfly, *Colias erate*. *Journal of Comparative Physiology A* **196**:501-517.
- Pureswaran, D. S., B. T. Sullivan, and M. P. Ayres. 2008. High individual variation in pheromone production by tree-killing bark beetles (Coleoptera: Curculionidae: Scolytinae). *Naturwissenschaften* **95**:33-44.
- Raffa, K. F., and A. A. Berryman. 1982. Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated microorganisms. *Environmental Entomology* **11**:486-492.
- Raffa, K. F., and A. A. Berryman. 1983. The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). *Ecological Monographs* **53**:27-49.
- Raffa, K. F., J.-C. Gregoire, and B. S. Lindgren. 2015. Natural history and ecology of bark beetles. Pages 1-40 *in* F. E. Vega and R. W. Hofstetter, editors. *Bark Beetles*. Elsevier.
- Roberds, J. H., B. L. Strom, and F. Hain. 2003. Genetic and phenotypic variability for constitutive oleoresin flow in loblolly pine. *in* Proceedings of the 27th Southern Forest Tree Improvement Conference. USDA, Stillwater, OK.
- Rocchini, L. A., B. S. Lindgren, and R. G. Bennett. 2000. Effects of resin flow and monoterpene composition on susceptibility of lodgepole pine to attack by the

- Douglas-fir pitch moth, *Synanthedon novaroensis* (Lep., Sesiidae). *Journal of Applied Entomology* **124**:87-92.
- Ryan, M. G. 2011. Tree responses to drought. *Tree Physiology* **31**:237-239.
- Safranyik, L., and A. L. Carroll. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. Pages 3-66 *in* L. Safranyik and W. R. Wilson, editors. *The mountain pine beetle: a synthesis of biology, management, and impacts on lodgepole pine*. Natural Resources Canada, Pacific Forestry Centre, Victoria, BC.
- Schmid, J., and R. H. Frye. 1977. Spruce beetle in the Rockies. Pages 1-38. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-49.
- Shepherd, R. 1965. Distribution of attacks by *Dendroctonus ponderosae* Hopk. on *Pinus contorta* Dougl. var. *latifolia* Engelm. *The Canadian Entomologist* **97**:207-215.
- Shepherd, R. F. 1966. Factors Influencing the Orientation and Rates of Activity of *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). *The Canadian Entomologist* **98**:507-518.
- Silva, L. C., A. Gómez-Guerrero, T. A. Doane, and W. R. Horwath. 2015. Isotopic and nutritional evidence for species- and site-specific responses to N deposition and elevated CO₂ in temperate forests. *Journal of Geophysical Research: Biogeosciences* **120**:1110-1123.
- Smith, R. H. 1967. Variations in the monoterpene composition of the wood resin of Jeffrey, Washoe, Coulter and lodgepole pines. *Forest Science* **13**:246-252.
- Smith, R. H. 2001. Xylem monoterpenes of pines: Distribution, variation, genetics, function. Pages i-x. USDA Forest Service, Pacific Southwest Research Station, General Technical Report, PSW-GTR-177.
- Stavenga, D. G. 2010. On visual pigment templates and the spectral shape of invertebrate rhodopsins and metarhodopsins. *Journal of Comparative Physiology A* **196**:869-878.
- Stocker, T., D. Qin, G. Plattner, M. Tignor, S. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. Midgley. 2013. IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.
- Strom, B., R. Goyer, and P. Shea. 2001. Visual and olfactory disruption of orientation by the western pine beetle to attractant-baited traps. *Entomologia Experimentalis et Applicata* **100**:63-67.

- Stuiver, M., R. Burk, and P. D. Quay. 1984. $^{13}\text{C}/^{12}\text{C}$ ratios in tree rings and the transfer of biospheric carbon to the atmosphere. *Journal of Geophysical Research* **89**:11731-11711,11748.
- Thompson, J. N. 2005. *The geographic mosaic of coevolution*. University of Chicago Press.
- Trapp, S., and R. Croteau. 2001. Defensive resin biosynthesis in conifers. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**:689-724.
- Wainhouse, D., D. J. Cross, and R. S. Howell. 1990. The role of lignin as a defense against the spruce bark beetle *Dendroctonus micans*: Effect on larvae and adults. *Oecologia* **85**:257-265.
- Wakakuwa, M., A. Terakita, M. Koyanagi, D. G. Stavenga, Y. Shichida, and K. Arikawa. 2010. Evolution and mechanism of spectral tuning of blue-absorbing visual pigments in butterflies. *PLoS ONE* **5**:e15015.
- Woodruff, D. R., F. C. Meinzer, and K. A. McCulloh. 2016. Forest canopy hydraulics. Pages 187-217 in K. Hikosaka, U. Niinemets, and N. P. R. Anten, editors. *Canopy photosynthesis: from basics to applications*. Springer.

2. PHYSIOLOGICAL, PHYSICAL, AND CHEMICAL
CHARACTERISTICS OF BLUE AND ENGELMANN SPRUCES IN
RELATION TO SPRUCE BEETLE COLONIZATION

Daniel Ott

2.1 Abstract

The spruce beetle, *Dendroctonus rufipennis*, is a specialist herbivore that completes much of its lifecycle in host spruce trees. Most spruces found within the geographic range of spruce beetle are susceptible to colonization. The primary host in the Rocky Mountains of the United States is Engelmann spruce, *Picea engelmanni*. Blue spruce, *Picea pungens*, occurs across much of the same range but, unlike Engelmann spruce, does not experience widespread mortality due to spruce beetle. In some areas, blue and Engelmann spruce hybridize, indicating a shared evolutionary history between the species. Understanding and comparing tree characteristics that affect spruce beetles throughout their lifecycle may help to explain why beetles preferentially select, colonize, and kill Engelmann spruce over blue spruce.

In this study, host tree physiological, physical, and chemical characteristics affecting spruce beetle during the host selection and colonization processes were measured and compared in blue and Engelmann spruces. Three sites consisting of 15 trees of each species were established representing 45 Engelmann spruce and 45 blue spruce (90 total). There were significant physiological differences in tree water potential among sites and over time, but not between species. Although blue spruce tended to be more negative than Engelmann. There were statistically significant differences in tree ring carbon isotope discrimination between species and site. Blue spruce had higher discrimination than Engelmann spruce. There were significant differences in resin flow and bark and phloem thickness between the two species. Resin flow was highly variable, but was greater in blue spruce, which may act as a defense mechanism to push out colonizing spruce beetles. Bark was thicker for blue spruce and phloem was thicker for Engelmann spruce. The thicker bark and thinner phloem of blue spruce may require more energy reserves for colonization and provide less nutrition for developing larvae. Secondary metabolites, specifically terpenes, were measured in volatiles and extracted from phloem. Engelmann spruce had higher concentrations of most terpenes and total terpenes in volatiles collected around branches. These compounds may contribute to spruce beetle aggregation. Blue spruce phloem terpene concentrations were more than double those for Engelmann

spruce for the majority of terpenes and total terpenes. The higher levels in blue spruce may be toxic to spruce beetles, acting as a defense mechanism. Synthetic pheromone lures were attached to nine trees of each species in 2014 and nine Engelmann and twelve blue spruce trees and nine and twelve trees, in 2015. Spruce beetle landed on significantly more Engelmann spruce than blue spruce trees and Engelmann spruce trees with lures were more likely to be colonized. Two blue spruce trees had evidence of colonization attempts. One blue spruce was successfully colonized while the other resulted in the beetles being encapsulated in resin. Overall, 23 of 34 Engelmann spruce trees at the two sites with spruce beetle present were successfully colonized by the final study year.

In conclusion, no defense mechanism appears to protect blue spruce from spruce beetle colonization, rather my research results indicate it's a combination of many tree attributes. Physiological, physical, and chemical characteristics relate and interact to create a suite of defensive characteristics that alter spruce beetle colonization success. Developing a better understanding of blue spruce traits affecting host selection by spruce beetle will aid land managers in predicting and reducing tree mortality during future bark beetle outbreaks.

2.2 Introduction

The spruce beetle (*Dendroctonus rufipennis* (Kirby)) is the primary cause of spruce tree mortality in North America (Massey and Wygant 1954, Schmid and Frye 1977, Maroja et al. 2007). Since the 1990s, spruce beetles have killed millions of Engelmann spruce (*Picea engelmannii*) across vast landscapes (Holsten et al. 1999, Ross et al. 2001, Maroja et al. 2007, Jenkins et al. 2014) in the Rocky Mountains and Alaska. However, spruce beetle colonization of blue spruce (*Picea pungens*), in the Rocky Mountains has remained low (Massey and Wygant 1954, Schmid and Frye 1977). In the United States, blue spruce is found primarily in Colorado and Utah, but its range also extends into parts of Idaho, Wyoming, Arizona, and New Mexico. The high elevational range of blue spruce may overlap the lower elevational range of Engelmann spruce in certain environments. While the species have convergent evolutionary traits, hybridization has only been shown under laboratory conditions

where Engelmann spruce is the female (Schaefer and Hanover 1986, Ernst et al. 1990, Stine and Keathley 1990, Ledig et al. 2006). Blue spruce is commonly found on mesic sites where water isn't limiting, while Engelmann spruce is more commonly found on drier sites than blue spruce.

Climate change-induced warming and drying in blue spruce elevational zones and habitats will likely continue (Chmura et al. 2011, Stocker et al. 2013, Intergovernmental Panel on Climate Change 2014, Anderegg et al. 2015). Therefore, blue spruce may experience increasing water stress events. Rising temperatures, are likely to expand spruce beetle range and increase outbreak population size in concert with a decrease in lifecycle timing, stimulated by more degree days per year (Price 1997, Bentz et al. 2010, Hansen et al. 2011, Anderegg et al. 2015). Because tree condition and vigor have been shown to affect bark beetle colonization (Moeck et al. 1981, Hebertson and Jenkins 2008, Hart et al. 2013), these changes should result in increased spruce beetle pressure on blue spruce and perhaps creating a more susceptible secondary host, further increasing spruce beetle range and outbreak intensity, severity, and size (Bentz et al. 2010). Whether environmental or specific physiological, physical, chemical, or other characteristics cause blue spruce to avoid high levels of spruce beetle colonization is unknown.

Tree defense, resistance, tolerance, and resilience to bark beetles have been studied extensively, including studies of spruce beetle in spruce-fir forests across North America. Some studies have linked changes in tree physiology, specifically tree response to drought, to bark beetle susceptibility (Hart et al. 2013, Gaylord et al. 2015). Other studies have shown tree physical attributes and chemistry to influence bark beetle host landing (selection) and colonization (Massey and Wygant 1954, Schmid and Frye 1977, Moeck et al. 1981, Raffa and Berryman 1983, Byers 1995, Holsten et al. 1999, Wallin and Raffa 1999, Wallin and Raffa 2004, Safranyik and Carroll 2006, Ott et al. 2011). While there are many potential factors that influence bark beetle colonization, the mechanisms underlying low beetle colonization of blue spruce remain unknown.

The objective of this study was to investigate and understand the mechanism(s) that affect spruce beetle colonization of Engelmann and blue spruces

and to infer how the relationship between blue spruce and spruce beetle may change in response to climate change. Several physiological, physical, and chemical characteristics related to spruce beetle colonization and survival were compared between Engelmann and blue spruces. Specifically, the variables measured and compared between the two species were: 1) water potential and physiological variables related to water use efficiency; 2) resin production; 3) bark and phloem thickness; 4) the quantity and quality of the most abundant constitutive terpenes from volatile and phloem collections; 5) bark reflectance and beetle vision; and 6) beetle landing and colonization in response to synthetic pheromone lures.

2.3 Materials and Methods

Study Sites and Design

This study was conducted at three study sites in the Uinta-Wasatch-Cache National Forest on the Salt Lake and Heber-Kamas Ranger Districts in Utah: Shingle Creek (40.61095,-111.11794), Silver Fork (40.63474,-111.61826), and Lost Mill (40.93021,-110.75278) (Figure 1). All sites consisted of blue and Engelmann spruce and ranged in elevation from about 2300 to 2750 m. Subalpine fir (*Abies lasiocarpa*) was the only other tree species present at all sites. All sample trees were free of mechanical, insect, and disease damage and were similar in size. Individual trees > 25 cm diameter at breast height (DBH) were selected for the study because spruce beetle prefers larger, mature trees (Schmid and Frye 1977). Fifteen study trees per species were selected at each site. All sample trees were located within 500 m of each other at Shingle Creek, 150 m at Silver Fork, and 1750 m at Lost Mill. The DBH and height of each study tree were measured. The mean DBH of Engelmann spruce was 58.4 cm (SD 13.8) and blue spruce was 56.6 cm (SD 15.4). The mean height of Engelmann spruce was 26.0 m (SD 4.3) and blue spruce was 23.6 m (SD 3.4). Spruce beetles were colonizing trees at Shingle Creek and Lost Mill but not at Silver Fork. Water potential, carbon isotope ratio, bark reflectance, bark and phloem thickness, resin flow, volatile terpenes, and phloem terpenes were measured on each study tree prior to spruce beetle dispersal, host selection, and colonization in the summers of 2014 and 2015. Spruce beetle response to different wavelengths of light

was assessed with an Electro-Retino Gram (ERG). Finally, a subset of both species of spruce trees were baited with synthetic pheromone lures in 2014 and 2015 to assess bark beetle landing (selection) and colonization at Lost Mill and Shingle Creek.

