EVALUATING GROWTH-DEFENSE TRADE-OFFS IN PONDEROSA PINE (*PINUS PONDEROSA*) IN RESPONSE TO STIMULATED BARK BEETLE ATTACK

By

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DEDICATION

This document is dedicated to my husband Kurt Jones, without his dedication and love none of this would be possible. As well as to my mother Annette Hull whose unconditional love and support has pushed me to persevere even when I wanted to give up.

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I would like to express my unending appreciation to Dr. Amy Trowbridge whose help has made this all possible. Without her guidance and support I would not have been able to construct this paper. Her expertise and attention to detail throughout this entire process has allowed me to construct and refine my writing skills into a paper that I am very proud of. I am very fortunate that I have had the opportunity to work with her and expand on a topic that I one day wish to pursue further as I continue my graduate education. Great thanks to the Trowbridge lab members for the collection and to Dr. Trowbridge and Dr. Tim Seipel for the data analysis and figures for this paper.

I would also like to thank the faculty and staff of the Department of Land Resources and Environmental Sciences, thank you for your support and feedback throughout the process of this degree.

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ABSTRACT

The mountain pine beetle (MPB; *Dentroctonus ponderosae,* Hopkins, Coleoptera: Curculionidae: Scolytinae) and its associated blue stain fungi are considered to be among the greatest natural threat to conifer ecosystems worldwide. In response to a rapidly changing climate, namely more frequent and hotter droughts, bark beetles benefit through a combination of higher reproductive capacity and greater availability of weakened and stressed host trees. Conifers have potent constitutive and induced chemical defenses to resist the bark beetle-fungi complex, but investment in these carbon-based defenses may be constrained by trade-offs associated with a tree's inherent growth rate. Although there are trade-offs that exist among all processes, it is unclear whether those particular growth-defense trade-offs are measurable and can be compared between individuals within various populations. Here we assess the concentration and composition of constitutive and induced terpene chemical defenses as a function of intraspecific variation in growth rates (slow-versus fast-growing mature ponderosa pines). We examine these relationships in the context of classical plant defense theories as well as the current state of the field. By determining the variation in chemical responses to stimulated bark beetle attack we can further our understanding of potential resistance trade-offs that might exist in stands that are being selectively bred for fast growth.

INTRODUCTION

Ecology of the mountain pine beetle in ponderosa pine habitats

Bark beetles (subfamily, Scolytinae) are obligate herbivores that have coevolved with conifer ecosystems since the early Mesozoic (Reeve et al. 2012). The mountain pine beetle (*Dentoctonus ponderosae, Hopkins*) is a native 'irruptive' species found throughout western North America and continues to be one of the leading causes of tree mortality throughout much of the US and Canada (Cooper et al. 2018) and is currently classified as one of the most destructive insect pests for a variety of conifer species (Balogh et al. 2019).

Beetles attack trees by burrowing into the trees bark and into the phloem tissue where they construct galleries for the purpose of mating and rearing offspring (Cooper et al. 2018). Successful attacks only occur when sufficient numbers of bark beetles are recruited to aid in the depletion of the tree's natural defenses. The use of aggregate pheromones are used by the initial attackers to recruit more beetles to help overcome these defenses (Byers et al. 1989). Semiochemicals found within the beetles' hindgut when combined with α-pinene from terpene vapors absorbed or ingested from the phloem tissue will then be converted into *cis*-verbenol or *trans*-verbenol which will aid in the metabolic production of aggregate pheromones (Byers et al. 1989). The ability to produce such pheromones is a vital adaptation for the success and colonization of the MPB (Cooper et al. 2018). Concurrent with mass attack, beetles also inoculate their host with associated blue stain fungi, which is often carried in specialized mycangia or on their exoskeletons (Balogh et al. 2019). Because the MPB and their associated fungi colonize within subcortical tissues, the reproduction and development of their young

typically impairs the trees ability to translocate water and nutrients, resulting in tree mortality (Keefover-Ring et al. 2016).