Water Potential

To determine the degree of water stress, water potential (Ψ_p) was measured in June and early July in 2014 during spruce beetle dispersal. Water potential was also measured on a subset of three randomly selected uncolonized trees per species at each site near the end of the spruce beetle flight period in August 2014 to ascertain cumulative seasonal drought effects. In the summer of 2015, water potential was measured on a random subsample of six uncolonized trees per species at each site every four weeks from June 15 through September 10.

Water potential was measured by sampling sunlit branches from the upper canopy of each tree by first using a Big Shot arborist's sling shot (Sherrill Tree, Greensboro, North Carolina). A sandbag and parachute cord was launched over a branch in the upper canopy of each tree, then pulling the cord repeatedly until a branch broke free from the upper canopy. The distal 5 cm to 8 cm of the branch was cut off with a razor blade, and the branch segment was placed in a pressure chamber (Model 600, PMS, Albany, Oregon) to measure water potential. Water potential was measured on three branches per tree at midday and predawn and averaged on each sampling date (Lorio Jr. 1993, Pears 2010). Predawn measurements in 2014 occurred between 01:00 and 06:00 hrs, while in 2015 measurements occurred between 04:00 and 06:00 hrs, progressing to 06:30 hrs by September to achieve time periods closest to dawn. Midday measurements occurred between 11:30 and 15:00 hrs. Mean Ψ_p at midday and predawn were compared to assess water stress.

Carbon Isotope Ratio

Carbon isotope ratio ($\delta^{13}\text{C}$) was calculated from xylem samples collected in 2014 to determine prolonged drought or water stress over the previous five years of tree growth. Less negative carbon isotope ratios were assumed to be indicative of drought effects on relative stomatal limitation of photosynthesis and related to species

historical evolutionary drivers such as temperature and water availability. A 5 mm diameter increment borer was used to remove the most recent five years of xylem growth at DBH on the northwest side of each tree. The length of each sample was measured (mm) and the sample placed in an individually labelled coin envelope. In the laboratory, xylem samples were ground into a fine powder with an electric ball mill. Samples were placed into heat sealed pouches in groups of six. Samples were then soaked in a 2000-ml beaker of distilled water in a 95°C water bath and agitated with a magnetic stir rod for eight hours to extract soluble sugars and increase the accuracy to measure carbon in structural polymers. Samples were dried at 50°C for 48 hours prior to isotope analysis.

Approximately 0.8 mg of ground sample was placed into a tin capsule and folded closed. Isotope analysis was performed with an Isotope Ratio Mass Spectrometer (IRMS) at the College of Earth Ocean and Atmospheric Sciences (CEOAS) Stable Isotope Laboratory, Oregon State University. Carbon isotope samples were combusted using an elemental analyzer, then carbon dioxide (CO₂) gas was analyzed by continuous-flow IRMS (EA-Delta Plus IRMS). Processed samples were calibrated daily using internal standards USGS40 (glutamic acid, $\delta^{13}\text{C} = -26.389$) and the in-house standard SIL Sucrose ($\delta^{13}\text{C} = -11.85$). Multiple internal standards calibrated the isotopic composition. The check standard, IAEA-600 (caffeine), had an expected $\delta^{13}\text{C}$ value of -27.771‰ . Typical error was $\pm 0.1\text{‰}$ or better. The isotope ratio of C was the heavier isotope carbon-13 over the lighter carbon-12 (Lambers et al. 2008, Taylor et al. 2008) and was represented by delta (δ) notation in parts per thousand (‰, per mil) as given by Equation 1 (McCarroll and Loader 2004):

$$\delta^{13}\text{C} = R_{\text{sample}}/R_{\text{standard}} - 1 (\text{‰}). \quad \text{Equation 1}$$

Samples were standardized to atmospheric carbon isotope ratio ($\delta^{13}\text{C}_{\text{air}}$) using Equation 2 (Farquhar et al. 1982, McCarroll and Loader 2004) to attain discrimination values (Δ):

$$\Delta^{13}\text{C}_{\text{cell}} = \delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{cell}}/1 + \delta^{13}\text{C}_{\text{cell}}. \quad \text{Equation 2}$$

Values for C_{air} were from Scripps CO₂ Program (<http://scrippsco2.ucsd.edu/>) and McCarroll and Loader (2004). The $\Delta^{13}\text{C}$ of carbon assimilated is related to c_i/c_a and fractionation against carbon-13 during photosynthesis:

$$\Delta^{13}\text{C} = a + (b - a) (c_i/c_a), \quad \text{Equation 3}$$

where a is the fractionation effect associated with diffusion of CO₂ through the stomata (4.4‰), b is fractionation associated with carboxylation by the enzyme RUBISCO (27‰) and c_i/c_a is the weighted mean ratio of intercellular CO₂ concentration (c_i) to the concentration in the atmosphere or air (c_a). Discrimination ($\Delta^{13}\text{C}$) is related to water stress and intrinsic water use efficiency (WUE) that may affect spruce beetle colonization (Farquhar et al. 1989a). Equation 3 relates variation in discrimination with c_i/c_a to A/g or photosynthesis and stomatal conductance respectively (Farquhar et al. 1989b).

Bark and Phloem Thickness and Resin Flow

To measure resin flow, a metal funnel was attached to the east and west sides of each tree at DBH in July of 2014. A 10 mm diameter bark punch was used to remove bark and phloem directly above the funnel, allowing resin to flow freely. A tared 15-ml plastic centrifuge vial was attached with a u-nail directly under each funnel for resin collection. A piece of folded duct tape was attached above each funnel to keep rain out of the vial. The trees were then mechanically damaged using the 10 mm diameter bark punch by removing bark and phloem in a spiral pattern centered at DBH to simulate spruce beetle attack (Pears 2010, Krokene et al. 2010, Christiansen et al. 1999). Trees 25 to 50 cm DBH received five bark punches, trees 50 to 75 cm DBH received 10 punches, and trees 75 to 100 cm DBH received 15 punches. Individual punches were 20 to 50 cm apart. Centrifuge vials were capped and taken to the laboratory after seven days. Vials were weighed, and empty vial weight was subtracted to calculate resin weight.

A 10 mm diameter bark punch and hammer were used to remove a piece of bark and phloem on the north and south sides of each tree at DBH in July of 2014. Bark and phloem thickness were measured with a sliding stage incremental micrometer on each bark section. The two measures of bark and phloem thickness were averaged for each tree.

Phloem Terpenes

The same phloem samples removed during bark and phloem thickness measurements were placed in individually labeled plastic vials that were stored on dry ice in a cooler for transport to the laboratory. Samples were stored at -20°C at the Forest Health Protection (FHP) Laboratory in Ogden, Utah until they were shipped to the Rocky Mountain Research Station Laboratory in Bozeman, Montana for terpene analysis.

Terpene extractions were similar to Powell and Raffa (2011). Samples were removed from individual vials and kept in liquid nitrogen until they were trimmed to approximately 5 x 5 mm squares. The samples were then finely chopped with a razor blade to increase surface area for extraction. Finally, samples were placed into 2-ml FastPrep tubes (MP Biodmedical, Solon, Ohio) with 1.5 ml of cyclohexane (Page et al. 2014). Samples were sonicated at room temperature for 30 minutes and then sat for 24 hours at room temperature. The upper of 300 µl of extract solution was transferred to a GC vial for analysis, and then 1000 ng of the internal standard n-nonyl-acetate was added.

Sample analysis was performed on an Agilent 7890A GC coupled with a 5975C mass spectrometer and separated on a HP-1ms (30 m x 0.25 i.d. 0.25 µm film thickness) column. With helium as a carrier gas, the GC started at 35°C for a duration of three minutes and incrementally increased by 5°C/min to 200°C and then 25°C/min to 250°C. The quantity of terpenes was compared to internal standards using ChemStation software (Agilent Technologies, Santa Clara, California), and compound identification was confirmed by comparison of retention times and mass spectra of commercial standards or by the NIST 08 Mass Spectral Search Program.

Phloem samples were dried at 25°C for one week and expressed on a dried mass of phloem basis.

Volatile Terpenes

In 2015, volatile terpenes were collected from one randomly selected lower branch on each of the three trees (Page et al. 2012, Gray et al. 2015, Giunta et al. 2016) per species at Silver Fork and Shingle Creek. Lost Mill was excluded due to time constraints and high mortality. On each tree, a lower branch was enclosed in a clear Teflon bag attached to a portable vacuum pump (SKC Airlite Sampler Model 110-100) with polyvinyl chloride tubing and a volatile trap (Volatile Assay Systems, Rensselaer, New York) containing 30 mg of adsorbent material HayeSep-Q (Restek, Bellefonte, Pennsylvania). Vacuum pumps sampled air at 0.5 l min⁻¹ for 30 minutes. Within-needle terpene concentrations, were quantified by removing needles and weighing them (nearest tenth in g). The needles were placed in plastic bags, and stored at -80°C until processing. Terpene analysis occurred at the Rocky Mountain Research Laboratory in Bozeman, Montana.

Following procedures in Page et al. (2012), 200 µl of dichloromethane and 1,000 ng of n-nonyl-acetate as an internal standard were used to elute volatiles from traps. An Agilent 7890A GC/ 5975 mass was used to analyze samples. The carrier gas was helium. The temperature program started at 35°C for three minutes then increased 5°C per minute until the temperature reached 125°C. Finally, temperature increased 25°C per minute up to 250°C. Volatiles were quantified by internal standard comparison using ChemStation software (Agilent Technologies, Santa Clara, California). Rates of volatile emissions were reported based on fresh needle weight. Terpenes were identified using the NIST 08 Mass Spectral Search Program (National Institute of Standards and Technology, Gaithersburg, Maryland) and confirmed by retention time comparison with mass spectra of commercial standards. When commercial standards were not available, compound identification was matched if probability was greater than 0.5 on NIST 08.

Terpenes in needles were extracted by freezing five grams of needles from each sample in liquid nitrogen and grinding the frozen needles into a fine powder

with a mortar and pestle. Approximately 0.1 g of sample powder was transferred into a 2-ml FastPrep tube (MP Biomedicals, Solon, Ohio), 1.5 ml of cyclohexane was added to the tube, and the tube was capped and put in the sonicator for 20 minutes at room temperature. FastPrep tubes were centrifuged for 1 min at 13,000 g, and the top 200 μ l of liquid sample was transferred into GC vials for analysis. Compounds were identified by comparison with retention times for known terpenes for within-needle terpene concentrations and are reported on fresh needle weight basis.

Bark Reflectance

Bark reflectance was measured on sample trees at the Lost Mill site to determine whether visual differences might account for spruce beetle preference for Engelmann spruce over blue spruce in the host selection process. In July 2015, a representative bark sample approximately 5 x 10 cm was removed from each tree at Lost Mill just below DBH. (Lost Mill alone was selected for sampling due to management constraints.) Bark samples were transported to the University of Idaho for analysis. Samples were placed on cardboard painted black with Krylon® Ultra-Flat paint (#KP1602) chosen because it does not cause reflectance readings with the spectrometer. Samples were tested outside in full sun and relative bark reflectance was measured using a FieldSpec Pro Full Range model spectrometer (ASD Inc., Boulder, Colorado) with a spectral measurement range of 350 to 2500 nanometers (nm). Relative reflectance was calculated as a percentage of a white standard from ASD Inc. A 3 cm distance was maintained between fiber optic cables and bark samples using a tri-pod at a 90-degree angle to the samples. Each sample's spectral relative reflectance (%) was recorded at each wavelength (nm). Each sample was measured five times, and readings were averaged to reduce potential measurement errors. Bark reflectance was averaged by species and integrated over peaks to make comparisons with spruce beetle Electro-Retino Gram (ERG) data.