Tree defenses include the production of resin as a means to physically expel the beetle from the tree or act as toxic or deterrent compounds. The production of such defenses, however requires a substantial investment of resources (Cooper et al. 2018). While bark beetles can spend a significant amount of time at low population densities the depletion of resources that aid in the production of these defenses can result in an increase in suitable hosts and thus a sudden MPB population increase. As a result, MPB outbreaks are commonly observed following an environmental trigger, for example when trees are stressed due to drought, disease, fire, or old age (Carmen et al. 2017).

Most MPB research focuses on lodgepole pine (*Pinus contorta*) ecosystems, as this is its most common and historical host (Maddens et al. 2012). However, MPB can also attack ponderosa pine (*Pinus ponderosa*). Ponderosa pine habitats such as those in western South Dakota, parts of Montana, and along the western region of Colorado currently experiencing substantial mortality due to MPB outbreak (Costella et al. 2013). The relatively warm and dry climate within ponderosa pine habitats makes them especially susceptible to eruptive MPB outbreaks. Ponderosa pines are relatively slow growing and intolerant to shade and dependent on adequate soil moisture and precipitation for successful regeneration (Briggs et al. 2015). When considering the direct effects of climatic change on ponderosa pine defenses we question if there is also evidence of associated growth-defense tradeoffs that may contribute to our understanding of defenses and susceptibility in this system.

Bark beetle symbionts

Bark beetles often carry a combination of fungal, bacterial, and mite associates that can influence the success of tree colonization and reproduction by depleting or helping beetles cope with trees defenses (Kausrud et al. 2011: Schowlter 2012) . The majority of these microorganisms are pathogenic fungal species, e.g. *Grosmannia clavigera Zipfel* or *Ophiostoma montium* that are capable of stimulating tree-induced defenses during their introduction (Gao et al. 2017). The tree typically responds to the fungi by allocating terpenoids to the site of infection in an attempt to prevent further fungal growth (Keefover-Ring et al. 2016). Some hypothesize that it is this mutualistic relationship between the fungi and the MPB that allows for such high beetle dispersal rates even when resources are considered to be depleted (Gohli and Jordal 2017). The blue stain fungi benefit the beetles by growing throughout a tree's parenchyma cells and can even act as a stored nitrogen budget for the MPB (Ayres et al. 2000), but the fungi can also modify host tree microclimates to favor brood development and protect adults from toxic defense compounds (Dario et al. 2017). Some species of bark beetles have even been known to participate in fungus farming such as that observed in terminates, ants, and two other beetle groups. These beetles transport the fungi from their birth site to new resources where spores are inoculated and will develop for future larval consumption (Gohli and Jordal 2017).

Herbivores and pathogens have major impacts on trees and are currently hypothesized to be some of the largest driving forces in their evolution of defenses and resilience (Ott et al. 2011). During MPB attacks not all trees will be attacked, there are trees that will not be landed upon, or are attacked but not successfully colonized. The eeasons why certain trees survive are still largely unknown, although genetic characteristics of the tree and their interactions with the environment and beetle symbionts are strongly influenced by any expression of resistance

mechanisms within the tree (Ott et al. 2011) Genetic resistance against bark beetles and their associated symbionts could prove to be a leading benefit for the development of management and prevention of bark beetle outbreaks.