Electro-Retino Gram

Spruce beetle response to different wavelengths of light was assessed with an ERG. In October 2014, eight 30-cm bolts were cut around DBH from spruce beetle

colonized Engelmann spruce in Utah, but not from study sites. The ends of the bolts were waxed to prevent desiccation and placed in cold storage (-4°C) for the winter to prevent spruce beetle emergence until ERG testing. In July and August of 2015, bolts were taken out of cold storage and held in emergence boxes under laboratory conditions (~21°C) for one to two months until adult beetles emerged. Beetles were collected, sexed, and put into a dark environment until ERG testing. Sixty-five beetles were tested for ERG response. Twenty-three beetles had no response. Twelve beetles were sexed as female and seven were sexed as male, for the remaining beetles sex was not determined. An electrode was inserted into the ommatidia of each beetle and another behind the head to create a full circuit. A full light spectrum was separated into individual wavelengths of light using a monochromator. Individual beetles were placed into the ERG system where they were exposed to light wavelengths from 300 to 1500 nm (from ultra violet to infrared), and wavelengths of beetle depolarization, where the full circuit signal drops, were recorded using electroantennogram EAG 2000 data recording software (Syntech®, Kirchzarten, Germany). Wavelengths of light that elicited a response from spruce beetles were counted and compared with integrated bark reflectance measurements.

Spruce Beetle Landing and Colonization

In July 2014, spruce beetle two-component pheromone lures consisting of frontalin and 1-methylcyclohex-2-en-1-ol (Synergy Semiochemicals Corp. Burnaby, British Columbia, Canada, product #3123) were attached to nine non-colonized study trees of each species at Lost Mill during spruce beetle flight. Lures were attached on the bole of each selected tree at eight feet. Trees were randomly selected for lure (bait) placement with the stipulation that a tree containing a lure had to be at least 100 m from trees without lures to decrease lure spill over onto other trees. At the end of the beetle flight period in September, all study trees were evaluated for spruce beetle colonization by looking for entrance holes with/without boring dust and/or pitch. At the end of June 2015, spruce beetle tree baiting and assessment of colonization success was repeated at Lost Mill with two Engelmann spruce and four blue spruce since many of the Engelmann spruce study trees died in 2014. Also, at the end of

June 2015, lures were attached to seven Engelmann spruce and eight blue spruce trees at Shingle Creek. No pheromone baiting was conducted at Silver Fork due to its proximity to a ski resort and concern from local businesses and stakeholders. In September 2015, all trees were evaluated for spruce beetle colonization as described above.

At the end of June 2014 and 2015 prior to attaching tree baits, sticky traps were placed on study trees just above DBH to quantify beetle landing. Sticky traps were constructed by applying 4 mm of insect adhesive on plastic transparencies (216 x 279 mm). Two transparencies were stapled on opposite sides of each tree at North and South, in June of each year. In 2014, Tanglefoot® (The Scotts Company, Maryville, Ohio) pest barrier was used and trap catches were low. Therefore, in 2015, sticky traps were constructed with Stickum Pro® (TangleTrap, Contech, Victoria, BC, Canada). In September of each year, the presence of spruce beetles on sticky traps was counted. Many traps contained one to two beetles, thus they were converted to presence or absence.

Statistical Analysis

To investigate the differences between blue spruce and Engelmann spruce related to spruce beetle colonization, linear mixed-effects models were developed. Species (blue spruce, Engelmann spruce), site (Lost Mill, Shingle Creek, Silver Fork), and the interaction of species and site were used as fixed effects. Sample tree was nested in the models as a random effect. The models assume that random effects from tree and random errors are independent and normally distributed. Response variables were Ψ_p , $\Delta^{13}\text{C}$, bark thickness, phloem thickness, resin flow, volatile terpene concentrations, and phloem terpene concentrations. Sites were far enough apart to assume independence among sites. Assumptions of constant variance and normality were met using Pearson standardized residual plots before interpretation of results. Model adjustments for particular variables are described below.

To account for temporal autocorrelation of the same trees being repeatedly measured for Ψ_p in 2015, three alternative models with varying correlation structures

were fit (compound symmetry, autoregressive lag 1, general). Bayesian information criteria (BIC) were used to select the appropriate model.

For resin flow and all terpene analyses, the assumption that the variances were constant within species was relaxed by adding a weights argument to the models using the varIdent function. This was necessary due to the high proportion of zeros for resin flow and the wide variance in terpene results. This process was used to select a more appropriate model rather than transform the data.

For bark thickness estimates of different factors, combinations were made using estimable() from the gmodels package in R (Warnes et al. 2015). A 95% Bonferroni correction was used to control for Type 1 error rate. Statistical analyses were performed using R version 3.5.1 statistical software (R Core Team 2018).

Chi-squared analyses were used to compare the binary variables of landing, lure, and colonization between species. Probabilities were used from Fisher's exact test due to its more robust and conservative test for low samples sizes.

2.4 Results

Water Potential

There were no significant differences in mean Ψ_p between Engelmann spruce and blue spruce for midday and predawn in June (midday Engelmann: N=36, -1.83 MPa, SE= 0.04, blue: N=37, -1.91 MPa, SE=0.05, predawn Engelmann: N=36, -0.69 MPa, SE= 0.02, blue: N=37, -0.70 MPa, SE=0.02) or August (midday Engelmann: N=9, -2.08 MPa, SE= 0.1, blue: N=9, -2.16 MPa, SE=0.07, predawn Engelmann: N=9, -0.73 MPa, SE= 0.04, blue: N=9, -0.67 MPa, SE=0.04) of 2014 (Table 1). There were statistically significant differences in water potential among sites at midday in June and at predawn in August of 2014. There was no significant interaction of species and site in either month at midday or predawn.

There was evidence that Ψ_p varied significantly over time in 2015, but again not by species or the interaction of species and time (Table 1) (Figure 2). Water potential at predawn was significantly different between sites. Water potential became more negative from June to September (Figure 2).

Carbon Isotope Ratio

Mean sapwood growth during the most recent five years was similar for both species (blue = 17.5 mm (SE = 1.1) and Engelmann = 17.2 mm (SE = 0.9)) (Species $F = 0.42$, $df = 1,82$, $p = 0.51$; Site $F = 6.35$, $df = 2,82$, $p < 0.01$; Species:Site $F = 1.8085$, $df = 2,82$, $p = 0.17$). There was convincing evidence that the change in mean carbon isotope discrimination $\Delta^{13}\text{C}$ was affected by species (blue = 17.73 ‰ SE = 0.12, Engelmann = 16.83 ‰ SE = 0.15) and site, but not by their interaction (Table 1). Blue spruce was estimated to have a 0.80 ‰ (SE = 0.15, Lower CI = 0.49, Upper CI = 1.10) higher $\Delta^{13}\text{C}$ ratio than Engelmann spruce ($t = 5.32$, $p < 0.0001$).

Bark and Phloem Thickness and Resin Flow

Resin flow was higher in blue spruce than Engelmann spruce ($p = 0.07$). Mean blue spruce resin flow was 0.37 g (SE = 0.16, N = 43) and Engelmann spruce was 0.07 g (SE = 0.05, N = 45). Resin flow had no significant effect by site or the interaction of species and site (Table 1).

There was evidence that blue spruce bark (13.6 mm SE = 0.63) was significantly thicker than Engelmann spruce bark (6.11 mm SE = 0.32), and there was a significant species by site interaction (Table 1). At all sites, confidence intervals did not cross zero and had significant p -values, indicating that blue spruce had thicker bark than Engelmann spruce (Figure 3).

Mean Engelmann spruce phloem (4.19 mm SE = 0.13) was significantly thicker than blue spruce phloem (2.97 mm SE = 0.12), and there were no significant effects of site or species by site interaction (Table 1).

Phloem Terpenes

Sabinene, myrcene, β -phellandrene, limonene, terpinolene, α -phellandrene, and total terpenes varied significantly by site (Table 2). There was strong evidence that α -pinene, sabinene, myrcene, β -phellandrene, limonene, terpinolene, linalool, and total terpenes varied significantly by species. 3-Carene also showed significant trends between species ($p = 0.09$). All terpenes that varied significantly by species were present in higher quantities in blue spruce compared to Engelmann spruce except for

β -Phellandrene and myrcene which were higher in blue spruce (Figure 4A). Alpha-pinene, sabinene, and limonene levels were significantly different based upon the interaction of species and site. β -Phellandrene/limonene, α -pinene/3-carene, and myrcene/terpinolene ratios varied significantly by species. The most abundant terpene α -pinene over 3-carene was more abundant in blue spruce (Figure 5A), while β -phellandrene/limonene and myrcene/terpinolene were present at higher concentrations in Engelmann spruce. The only ratio that did not vary by species, 3-carene/limonene, varied significantly by site. Only β -phellandrene/limonene varied significantly by the interaction of species and site.

Volatiles

All volatile terpenes, except for myrcene and β -phellandrene, varied significantly by site (Table 2). No volatile terpene varied by the interaction of species and site. β -Pinene was the only terpene to vary significantly by species. α -Pinene and total terpenes had marginally significant effects by species. β -Pinene, α -pinene, and total terpene concentrations were higher in Engelmann spruce (Figure 4B). For selected terpene ratios, no volatiles varied significantly by species, site, or the interaction of species and site (Figure 5B).

Bark Reflectance

Although blue and Engelmann spruce bark reflectance were similar, there were spectral differences in the amplitude of reflectance. The highest amplitudes of reflectance for blue and Engelmann spruce occurred in the 450 to 650 nm wavelength at 150,000 to 250,000. Engelmann spruce had higher bark reflectance than blue spruce (Figure 6).

Electro-Retino Gram

Of the beetles that elicited a response, only one beetle responded to the spectrum below 400 nm, and that beetle had a response that was not distinct, rather a depolarization from 380 to 430 nm in the violet/purple spectrum. Five beetles depolarized at ~450 nm in the blue spectrum. Eight beetles depolarized at ~550 nm in

the green spectrum. Four beetles depolarized at ~650 nm in the red regions of the spectrum. Six beetles depolarized at ~750 nm in the red region of the spectrum, but two were sexed as male and the others had multiple depolarizations rather than one or two. Six beetles depolarized outside the visible spectrum in the infrared at ~850 nm, but half of those had a noisy signal response with multiple depolarizations. Some beetles had second or third depolarizations between 900 and 1300 nm. No beetle had reliable depolarizations above 1300 nm. Primary spruce beetle sensitivity occurred in the visual spectrum between 400 to 800 nm.

Spruce Beetle Landing and Colonization

In 2014, there was evidence that spruce beetle lures caused spruce beetles to land and colonize Engelmann spruce but not blue spruce (Table 3). Eight of the nine Engelmann spruce trees with lures had beetles land and attempt colonization. Only two of nine blue spruce trees with lures had beetles land on the bark, and of those two only one attempted colonization. That beetle was determined unsuccessful due to resin flow and no boring dust and the tree survived the colonization attempt. In 2015, all Engelmann spruce with lures at both sites were colonized. Another 14 Engelmann spruce trees in the study without lures were landed upon and colonized. In 2015, half of the baited blue spruce trees at Lost Mill had beetles on the sticky surface area and colonization was attempted as determined by resin flow and frass. At Shingle Creek, none of the blue spruce with lures were landed upon or colonized.

2.5 Discussion

In this study, several tree traits related to colonization success of spruce beetle were compared in blue and Engelmann spruce. Engelmann spruce naturally experiences higher rates of colonization and resulting tree mortality than blue spruce. The reasons for this difference in colonization and ensuing mortality have not been fully explored. Tree condition, damage, and stress may play an important role. Water stress can limit a tree's ability to tolerate other stressors. Factors such as tree bark and phloem thickness may provide barriers or limited habitat for spruce beetles to reproduce. Defense mechanisms such as resin flow and constitutive terpene

concentration may be beneficial in tree defense as well as aggregation and host tree selection.

Water potential

Water potential was measured during two summers on a subset of trees from the sample population. Water potential varied by site and over time, but it did not vary significantly between the two species. The reasons for this result are likely related to site condition. The two species were compared in mesic sites along creeks and wet meadows. Engelmann spruce is often present at drier sites, but the elevational zone and environmental conditions where both species co-occur are more typical of sites where blue spruce is found. Due to the general lack of water stress of trees growing in mesic site conditions, there may not have been identifiable differences in Ψ_p (Lambers et al. 2008). Both species followed expected seasonal trends for Ψ_p , where Ψ_p became increasingly more negative over the course of the dry season likely decreasing stomatal conductance and carbon assimilation (Figure 2). Alternatively, both trees species may not show a difference in Ψ_p due to water use strategy and life history. Engelmann spruce, commonly found in more arid environments relative to blue spruce, may have a more conservative water use strategy, maintaining Ψ_p by earlier stomatal closure in response to water stress. This may also be related to tracheid size, as Engelmann spruce may have smaller tracheids that would be more resistant to embolism, but also resulting in reduced hydraulic conductivity per unit of sapwood area (Pfausch et al. 2016). This is likely since smaller tracheids are more resistant to freeze-thaw induced embolism and Engelmann spruce occurs at higher elevations and colder temperatures than blue spruce (Schmid and Frye 1977). Blue spruce may have a riskier water use strategy, allowing its stomata to remain open longer during water stress maintaining carbon assimilation. Since blue spruce in a forest environment are likely to grow on sites that have more available water, its water column does not experience substantial periods of tension. However, under current climate change scenarios, both species may experience more severe and prolonged periods of water stress (Kolb et al. 2016). The riskier water strategy of blue spruce may result in cavitation or enough water stress to cause carbon

assimilation and defenses to decrease. Drought has been linked to colonization success in Engelmann spruce and to decreases in growth (Hebertson and Jenkins 2008, Hart et al. 2013). Hard (1985) found that fast-growing white spruce experienced less spruce beetle colonization relative to slow-growing white spruce. While conifers tend to be less sensitive to environmental stressors than deciduous trees, their ability to acclimate to environmental stress may be pushed to extremes under a warming climate regime (Oliver and Larson 1996). Spruce water use strategies were further explored with $\Delta^{13}\text{C}$.

Carbon isotope ratio

Carbon isotope ratio was measured over the most recent five years of sapwood growth to estimate drought or water stress effects in recent years that might have weakened and predisposed trees to bark beetle colonization. McCarroll and Loader (2004) state that stable carbon isotopes record the balance between stomatal conductance and photosynthetic rate, dominated at dry sites by relative humidity and soil water status and at moist sites by summer irradiance and temperature. Mean sapwood growth did not vary significantly by species, indicating that the species put similar amounts of carbon resources towards growth. However, $\Delta^{13}\text{C}$ results indicate that carbon isotope ratios varied by species and by site. Blue spruce tended to have higher $\Delta^{13}\text{C}$ values than Engelmann spruce. This can result in more water loss with more stomatal conductance, resulting in lower intrinsic WUE (Farquhar et al. 1989a). Higher $\Delta^{13}\text{C}$ means higher stomatal conductance or lower photosynthesis. Similarities in growth between the two species may be related to blue spruce allocating more assimilated carbon towards defenses such as preformed resin flow. Due to limited historical evidence of blue spruce colonization by spruce beetle this result seems unlikely (Herms and Mattson 1992), unless explained by lack of nutrients such as nitrogen limiting growth. Future research comparing available nitrogen with dual isotope techniques may better explain these results (Mayer and Wassenaar 2012).

Under current climate and environmental conditions, blue spruce grows in mesic areas where water generally isn't limiting. Under a warming climate, as predicted in the IPCC climate change models, blue spruce will likely have less

available water and with a lower intrinsic WUE, may become more susceptible to future spruce beetle outbreaks. In addition, Kolb et al. (2016) discusses nitrogen build up in trees experiencing drought. Since nitrogen is an important building block for insect growth and maintenance requirements (Ayres et al. 2000), drought affected trees may become a better nutritional habitat for spruce beetle development. Spruce beetle preference for Engelmann spruce may be related to nitrogen levels (Cates et al. 1987, Ayres et al. 2000). Under a future with a warming climate, blue spruce may be more water stressed, have less available carbon for defense allocation, and have higher levels of nitrogen as drought effects become more common.

Resin flow and Bark and Phloem Thickness

Studies have shown that preformed resin is the most important defense against bark beetles (Raffa and Berryman 1982, Raffa and Berryman 1983, Wainhouse et al. 1990, Ruel et al. 1998, Ayres and Lombardero 2000, Lombardero et al. 2000, Wallin and Raffa 2001, Safranyik and Carroll 2006). The difference in resin flow between species was significant where blue spruce tended to have higher resin flow following wounding. Blue spruce and Engelmann spruce both had lower mean resin flow than loblolly pine (Klepzig et al. 2005). Many resin flow studies have been conducted in loblolly pine, Norway spruce, and lodgepole pine (Cook and Hain 1986, Ruel et al. 1998, Lombardero et al. 2000, Rocchini et al. 2000, Roberds et al. 2003) and most studies indicate higher mean resin flow rates than observed for either Engelmann or blue spruce. The absence of resin flow on many trees confounds this result. Blue spruce had trees with resin flow triple the means Klepzig et al. (2005) reported in approximately half the time with this study collecting resin for seven days. Since the tree species in this study have similar growth perhaps Engelmann spruce trees do not allocate carbon towards resin flow because the carbon is used in growth, maintenance, and non-structural carbohydrate storage. This may indicate there are nutrient limits in this environment for blue spruce as it isn't outgrowing Engelmann spruce and has higher resin flow. Lombardero et al. (2000) found resin flow in loblolly pine, which grows faster than spruce and in warmer environments, to be lower in fast-growing trees and higher in slower growing trees. In fact, trees

experiencing drought had the highest resin flow. This is contrary to Hart et al. (2013), which suggests drought is related to spruce beetle colonization, because it reduces resin flow in Engelmann spruce. It is not known how drought will affect resin flow in blue spruce and whether resistance to spruce beetle may remain.

In addition to higher resin flow, blue spruce bark was thicker than Engelmann spruce (Figure 3), meaning the adult beetles must tunnel through more bark when attempting to colonize blue spruce thus using more of their energy reserves to reach the phloem (Safranyik and Carroll 2006, Graf et al. 2012, Raffa et al. 2015). Blue spruce also has thinner phloem than Engelmann spruce, which may provide less available food for growth and development of spruce beetle larvae (Cole and Amman 1969, Amman 1972). Further, there is less available phloem substrate for the spruce beetle symbiotic fungus, *Leptographium abietinum*, to propagate through the tree concentrating nitrogen, phosphorus, and protein near bark beetle tunnels as a food source (Ayres et al. 2000, Davis et al. 2019). Spruce beetle symbiotic fungi have also been shown to decrease spruce beetle antagonistic microbes and decrease toxic terpenes like 3-carene (Davis et al. 2019).

Phloem Terpenes

The most abundant phloem based terpenes; including α -pinene, limonene, and 3-carene, were all higher in blue spruce than Engelmann spruce (Figure 4A). Higher levels of these terpenes may indicate a higher level of defense for blue spruce compared to Engelmann spruce. Limonene and 3-carene are known to have toxic effects on beetles (Smith 1965, Raffa and Smalley 1995, Werner 1995, Lindgren et al. 1996, Rocchini et al. 2000, Ott et al. 2011). Terpenes may interfere with insect digestion and inhibit germination and growth of fungi (Klepzig et al. 1996, Klepzig and Six 2004, Davis et al. 2018). Davis et al. (2018) found linalool and terpinolene to suppress *Leptographium abietinum* growth. Linalool levels were significantly higher in Engelmann spruce while terpinolene levels were significantly higher in blue spruce (Table 2). Some terpenes are potentially beneficial to spruce beetle; myrcene and terpinolene are precursors of bark beetle pheromones affecting colonization (Borden 1982, Raffa and Berryman 1983, Safranyik and Carroll 2006) and were significantly

different by species. Myrcene was higher in Engelmann spruce. α -Pinene was in highest abundance in blue spruce and has been shown to be important in the production of both anti-aggregation pheromones (Pureswaran and Borden 2005) and aggregation pheromones (Borden 1982, Seybold et al. 1995, Raffa 2001).

Volatiles

Volatile terpene results indicate the most abundant and total terpenes were higher in Engelmann spruce than blue spruce. The rate of α -Pinene emission from Engelmann spruce was approximately double that from blue spruce. Wallin and Raffa (2004) found that high levels of the most abundant terpene, α -pinene, on a host media repelled spruce beetle while intermediate levels were attractive. Blue spruce had lower levels of emitted α -pinene, but more than twice the mean abundance found in Engelmann spruce phloem (Figure 4B). Gray et al. (2015) found that rather than one terpene alone, multiple compounds affected mountain pine beetle colonization behavior. Gray et al. (2015) found limonene to disturb tree species identification between the host limber pine and non-host Great Basin bristlecone pine. While there was no significant difference between blue spruce and Engelmann spruce for limonene (Figure 4) (Table 2), ratios of limonene with the terpenes 3-carene and β -phellandrene were highly variable. There were no significant differences between tree species in levels of terpenes thought to affect beetle aggregation except α -pinene that was significant at $p = 0.09$. Perhaps higher concentrations of volatiles combined with terpenes attract spruce beetle to Engelmann spruce either by themselves or by synergizing with pheromones.

Bark Reflectance and Electro-Retino Gram

While reflectance profiles for both spruce species seem to indicate similar trends in reflectance, Engelmann spruce reflected more of the full visible light spectrum than blue spruce. One wavelength that most spruce beetles responded to along the full spectrum of light was at ~ 760 nm and both tree species had similar reflectance values at that wavelength (Figure 6). Results from the ERG indicate multiple beetle depolarization cues, meaning a sensitivity for beetles at multiple

wavelengths of light, but six beetles depolarized at approximately 750 nm. Contrary to other studies reported in Byers (1995), these results suggest that spruce beetles have photoreceptors in other wavelengths than blue and green. Overall, there were differences in reflectance amplitude between the bark of the two species, but spruce beetle did not show a consistent sensitivity to particular wavelengths of light. Consequently, there was no evidence from these data of any visual basis for spruce beetle avoiding blue spruce. The variation in the amplitude of bark reflectance may play a part in spruce beetle host selection although random landing on larger hosts as a visual cue may be a more reasonable assumption on finding a suitable host. More research needs to be conducted to enhance our knowledge of host finding behavior associated with visual cues. However, many studies have indicated bark structure is important for successful bark beetle colonization (Schmid and Frye 1977, Safranyik and Carroll 2006). Future research into fine-scale bark structural differences between blue and Engelmann spruces may provide additional information on bark structure and beetle preference.

Spruce Beetle Landing and Colonization

Engelmann spruce gets landed on and colonized more often than blue spruce. Even with lures present, beetle landing was low upon blue spruce, and of the two trees with colonization attempts only one tree was successfully colonized and experienced mortality (Table 3). This is consistent with previous studies for blue spruce (Massey and Wygant 1954, Schmid and Frye 1977, Colorado State Forest Service 2017). Most Engelmann spruce at the two sites with spruce beetle present, regardless of spruce beetle baiting, were colonized by the end of the study.

2.6 References

- Amman, G. D. 1972. Mountain pine beetle brood production in relation to thickness of lodgepole pine phloem. *Journal of Economic Entomology* **65**:138-140.
- Anderegg, W. R., J. A. Hicke, R. A. Fisher, C. D. Allen, J. Aukema, B. Bentz, S. Hood, J. W. Lichstein, A. K. Macalady, and N. McDowell. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* **208**:674-683.

- Ayres, M. P., and M. J. Lombardero. 2000. Assessing the consequences of global change for forest disturbance from herbivores and pathogens. *Science of the Total Environment* **262**:263-286.
- Ayres, M. P., R. T. Wilkens, J. J. Ruel, M. J. Lombardero, and E. Vallery. 2000. Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi. *Ecology* **81**:2198-2210.
- Bentz, B. J., J. Regniere, C. J. Fettig, E. M. Hansen, J. L. Hayes, J. A. Hicke, R. G. Kelsey, J. F. Negron, and S. J. Seybold. 2010. Climate Change and Bark Beetles of the Western United States and Canada: Direct and Indirect Effects. *BioScience* **60**:602-613.
- Borden, J. 1982. Aggregation pheromones. *Bark Beetles in North American Conifers*. University of Texas Press, Austin, Texas:74-139.
- Byers, J. A. 1995. Host-tree chemistry affecting colonization in bark beetles. *Chemical Ecology of Insects* **2**:154-213.
- Cates, R. G., C. B. Henderson, and R. A. Redak. 1987. Responses of the western spruce budworm to varying levels of nitrogen and terpenes. *Oecologia* **73**:312-316.
- Chmura, D. J., P. D. Anderson, G. T. Howe, C. A. Harrington, J. E. Halofsky, D. L. Peterson, D. C. Shaw, and J. B. S. Clair. 2011. Forest responses to climate change in the northwestern United States: ecophysiological foundations for adaptive management. *Forest Ecology and Management* **261**:1121-1142.
- Cole, W. E., and G. D. Amman. 1969. Mountain pine beetle infestations in relation to lodgepole pine diameters. US Dept. of Agriculture, Forest Service, Intermountain Forest & Range Experiment Station.
- Colorado State Forest Service. 2017. The Health of Colorado's Forests.
- Cook, S. P., and F. P. Hain. 1986. Defensive mechanisms of loblolly and shortleaf pine against attack by southern pine beetle, *Dendroctonus frontalis* Zimmerman, and its fungal associate, *Ceratocystis minor* (Hedgecock) Hunt. *Journal of Chemical Ecology* **12**:1397-1406.
- Davis, T. S., F. B. Horne, J. C. Yetter, and J. E. Stewart. 2018. Engelmann Spruce Chemotypes in Colorado and their Effects on Symbiotic Fungi Associated with the North American Spruce Beetle. *Journal of Chemical Ecology* **44**:601-610.
- Davis, T. S., J. E. Stewart, A. Mann, C. Bradley, and R. W. Hofstetter. 2019. Evidence for multiple ecological roles of *Leptographium abietinum*, a symbiotic fungus associated with the North American spruce beetle. *Fungal Ecology* **38**:62-70.