Tree bark beetle interactions: Chemical defenses

Resin provides an important physical barrier against bark beetle attack; it also contains compounds that can prove to be both toxic, repellent, or even inhibit the attraction of released aggregation pheromones by attacking beetles (Keefover-Ring et al. 2016). Resin flow can delay entering beetles providing critical time for the biosynthesis of insecticidal and antimicrobial compounds. Such defenses among conifers are primarily carbon based including phenolics, terpenoids, and alkaloids. Conifer defense reactions are complex and involve several metabolic pathways, including the isoprenoid and phenylpropanoid pathways (Pan et al. 2018). Conifers rely heavily on monoterpenes and other products of the isoprenoid pathway for the defense against bark beetle attack (Pan et al. 2018). Monoterpenes that are stored within the resin ducts, phloem, and sapwood are the first layer of defense. After beetle attack and fungal infection monoterpene concentrations increase dramatically within necrotic lesions formed by the trees induced defenses (Novak et al. 2014; Keefover-Ring et al. 2016; Pan et al. 2018). For example, elevated levels of monoterpenes such as β-pinene, 3-carene, sabinene following artificial fungal inoculations have previously been observed (Novak et al. 2014). Monoterpenes although advantageous at high levels do inhibit bark beetles at lower concentrations where they can be beneficial by serving as a precursor for aggregate pheromone production (Raffa et al. 2014).

Phenolics often occur in trees as polymers, acids, or glycosylated esters (Harborne et al. 1979). Lignin is a phenolic known as a phenylpropane heteropolymer which provides a physical

defense by providing structural strength to the wood. Induced production of phenols is a common physiological response to injury and infection among plants (Arango-Velez et al. 2018). Phenolics can also act as fungistatic compounds that respond with the anatomical changes within the plant cells surrounding the site of fungal infection (Arango-Velez et al. 2018). Such responses limit the spread of fungal infection and damage to the tree's subcortical tissues.

Conifer defenses are characterized as either constitutive or induced, each having their own level of fitness and ecological costs. Constitutive defenses are present regardless of the presence of an ongoing attack whereas inducible defenses are only triggered after pathogen or herbivore activity (Villari et al. 2014). Induced defenses are expected to be more-cost efficient than constitutive defenses as they are only produced in response to stress and can be tailored to specific elicitors via stress-induced hormones (Moreira et al. 2015).

Conifer defenses are a combination of high concentrations of carbon-based chemical s and physical structures (Moreira et al. 2015). Investment in these carbon-based compounds are dictated by the level of environmental stress, such as drought or increased temperatures, and herbivore pressure. Conifer constitutive or inducible defenses have presumably evolved in response to these environmental extremes as well as the differences in the strength and variability of challenging elements (Moreira et al. 2015). Constitutive chemical defenses vary based on responses to a diversity of abiotic factors including soil nutrient availability - higher levels of energy are invested in constitutive defenses when living in resource poor conditions than when resources are rich (Moreira et al. 2012). Thus, the level of defense and its associate costs both vary depending on the abiotic and biotic context in which the plant is embedded (Moreira et al. 2016).

Moreira et al. (2012) discuss the evidence of trade-offs between induced and constitutive

expression of chemical defenses across pine species. There is a strong phylogenetic signal found in constitutive concentrations of phenolics within the needles and the stem resin. Evidence also suggests constitutive concentrations of resin within stems are negatively correlated with induced concentrations of resin and growth rate, resulting in slow growing species investing more in constitutive defenses when compared to that of induced levels of resin production (Moreira et al. 2016). More specifically slow growing species tend to prioritize investment in higher levels of constitutive resin within needles and stems, compared to that of fast-growing species that prioritize high inducibility of resin and phenolics within needles (Moreira et al. 2016). We question if similar patterns are true for ponderosa pine when under induced bark beetle attack.

Climate change impacts on tree-beetle interactions

A combination of global climate change and population dynamics among conifer ecosystems has increased the abundance of eruptive bark beetle outbreaks (Kausrud et al. 2011). Bark beetles are an obligate herbivore whose development and host selection are extremely sensitive to climatic change. As a result, bark beetle distribution around the globe has already been documented to have increased as a response to elevated temperature (Seidl et al. 2016). Warmer and drier conditions stress host trees and provide longer periods of temperatures suitable for beetle reproduction (Cooper et al. 2018). Temperature directly influences the rate of beetle development, affecting both phenotype (size, fecundity) and phenology (timing of emergence) (Lombardo et al. 2018). With increases in climatic shifts beetles are now both more capable of reproducing rapidly (creating more generations per year) and can more easily overwhelm tree defenses (Cooper et al. 2018).