- Ernst, S., J. Hanover, and D. Keathley. 1990. Assessment of natural interspecific hybridization of blue and Engelmann spruce in southwestern Colorado. *Canadian journal of botany* **68**:1489-1496.
- Farquhar, G., K. Hubick, A. Condon, and R. Richards. 1989a. Carbon isotope fractionation and plant water-use efficiency. Pages 21-40 *Stable Isotopes in Ecological Research*. Springer.
- Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989b. Carbon isotope discrimination and photosynthesis. *Annual review of plant biology* **40**:503-537.
- Farquhar, G. D., M. H. O'Leary, and J. A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Functional Plant Biology* **9**:121-137.
- Gaylord, M. L., T. E. Kolb, and N. G. McDowell. 2015. Mechanisms of piñon pine mortality after severe drought: a retrospective study of mature trees. *Tree Physiology* **35**:806-816.
- Giunta, A. D., J. B. Runyon, M. J. Jenkins, and M. Teich. 2016. Volatile and Within-Needle Terpene Changes to Douglas-fir Trees Associated With Douglas-fir Beetle (Coleoptera: Curculionidae) Attack. *Environmental Entomology* **45**:920-929.
- Graf, M., M. Reid, B. Aukema, and B. Lindgren. 2012. Association of tree diameter with body size and lipid content of mountain pine beetles. *The Canadian Entomologist* **144**:467-477.
- Gray, C. A., J. B. Runyon, M. J. Jenkins, and A. D. Giunta. 2015. Mountain pine beetles use volatile cues to locate host limber pine and avoid non-host great basin bristlecone pine. *PLoS ONE* **10**:e0135752.
- Hansen, E. M., B. J. Bentz, J. A. Powell, D. R. Gray, and J. C. Vandygriff. 2011. Prepupal diapause and instar IV developmental rates of the spruce beetle, *Dendroctonus rufipennis* (Coleoptera: Curculionidae, Scolytinae). *Journal of insect physiology* **57**:1347-1357.
- Hard, J. S. 1985. Spruce beetles attack slowly growing spruce. *Forest Science* **31**:839-850.
- Hart, S. J., T. T. Veblen, K. S. Eisenhart, D. Jarvis, and D. Kulakowski. 2013. Drought induces spruce beetle (*Dendroctonus rufipennis*) outbreaks across northwestern Colorado. *Ecology* **95**:930-939.
- Hebertson, E. G., and M. J. Jenkins. 2008. Climate factors associated with historic spruce beetle (Coleoptera: Curculionidae) outbreaks in Utah and Colorado. *Environmental Entomology* **37**:281-292.

- Hermes, D. A., and W. J. Mattson. 1992. The Dilemma of Plants: To Grow or Defend. *The quarterly review of biology* **67**:283-335.
- Holsten, E. H., R. Their, A. S. Munson, and K. E. Gibson. 1999. The spruce beetle. *The Bark Beetles, Fuels, and Fire Bibliography*:1-7.
- Intergovernmental Panel on Climate Change. 2014. IPCC Fifth Assessment Synthesis Report. IPCC 5th Assessment Synthesis Report.
- Jenkins, M., E. Hebertson, and A. Munson. 2014. Spruce beetle biology, ecology and management in the Rocky Mountains: an addendum to spruce beetle in the Rockies. *Forests* **5**:21-71.
- Klepzig, K. D., D. J. Robison, G. Fowler, P. R. Minchin, F. P. Hain, and H. L. Allen. 2005. Effects of mass inoculation on induced oleoresin response in intensively managed loblolly pine. *Tree Physiology* **25**:681-688.
- Klepzig, K. D., and D. Six. 2004. Bark beetle-fungal symbiosis: context dependency in complex associations. *Symbiosis* **37**:189-205.
- Klepzig, K. D., E. B. Smalley, and K. F. Raffa. 1996. Combined chemical defenses against an insect-fungal complex. *Journal of Chemical Ecology* **22**:1367.
- Kolb, T. E., C. J. Fettig, M. P. Ayres, B. J. Bentz, J. A. Hicke, R. Mathiasen, J. E. Stewart, and A. S. Weed. 2016. Observed and anticipated impacts of drought on forest insects and diseases in the United States. *Forest Ecology and Management* **380**:321-334.
- Lambers, H., F. S. Chapin, and T. L. Pons. 2008. Plant water relations. Pages 163-223 *in* Plant physiological ecology, editor. Springer, New York, NY.
- Ledig, F. T., P. D. Hodgskiss, and D. R. Johnson. 2006. The structure of genetic diversity in Engelmann spruce and a comparison with blue spruce. *Botany* **84**:1806-1828.
- Lindgren, B., G. Nordlander, and G. Birgersson. 1996. Feeding deterrence of verbenone to the pine weevil, *Hylobius abietis* (L.)(Col., Curculionidae). *Journal of Applied Entomology* **120**:397-403.
- Lombardero, M. J., M. P. Ayres, P. L. Lorio Jr., and J. J. Ruel. 2000. Environmental effects on constitutive and inducible resin defenses of *Pinus taeda*. *Ecology Letters* **3**:329-339.
- Lorio Jr., P. L. 1993. Environmental stress and whole tree physiology. Pages 81-100 *in* T. D. Schowalter and G. M. Filip, editors. *Beetle-Pathogen Interactions in Conifer Forests*. Academic Press Inc., San Diego.

- Maroja, L. S., S. M. Bogdanowicz, K. F. Wallin, K. F. Raffa, and R. G. Harrison. 2007. Phylogeography of spruce beetles (*Dendroctonus rufipennis* Kirby) (Curculionidae: Scolytinae) in North America. *Molecular Ecology* **16**:2560-2573.
- Massey, C., and N. Wygant. 1954. Biology and control of the Engelmann spruce beetle in Colorado. US Department of Agriculture, Washington.
- Mayer, B., and L. I. Wassenaar. 2012. Isotopic characterization of nitrate sources and transformations in Lake Winnipeg and its contributing rivers, Manitoba, Canada. *Journal of Great Lakes Research* **38**:135-146.
- McCarroll, D., and N. J. Loader. 2004. Stable isotopes in tree rings. *Quaternary Science Reviews* **23**:771-801.
- Moeck, H. A., D. L. Wood, and K. Q. Lindahl Jr. 1981. Host selection behavior of bark beetles (Coleoptera: Scolytidae) attacking *Pinus ponderosa*, with special emphasis on the western pine beetle, *Dendroctonus brevicomis*. *Journal of Chemical Ecology* **7**:49-83.
- Oliver, C. D., and B. C. Larson. 1996. Forest stand dynamics: updated edition. John Wiley and sons.
- Ott, D. S., A. D. Yanchuk, D. P. Huber, and K. F. Wallin. 2011. Genetic variation of lodgepole pine, *Pinus contorta* var. *latifolia*, chemical and physical defenses that affect mountain pine beetle, *Dendroctonus ponderosae*, attack and tree mortality. *Journal of Chemical Ecology* **37**:1002-1012.
- Page, W. G., M. J. Jenkins, and J. B. Runyon. 2012. Mountain pine beetle attack alters the chemistry and flammability of lodgepole pine foliage. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **42**:1631-1647.
- Page, W. G., M. J. Jenkins, and J. B. Runyon. 2014. Spruce beetle-induced changes to Engelmann spruce foliage flammability. *Forest Science* **60**:691-702.
- Pears, S. L. 2010. Interactions of *Amylostereum areolatum* (FR.) Boiden (Russulales:Stereaceae), The Fungal Symbiont of *Sirex noctilio* Fabricus (Hymenoptera: Siricidae), With North American *Pinus* Hosts and Native Herbivorous Insect-Fungal Complexes. University of Vermont.
- Pfautsch, S., M. Harbusch, A. Wesolowski, R. Smith, C. Macfarlane, M. G. Tjoelker, P. B. Reich, and M. A. Adams. 2016. Climate determines vascular traits in the ecologically diverse genus *Eucalyptus*. *Ecology Letters* **19**:240-248.
- Powell, E. N., and K. F. Raffa. 2011. Fire injury reduces inducible defenses of lodgepole pine against mountain pine beetle. *Journal of Chemical Ecology* **37**:1184-1192.

- Price, P. W. 1997. Insect ecology. John Wiley & Sons.
- Pureswaran, D. S., and J. H. Borden. 2005. Primary attraction and kairomonal host discrimination in three species of *Dendroctonus* (Coleoptera: Scolytidae). *Agricultural and Forest Entomology* **7**:219-230.
- R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Raffa, K. F. 2001. Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. *Chemoecology* **11**:49-65.
- Raffa, K. F., and A. A. Berryman. 1982. Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated microorganisms. *Environmental Entomology* **11**:486-492.
- Raffa, K. F., and A. A. Berryman. 1983. The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). *Ecological Monographs* **53**:27-49.
- Raffa, K. F., J.-C. Gregoire, and B. S. Lindgren. 2015. Natural history and ecology of bark beetles. Pages 1-40 *in* F. E. Vega and R. W. Hofstetter, editors. *Bark Beetles*. Elsevier.
- Raffa, K. F., and E. B. Smalley. 1995. Interaction of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle-fungal complexes. *Oecologia* **102**:285-295.
- Roberds, J. H., B. L. Strom, and F. Hain. 2003. Genetic and phenotypic variability for constitutive oleoresin flow in loblolly pine. *in* Proceedings of the 27th Southern Forest Tree Improvement Conference. USDA, Stillwater, OK.
- Rocchini, L. A., B. S. Lindgren, and R. G. Bennett. 2000. Effects of resin flow and monoterpene composition on susceptibility of lodgepole pine to attack by the Douglas-fir pitch moth, *Synanthedon novaroensis* (Lep., Sesiidae). *Journal of Applied Entomology* **124**:87-92.
- Ross, D. W., G. Determan, J. L. Boughton, and T. M. Quigley. 2001. Forest health restoration in south-central Alaska: a problem analysis. Pages 1-38, United States Department of Agriculture Forest Service General Technical Report, PNW-GTR-523.
- Ruel, J. J., M. P. Ayres, and P. L. Lorio Jr. 1998. Loblolly pine responds to mechanical wounding with increased resin flow. *Canadian Journal of Forest Research* **28**:596-602.
- Safranyik, L., and A. L. Carroll. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. Pages 3-66 *in* L. Safranyik and W. R.