Similar patterns have also been observed with respect to MPB distribution. Beetles that do not currently occupy the full extent of their host tree range are considered to be largely

limited by climate (Bentz et al. 2010). With an increase of annual temperatures beetles are no longer constrained by such limitations. This is why the expansion of MPB can now be seen as far north as parts of British Columbia and eastward into the boreal forests of north-central Alberta. Stressed host trees coupled with warm summers and warmer winters can result in outbreak progression across landscapes of now suitable hosts.

Carbon allocation to tree defense

The allocation of a plant's carbon among the different sink organs and functions determines the relative growth rates, biomass accumulation, and accumulation of chemical defenses (Lacointe 2000). Carbon allocations flows from "source" areas such as leaves to "sink" areas including chemical and morphological defenses. Effects of minor nutrients or temperatures on assimilate allocation are considered to be extremely variable, however, major factors such as water, mineral nutrients, and light availability will affect the relative carbon allocation and defenses to various parts of the plant (Lacointe 2000).

The secondary metabolites of woody plants are largely controlled by resource availability. This proposes a hierarchical model of carbon allocation to growth as well as the storage of carbon-based defensive compounds (Villari et al. 2014). One of the most commonly invoked conceptual frameworks for assessing investment in defense under stress is the growth-differentiation balance hypothesis (GDBH). The GDBH predicts that under mild to moderate resource limitations, growth is more than photosynthesis and the higher available levels of carbohydrates that will thus be allocated to defense (Moreira et al. 2015). The Resource Availability Hypothesis is another useful theory that suggests that unfavorable environments that favor slow growth strategies should select for increased allocation to constitutive chemical

defenses for plants that cannot easily replace damaged tissues that normally represents a large portion of their growth (Moreira et al. 2016).

We can use these plant defense theories to construct a stronger frame work of the investment in growth and defense in response to various global factors. Increases in air temperature and drought are considered to act as globally important drivers of forest change, largely due to the potential effects on tree growth and forest productivity (Duan et al. 2018). With additions to atmospheric CO_2 and elevated temperatures frequent and more extreme droughts are more likely to occur. These droughts quantify tree growth responses to a changing environment and are a key factor in determining how forests cope and function during climatic change (Duan et al. 2018). However, past research does indicate disparities that relate to the context of existing resilience and resistance among drought stressed trees that could relate to the existence of a threshold of water stress intensity (Gao et al. 2017). The growth-differentiation-balance hypothesis (GDBH) predicts that moderately water-stressed trees may actually be more resistant to bark beetle attacks as moderate water stress constrains growth sinks more than defensive compound sinks, also leading to a relative increase in overall resin production (Macalady et al. 2013). If true evidence would suggest that there would be a potential trade-off between defense compounds and relative growth rate when under environmental or herbivore stress.

For this study, my objective was to investigate the potential differences in investment of constittutive and induced mono- and sesquiterpenes that might result from trade-offs with relative growth-rates of fast-and slow-growing families of ponderosa pine. I hypothesized that fast-growing trees would allocate more resources towards growth than physical defenses resulting in reduced constitutive levels of terpene defenses while slow growing trees would

respectively allocate more resources towards constitutive chemical defenses as a part of a trade-off with respect to growth. I also hypothesize that there will be an associated trade-off between constitutive and induced chemical defenses with fast-growing trees exhibiting higher levels of induced defenses when compared to that of slow-growing trees. I will aim to explore how changes in the ratio of different classes of terpenes alters resin composition and how inherent growth rates may shift both chemical toxicity and physical defenses as part of the tree's comprehensive defense syndrome. The data that was used for this paper was collected by Trowbridge lab members, and all statistical analyses and figures were completed by Dr. Trowbridge and Dr. Seipel and then shared with me for interpretation for this paper.