- Wilson, editors. The mountain pine beetle: a synthesis of biology, management, and impacts on lodgepole pine. Natural Resources Canada, Pacific Forestry Centre, Victoria, BC.
- Schaefer, P. R., and J. W. Hanover. 1986. Taxonomic implications of monoterpene compounds of blue and Engelmann spruces. *Forest Science* **32**:725-734.
- Schmid, J., and R. H. Frye. 1977. Spruce beetle in the Rockies. Pages 1-38. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, General Technical Report RM-49.
- Seybold, S., D. Quilici, J. Tillman, D. Vanderwel, D. Wood, and G. Blomquist. 1995. De novo biosynthesis of the aggregation pheromone components ipsenol and ipsdienol by the pine bark beetles *Ips paraconfusus* Lanier and *Ips pini* (Say)(Coleoptera: Scolytidae). *Proceedings of the National Academy of Sciences* **92**:8393-8397.
- Smith, R. H. 1965. Effect of monoterpene vapors on the western pine beetle. *Journal of Economic Entomology* **58**:509-510.
- Stine, M., and D. Keathley. 1990. Paternal inheritance of plastids in Engelmann spruce x blue spruce hybrids. *Journal of Heredity* **81**:443-446.
- Stocker, T., D. Qin, G. Plattner, M. Tignor, S. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. Midgley. 2013. IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.
- Taylor, A. M., J. Renée Brooks, B. Lachenbruch, J. J. Morrell, and S. Voelker. 2008. Correlation of carbon isotope ratios in the cellulose and wood extractives of Douglas-fir. *Dendrochronologia* **26**:125-131.
- Wainhouse, D., D. J. Cross, and R. S. Howell. 1990. The role of lignin as a defense against the spruce bark beetle *Dendroctonus micans*: Effect on larvae and adults. *Oecologia* **85**:257-265.
- Wallin, K. F., and K. F. Raffa. 1999. Altered constitutive and inducible phloem monoterpenes following natural defoliation of jack pine: Implications to host mediated interguild interactions and plant defense theories. *Journal of Chemical Ecology* **25**:861-880.
- Wallin, K. F., and K. F. Raffa. 2001. Effects of folivory on subcortical plant defenses: can defense theories predict interguild processes? *Ecology* **82**:1387-1400.
- Wallin, K. F., and K. F. Raffa. 2004. Feedback between Individual Host Selection Behavior and Population Dynamics in an Eruptive Herbivore. *Ecological Monographs* **74**:101-116.

- Warnes, G. R., B. Bolker, T. Lumley, and R. C. Johnson. 2015. Package gmodels: Various R Programming Tools for Model Fitting. R package version 2.16. 2.
- Werner, R. A. 1995. Toxicity and Repellency of 4-Allylanisole and Monoterpenes from White Spruce and Tamarack to the Spruce Beetle and Eastern Larch Beetle (Coleoptera: Scolytidae). *Environmental Entomology* **24**:372-379.



Figure 1. Map of research sites.

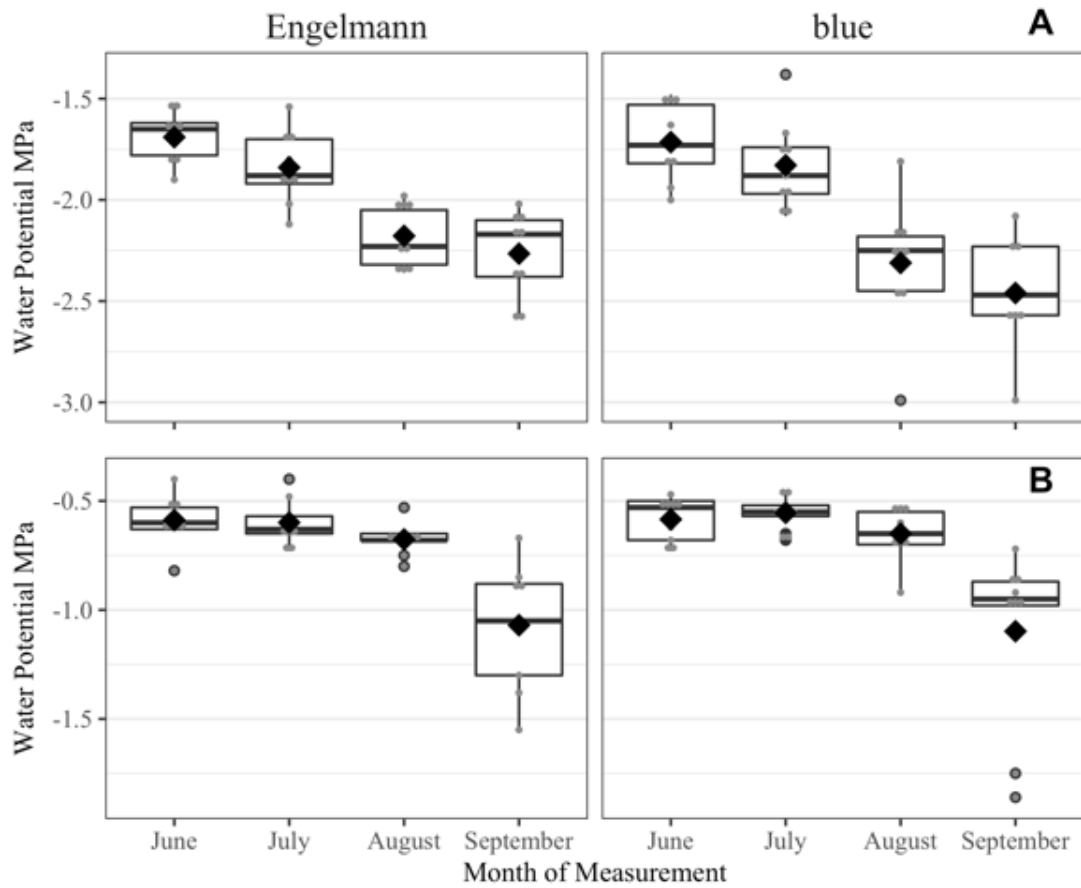


Figure 2. Water potential in (MPa) for Engelmann and blue spruces at midday (A) and predawn (B) during the summer of 2015. Black diamonds represent the mean for each species by month.

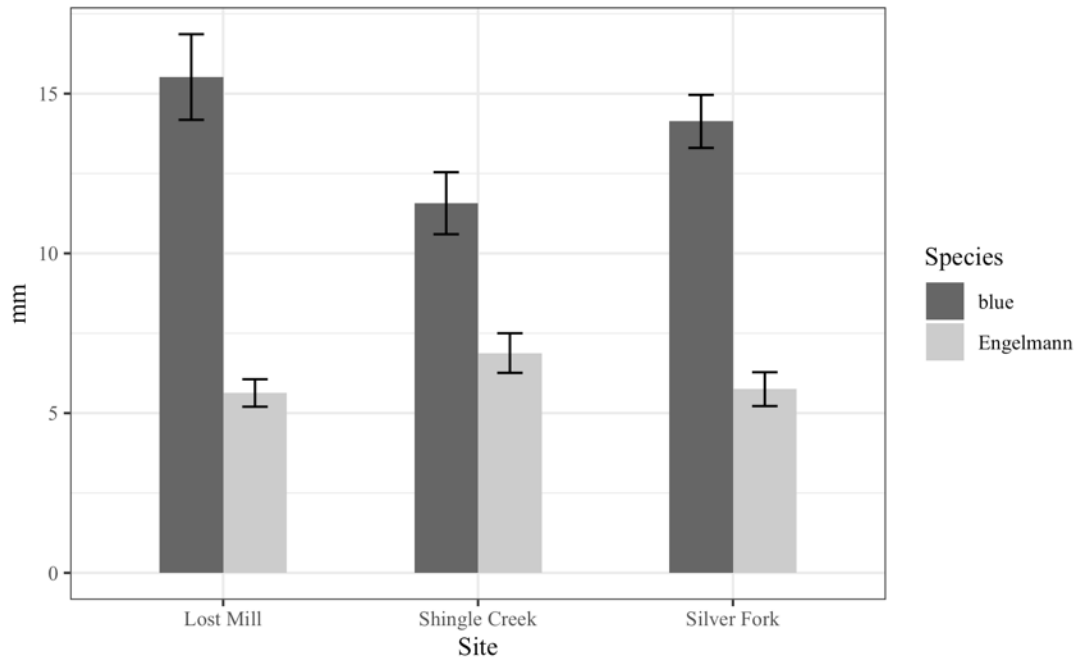


Figure 3. Mean bark thickness of blue spruce and Engelmann spruce separated at three study sites in Utah and Wyoming. Error bars (SE).

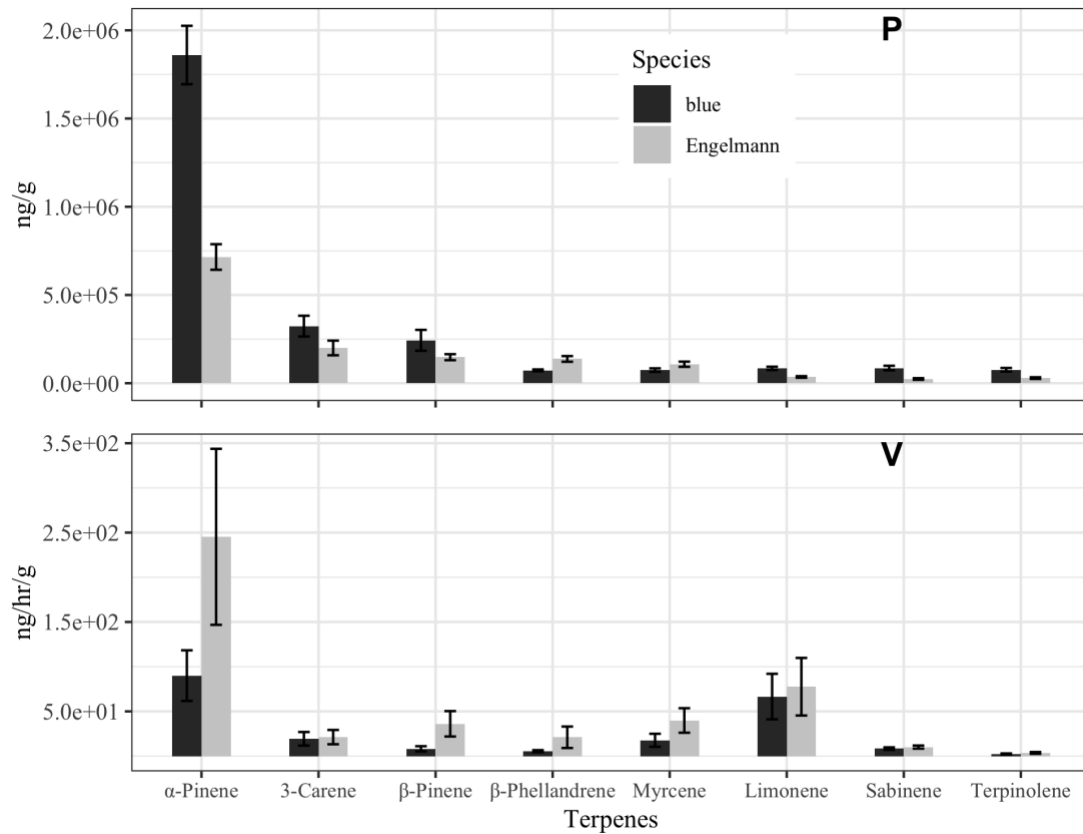


Figure 4. Phloem and volatile terpenes in blue and Engelmann spruces. A. Mean phloem terpenes (bars=SE) (n=41 blue, n=39 Engelmann). B. Mean volatile terpenes (n=12). (bars=SE)

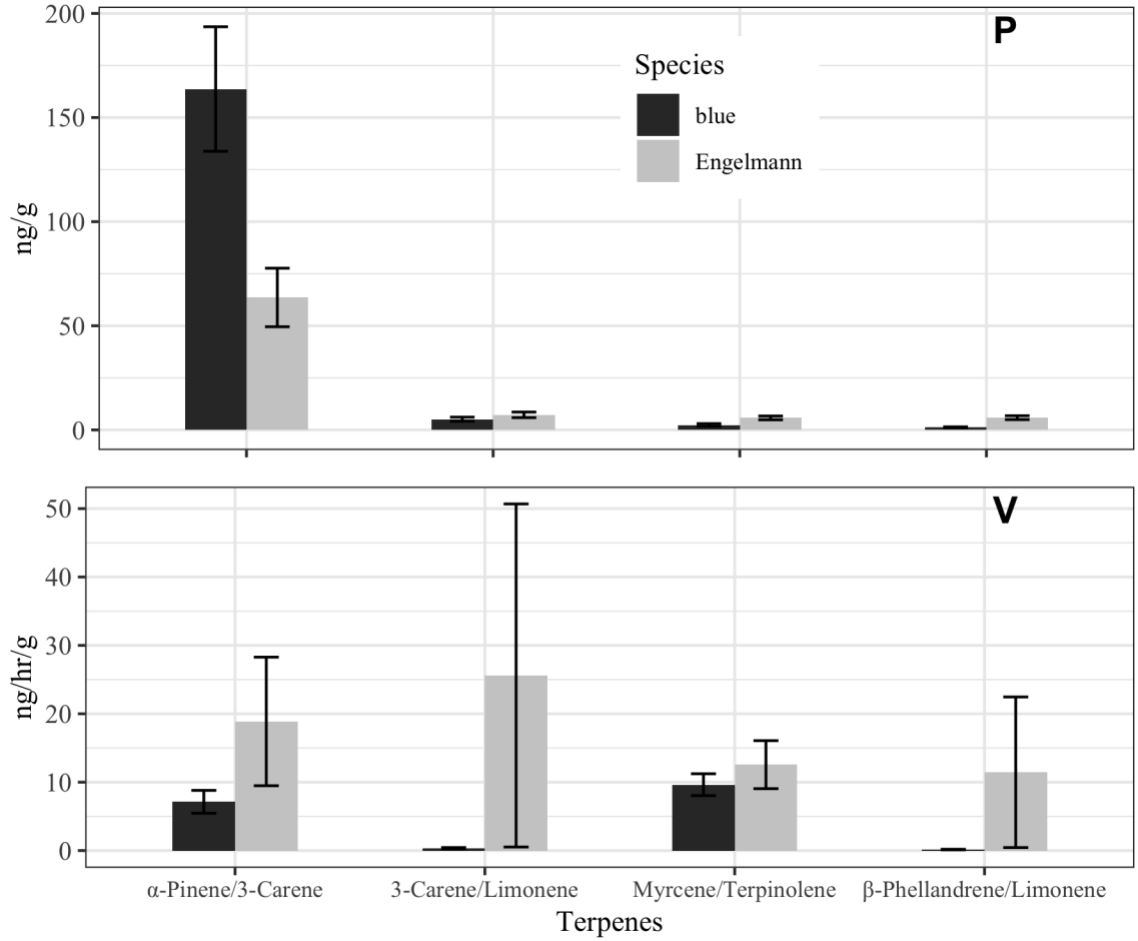


Figure 5. Selected ratios of phloem and volatile terpenes in blue and Engelmann spruces. A. Ratios of mean phloem terpenes (n=41 blue, n=39 Engelmann). B. Ratios of mean volatile terpenes (n=12). (bars=SE)

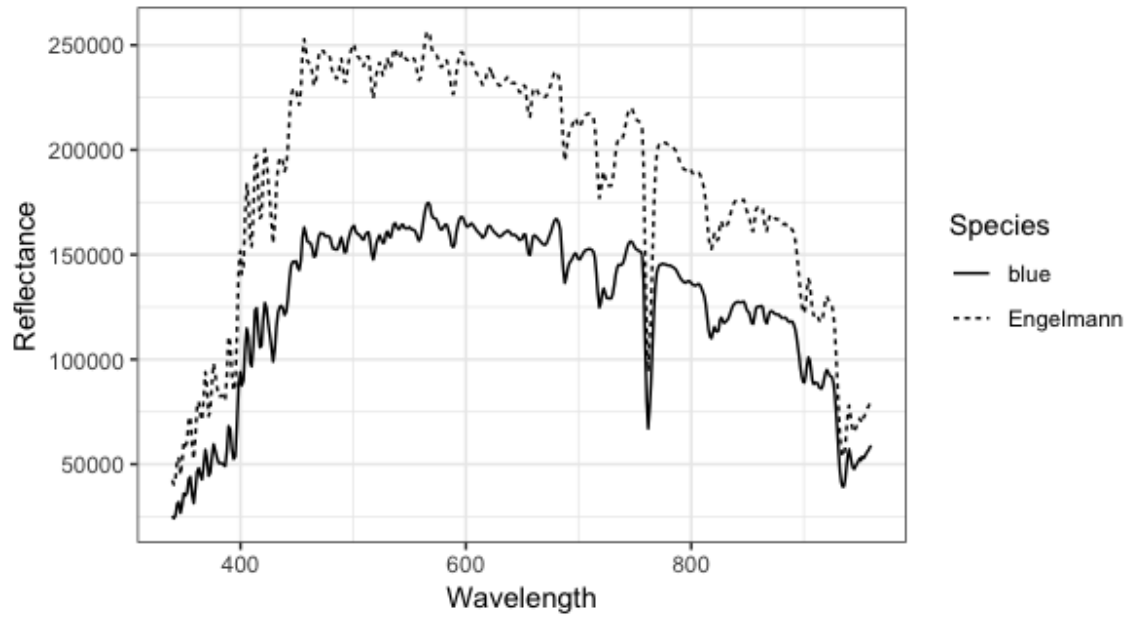


Figure 6. Mean bark reflectance of blue and Engelmann spruces for the visual spectrum into infrared.

Table 1. Marginal F test for Ψ_p (repeated in 2015) (MPa), $\Delta^{13}\text{C}$, bark thickness (mm), phloem thickness (mm), and resin amount (g) for Engelmann spruce and blue spruce at three sites in Utah and Wyoming. Statistically significant results are in bold.

| | | Ψ_p | | | | Variables | | | | |
|---------------------------------|--------------|----------|-------|------------|-------------------|-----------------------|-------|------------|------------|-------------------|
| | | numDF | denDF | F -value | p -value | numDF | denDF | F -value | p -value | |
| Midday June 2014 | Intercept | 1 | 67 | 3551.83 | <0.0001 | $\Delta^{13}\text{C}$ | 1 | 82 | 33917.44 | <0.0001 |
| | Species | 1 | 67 | 1.61 | 0.21 | | 1 | 82 | 0.43 | 0.51 |
| | Site | 2 | 67 | 3.40 | <0.05 | | 2 | 82 | 12.87 | <0.0001 |
| | Species:Site | 2 | 67 | 0.31 | 0.74 | | 2 | 82 | 3.14 | <0.05 |
| Predawn June 2014 | Intercept | 1 | 67 | 1839.84 | <0.0001 | Bark Thickness | 1 | 82 | 867.71 | <0.0001 |
| | Species | 1 | 67 | 0.06 | 0.81 | | 1 | 82 | 126.53 | <0.0001 |
| | Site | 2 | 67 | 1.83 | 0.17 | | 2 | 82 | 1.19 | 0.31 |
| | Species:Site | 2 | 67 | 0.45 | 0.64 | | 2 | 82 | 5.46 | <0.01 |
| Midday August 2014 | Intercept | 1 | 12 | 1089.84 | <0.0001 | Phloem Thickness | 1 | 58 | 1469.20 | <0.0001 |
| | Species | 1 | 12 | 0.38 | 0.55 | | 1 | 58 | 42.37 | <0.0001 |
| | Site | 2 | 12 | 0.40 | 0.68 | | 1 | 58 | 0.72 | 0.40 |
| | Species:Site | 2 | 12 | 0.79 | 0.48 | | 1 | 58 | 0.46 | 0.50 |
| Predawn August 2014 | Intercept | 1 | 12 | 1368.95 | <0.0001 | Resin | 1 | 82 | 4.36 | <0.05 |
| | Species | 1 | 12 | 2.61 | 0.13 | | 1 | 82 | 3.50 | 0.07 |
| | Site | 2 | 12 | 11.09 | <0.01 | | 2 | 82 | 1.11 | 0.33 |
| | Species:Site | 2 | 12 | 0.52 | 0.61 | | 2 | 82 | 1.61 | 0.21 |
| Repeated Measures Ψ_p 2015 | | | | | | | | | | |
| Midday | Intercept | 1 | 48 | 4104.48 | <0.0001 | Predawn | 1 | 48 | 713.23 | <0.0001 |
| | Species | 1 | 14 | 1.80 | 0.20 | | 1 | 14 | 0.05 | 0.84 |
| | Site | 2 | 14 | 1.40 | 0.28 | | 2 | 14 | 3.97 | 0.04 |
| | Time | 3 | 48 | 48.95 | <0.0001 | | 3 | 48 | 38.34 | <0.0001 |
| | Species:Time | 3 | 48 | 1.10 | 0.36 | | 3 | 48 | 0.16 | 0.92 |

Table 2. Marginal *F* Test for volatile and phloem based terpenes and ratios of specific terpenes by species, site, and the interaction of species by site. For phloem terpenes, same variable as volatile unless indicated. Statistically significant results are in bold.

| | | Volatile Terpenes | | | | Phloem Terpenes | | | |
|------------------------|--------------|-------------------|-------|-----------------|-------------------|-----------------|-------|-----------------|-------------------|
| | | numDF | lenDF | <i>F</i> -value | <i>p</i> -value | numDF | lenDF | <i>F</i> -value | <i>p</i> -value |
| <i>α</i> -Pinene | Intercept | 1 | 18 | 23.30 | <0.0001 | 1 | 74 | 204.96 | <0.0001 |
| | Species | 1 | 18 | 3.59 | 0.07 | 1 | 74 | 48.21 | <0.0001 |
| | Site | 2 | 18 | 6.45 | <0.01 | 2 | 74 | 2.57 | 0.08 |
| | Species:Site | 2 | 18 | 2.48 | 0.11 | 2 | 74 | 3.98 | <0.05 |
| Sabinene | Intercept | 1 | 18 | 175.62 | <0.0001 | 1 | 74 | 60.76 | <0.0001 |
| | Species | 1 | 18 | 1.34 | 0.26 | 1 | 74 | 21.71 | <0.0001 |
| | Site | 2 | 18 | 17.82 | <0.0001 | 2 | 74 | 4.87 | <0.01 |
| | Species:Site | 2 | 18 | 0.49 | 0.62 | 2 | 74 | 3.30 | <0.05 |
| <i>β</i> -Pinene | Intercept | 1 | 18 | 17.79 | <0.0001 | 1 | 74 | 88.58 | <0.0001 |
| | Species | 1 | 18 | 4.40 | <0.05 | 1 | 74 | 2.56 | 0.11 |
| | Site | 2 | 18 | 6.18 | <0.001 | 2 | 74 | 0.59 | 0.56 |
| | Species:Site | 2 | 18 | 1.24 | 0.31 | 2 | 74 | 2.29 | 0.11 |
| Myrcene | Intercept | 1 | 18 | 12.51 | <0.001 | 1 | 74 | 117.20 | <0.0001 |
| | Species | 1 | 18 | 1.96 | 0.18 | 1 | 74 | 3.80 | <0.05 |
| | Site | 2 | 18 | 2.19 | 0.14 | 2 | 74 | 4.88 | <0.01 |
| | Species:Site | 2 | 18 | 0.13 | 0.88 | 2 | 74 | 0.64 | 0.53 |
| 3-Carene | Intercept | 1 | 18 | 17.20 | <0.001 | 1 | 74 | 49.90 | <0.0001 |
| | Species | 1 | 18 | 0.03 | 0.86 | 1 | 74 | 2.89 | 0.09 |
| | Site | 2 | 18 | 4.67 | <0.05 | 2 | 74 | 0.89 | 0.42 |
| | Species:Site | 2 | 18 | 0.45 | 0.64 | 2 | 74 | 0.90 | 0.41 |
| <i>β</i> -Phellandrene | Intercept | 1 | 18 | 28.53 | <0.0001 | 1 | 74 | 283.08 | <0.0001 |
| | Species | 1 | 18 | 1.39 | 0.25 | 1 | 74 | 16.35 | <0.001 |
| | Site | 2 | 18 | 1.87 | 0.18 | 2 | 74 | 3.78 | <0.05 |
| | Species:Site | 2 | 18 | 0.13 | 0.88 | 2 | 74 | 1.87 | 0.16 |
| Limonene | Intercept | 1 | 18 | 16.77 | <0.001 | 1 | 74 | 143.43 | <0.0001 |
| | Species | 1 | 18 | 0.10 | 0.76 | 1 | 74 | 26.31 | <0.0001 |
| | Site | 2 | 18 | 5.31 | <0.05 | 2 | 74 | 6.18 | <0.01 |
| | Species:Site | 2 | 18 | 0.42 | 0.66 | 2 | 74 | 3.30 | <0.05 |

Table 2. Continued

| | | Volatile Terpenes | | | | Phloem Terpenes | | | | |
|-----------------------------|--------------|-------------------|-------|-----------------|------------------|-----------------|-------|-----------------|-----------------|-------------------|
| | | numDF | lenDF | <i>F</i> -value | <i>p</i> -value | numDF | lenDF | <i>F</i> -value | <i>p</i> -value | |
| Terpinolene | Intercept | 1 | 18 | 39.37 | <0.0001 | | 1 | 74 | 81.24 | <0.0001 |
| | Species | 1 | 18 | 1.64 | 0.22 | | 1 | 74 | 17.77 | <0.0001 |
| | Site | 2 | 18 | 11.36 | <0.001 | | 2 | 74 | 3.08 | <0.05 |
| | Species:Site | 2 | 18 | 0.12 | 0.88 | | 2 | 74 | 1.77 | 0.18 |
| Total Terpenoids | Intercept | 1 | 18 | 29.19 | <0.0001 | | 1 | 74 | 330.86 | <0.0001 |
| | Species | 1 | 18 | 3.12 | 0.09 | | 1 | 74 | 42.47 | <0.0001 |
| | Site | 2 | 18 | 6.64 | <0.01 | | 2 | 74 | 5.08 | <0.01 |
| | Species:Site | 2 | 18 | 1.67 | 0.22 | | 2 | 74 | 2.14 | 0.12 |
| Verbenone | Intercept | 1 | 18 | 36.53 | <0.0001 | Linalool | 1 | 74 | 169.65 | <0.0001 |
| | Species | 1 | 18 | 1.86 | 0.19 | | 1 | 74 | 5.41 | <0.05 |
| | Site | 2 | 18 | 0.11 | 0.90 | | 2 | 74 | 3.07 | 0.05 |
| | Species:Site | 2 | 18 | 1.08 | 0.36 | | 2 | 74 | 0.81 | 0.45 |
| Phellandrene/ Limonene | Intercept | 1 | 18 | 13.32 | <0.01 | | 1 | 74 | 66.43 | <0.0001 |
| | Species | 1 | 18 | 0.95 | 0.34 | | 1 | 74 | 27.18 | <0.0001 |
| | Site | 2 | 18 | 2.08 | 0.15 | | 2 | 74 | 1.77 | 0.18 |
| | Species:Site | 2 | 18 | 0.45 | 0.65 | | 2 | 74 | 3.97 | <0.05 |
| 3-Carene/ Limonene | Intercept | 1 | 18 | 17.63 | <0.001 | | 1 | 74 | 60.91 | <0.0001 |
| | Species | 1 | 18 | 0.91 | 0.35 | | 1 | 74 | 1.77 | 0.19 |
| | Site | 2 | 18 | 1.44 | 0.26 | | 2 | 74 | 4.45 | <0.05 |
| | Species:Site | 2 | 18 | 0.45 | 0.64 | | 2 | 74 | 2.37 | 0.10 |
| α -Pinene/ Carene | Intercept | 1 | 18 | 21.44 | <0.001 | | 1 | 74 | 42.93 | <0.0001 |
| | Species | 1 | 18 | 1.83 | 0.19 | | 1 | 74 | 9.92 | <0.01 |
| | Site | 2 | 18 | 0.81 | 0.46 | | 2 | 74 | 0.54 | 0.59 |
| | Species:Site | 2 | 18 | 2.41 | 0.12 | | 2 | 74 | 2.78 | 0.07 |
| Myrcene/ Terpinolene | Intercept | 1 | 18 | 49.37 | <0.0001 | | 1 | 74 | 43.88 | <0.0001 |
| | Species | 1 | 18 | 0.60 | 0.45 | | 1 | 74 | 9.10 | <0.01 |
| | Site | 2 | 18 | 1.46 | 0.26 | | 2 | 74 | 0.67 | 0.52 |
| | Species:Site | 2 | 18 | 0.85 | 0.44 | | 2 | 74 | 0.26 | 0.77 |

Table 3. Species frequency tests (chi squared, Fisher's exact) of binary variables for Engelmann and blue spruces. Lure 2014 (9 per species), Landing 2014, Colonization 2014 (trees with attempted colonization), Lure 2015, Landing 2015, Colonization 2015. Site specific data is also included (Lost Mill and Shingle Creek). Lures were used at both Lost Mill (Engelmann = 2 Lures, Blue = 4 Lures) and Shingle Creek (Engelmann = 7 Lures, Blue = 8 Lures) in 2015. Percentage (%) is for trees if variable A and variable B (e.g. the likelihood if a lure was placed on a tree then it was landed upon). Count (#) is data for if variable A and variable B (as described for percentage). Degrees of freedom (*df*) is 1 for all variables. No statistical test could be complete for blue at Shingle Creek in 2015 because there was no variation (represented by “.”) Statistically significant results in bold.

| Variable A x B | Engelmann | | | | | Blue | | | | |
|--|-----------|----------|-----------------------------|-----|-------|----------|----------|-----------------------------|-----|------|
| | <i>N</i> | χ^2 | Fisher's Exact $P > \chi^2$ | % | # | <i>N</i> | χ^2 | Fisher's Exact $P > \chi^2$ | % | # |
| Lost Mill Lure 2014 x Landing 2014 | 18 | 14.40 | <0.001 | 89 | 8,9 | 16 | 1.78 | 0.30 | 22 | 2,9 |
| Lost Mill Lure 2014 x Colonization 2014 | 18 | 14.40 | <0.001 | 89 | 8,9 | 16 | 0.83 | 0.56 | 11 | 1,9 |
| Lost Mill Landing 2014 x Colonization 2014 | 18 | 18.00 | <0.0001 | 100 | 8,8 | 16 | 7.47 | 0.13 | 50 | 1,2 |
| Lure 2014 x Landing 2014 | 34 | 29.06 | <0.0001 | 89 | 8,9 | 32 | 5.45 | 0.07 | 22 | 2,9 |
| Lure 2014 x Colonization 2014 | 34 | 29.06 | <0.0001 | 89 | 8,9 | 32 | 2.64 | 0.28 | 11 | 1,9 |
| Landing 2014 x Colonization 2014 | 34 | 34.00 | <0.0001 | 100 | 8,8 | 32 | 15.48 | 0.06 | 50 | 1,2 |
| Lost Mill Lure 2015 x Landing 2015 | 18 | 0.45 | 0.69 | 100 | 2,2 | 16 | 6.86 | 0.05 | 50 | 2,4 |
| Lost Mill Lure 2015 x Colonization 2015 | 18 | 0.45 | 0.69 | 100 | 2,2 | 16 | 6.86 | 0.05 | 50 | 2,4 |
| Lost Mill Landing 2015 x Colonization 2015 | 18 | 18.00 | <0.0001 | 100 | 15,15 | 16 | 16.00 | <0.001 | 100 | 2,2 |
| Shingle Creek Lure 2015 x Landing 2015 | 16 | 12.44 | <0.001 | 100 | 7,7 | 16 | . | . | 0 | 0,8 |
| Shingle Creek Lure 2015 x Colonization 2015 | 16 | 12.44 | <0.001 | 100 | 7,7 | 16 | . | . | 0 | 0,8 |
| Shingle Creek Landing 2015 x Colonization 2015 | 16 | 16.00 | <0.0001 | 100 | 8,8 | 16 | . | . | 0 | 0,16 |
| Lure 2015 X Landing 2015 | 34 | 5.85 | <0.05 | 100 | 9,9 | 32 | 3.56 | 0.13 | 17 | 2,12 |
| Lure 2015 X Colonization 2015 | 34 | 5.85 | <0.05 | 100 | 9,9 | 32 | 3.56 | 0.13 | 17 | 2,12 |
| Landing 2015 X Colonization 2015 | 34 | 34.00 | <0.0001 | 100 | 23,23 | 32 | 32.00 | <0.001 | 100 | 2,2 |

3 CONCLUSION

Given that the warming climate is affecting forests and insects across the United States, (Bentz et al. 2010, Chmura et al. 2011, Anderegg et al. 2015, Kolb et al. 2016) it is uncertain whether blue spruce, which commonly experiences little to no colonization by spruce beetle, will experience future colonization and outbreaks. Bark beetle outbreak size and the extent of the impact on forests are increasing. Trees growing in forests that formerly had little to no beetle-caused mortality now have active colonization with warming winter temperatures (Carroll et al. 2003). By employing a multi-discipline approach to better understand relationships between spruce beetle and its host species, this dissertation suggests how drought and higher beetle populations may affect spruce beetle interactions between a preferred host and one less suitable including host selection and colonization.

There are many characteristics of blue spruce associated with low spruce beetle colonization. Bark and phloem thickness, resin flow, phloem and volatile terpenes, and tree physiology likely affect blue spruce colonization. Blue spruce and Engelmann spruce growing in moist mixed conifer stands follow predictable seasonal trends in water potential. However, blue spruce has a different intrinsic water use efficiency, assimilating higher levels of carbon-12 than carbon-13. Whether this water use strategy will be successful for blue spruce with global warming and corresponding spruce beetle susceptibility is dependent upon future precipitation, which is uncertain though predicted to decrease in many future climate models (Intergovernmental Panel on Climate Change 2014). These differences may affect tree defensive traits, where in a warming and drying climate both Engelmann spruce and blue spruce colonization may increase.

Overall, this study demonstrates variation in structure, function, and host chemistry between blue spruce and Engelmann spruce related to spruce beetle colonization. In addition, this study indicates a tradeoff between growth and defense that may be susceptible to drought and climate change related warming for blue spruce stomatal conductance. Warming climate predictions indicate increases in the rate of spruce beetle reproduction and advances into once climatically unsuitable regions that were either too cold or trees were too vigorous for spruce beetle colonization. Spruce

beetle will certainly be a recurring problem for land managers in the future (Carroll et al. 2003, Negron et al. 2008, Bentz et al. 2010).

Further research related to bark and phloem nitrogen, beetle vision and host tree reflectance, no resin flow, and other physical characteristics such as antifeedant structures (e.g. calcium oxalate crystals, stone cells, phenolics, lignin) may help explain the differences between blue spruce and Engelmann spruce colonization by spruce beetle. Other gaps in research, not addressed in this study, related to spruce beetle host defenses include induced resin duct formation (Lombardero et al. 2000, Martin et al. 2002, Faldt et al. 2003) and induced terpenes as indicated in Davis et al. (2018).

3.1 References

- Anderegg, W. R., J. A. Hicke, R. A. Fisher, C. D. Allen, J. Aukema, B. Bentz, S. Hood, J. W. Lichstein, A. K. Macalady, and N. McDowell. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* **208**:674-683.
- Bentz, B. J., J. Regniere, C. J. Fettig, E. M. Hansen, J. L. Hayes, J. A. Hicke, R. G. Kelsey, J. F. Negron, and S. J. Seybold. 2010. Climate Change and Bark Beetles of the Western United States and Canada: Direct and Indirect Effects. *BioScience* **60**:602-613.
- Carroll, A. L., S. W. Taylor, J. Regniere, and L. Safranyik. 2003. Effects of climate change on range expansion by the mountain pine beetle in British Columbia. Pages 223-232 in *Mountain Pine Beetle Symposium: Challenges and Solutions*. Natural Resources Canada, Information Report BC-X-399, Victoria, Kelowna, BC.
- Chmura, D. J., P. D. Anderson, G. T. Howe, C. A. Harrington, J. E. Halofsky, D. L. Peterson, D. C. Shaw, and J. B. S. Clair. 2011. Forest responses to climate change in the northwestern United States: ecophysiological foundations for adaptive management. *Forest Ecology and Management* **261**:1121-1142.
- Davis, T. S., F. B. Horne, J. C. Yetter, and J. E. Stewart. 2018. Engelmann Spruce Chemotypes in Colorado and their Effects on Symbiotic Fungi Associated with the North American Spruce Beetle. *Journal of Chemical Ecology* **44**:601-610.
- Faldt, J., D. Martin, B. Miller, S. Rawat, and J. Bohlmann. 2003. Traumatic resin defense in Norway spruce (*Picea abies*): Methyl Jasmonate-induced terpene synthase gene expression, and cDNA cloning and functional characterization of (+)-3-carene synthase. *Plant Molecular Biology* **51**:119-133.

Intergovernmental Panel on Climate Change. 2014. IPCC Fifth Assessment Synthesis Report. IPCC 5th Assessment Synthesis Report.

Kolb, T. E., C. J. Fettig, M. P. Ayres, B. J. Bentz, J. A. Hicke, R. Mathiasen, J. E. Stewart, and A. S. Weed. 2016. Observed and anticipated impacts of drought on forest insects and diseases in the United States. *Forest Ecology and Management* **380**:321-334.

Lombardero, M. J., M. P. Ayres, P. L. Lorio Jr., and J. J. Ruel. 2000. Environmental effects on constitutive and inducible resin defenses of *Pinus taeda*. *Ecology Letters* **3**:329-339.

Martin, D., D. Tholl, J. Gershenzon, and J. Bohlmann. 2002. Methyl Jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. *Plant Physiology* **129**:1003-1018.

Negron, J. F., B. J. Bentz, C. J. Fettig, N. Gillette, E. M. Hansen, J. L. Hays, R. G. Kelsey, J. E. Lundquist, A. M. Lynch, R. A. Progar, and S. J. Seybold. 2008. US Forest Service bark beetle research in the western United States: Looking toward the future. *Journal of Forestry* **106**:325-331.