MATERIALS AND METHODS

Study Site

This study took place at the University of Montana's Lubrecht Experimental Forest in western Montana (46.8874° N, 113.4753° W). The experiment leveraged an existing common garden that consisted of half-sibling trees part of a genetic trial initiated in 1974 by the Inland Empire Tree Improvement Cooperative (see de la Mata *et al.*, 2017). Across 44 natural stands in western Montana and northern Idaho, seeds were collected from 204 open-pollinated, unrelated, wild mother trees that were selected for enhanced growth and improved morphology. Seeds were then planted and reared at the US Department of Agriculture Forest Service tree nursery in Coeur d'Alene, Idaho. One-year-old bare-root seedlings were planted on 3 × 3-m spacing using a randomized complete block design with four-tree family row plots in each of five blocks for a total of 20 trees per family (4,025 trees in total). Additional planting of 2-y-old seedlings occurred in 1975 to replace mortality during the first year. The mean annual air temperature at the site is 7 °C and mean annual precipitation is 500 mm, with 44% falling as snow.

For this experiment lab members selected five of the fastest and slowest growing families (herein referred to as "fast" and "slow" families) and based this on the percentage total present as opposed to the percentage live within a stand. This was because the long-term monitoring plots were mass attacked and killed by bark beetles between 2005 and 2012 and they could not be sure that the beetles did not attack live trees because they could not get to them or because there was some sort of defensive strategy that was advantageous and allowed for active avoidance. Using a random number generator, they chose four target trees to sample within each family ($n = 5$) in the fast and slow groups. Within the fast families that were chosen, at least one tree was observed to have pitch tubes, suggesting that beetles attempted to colonize but were unsuccessful. Interestingly, only seven total trees were observed to have pitch tubes in these fast families, and of the seven trees, three were in family 32. Thus, for this particular family we selected two unattacked trees and two trees containing pitch tubes so not to bias the sampling.

Measurements

To assess constitutive levels of terpenes, the Trowbridge lab collected phloem samples from 40 trees on the 24th and 25th of August 2016. Sample locations (cardinal direction) on target trees were determined using a random number generator and a 1 x 4 cm piece of phloem was collected at the initial time point (T_0) by removing the outer bark with a chisel and cutting the phloem using a scalpel. To examine the induced effect of bark beetle attack they followed the methods described in Keefover-Ring *et al.*, (2016). Trees were wounded laterally to where the constitutive sample was taken by removing a bark plug using a 6 mm tree borer and were immediately inoculated with the mount pine beetle's primary symbiont, *Grosmannia clavigera* (Provided by Dr. Six and the University of Montana). The fungal inoculation was applied by procuring a 5 mm plug of actively growing mycelium, administering it directly to the phloem, and reinserting the

plug (see Boone *et al.*, (2011)). Trees were then resampled on 8 and 9 September 2016 (T_1) approximately 15 d following the wounding + inoculation treatments by removing lesions from the damaged areas with a scalpel. Samples were placed in glass vials and immediately stored in a cooler over dry ice before being brought to Montana State University. They were stored at -80 C until they could be processed for chemical analysis.

Chemical extraction and analysis

After removing samples from the freezer as needed, the phloem was cut into small cubes (~2-3 mm) and divided into two portions and each was immediately submerged into 1 ml of 95% n-hexane with 0.1 um ml-1 of fenchone as an internal standard in 2 ml GC vials with PTFE screw caps for mono- and sesquiterpene extraction. All vials were placed in a sonication bath for 10 min, vortexed, and allowed to shake overnight on an orbital mixer. Due to the high concentration, all induced samples were diluted 1:11 with the hexane + internal standard solvent prior to chemical analysis. Identification and concentrations of monoterpenes was achieved using available standards, all terpenoids were analyzed according to the methods described in (Keefover-Ring *et al.*, (2016). The lab analyzed the concentration and composition of mono- and sesquiterpenes using gas chromatography-mass spectrometry (GC-MS). The Agilent 5977B GC-MS system was equipped with a Cyclodex-B capillary column (30m x 0.25 mm I.D., film thickness 0.25 μm; Agilent Technologies) with helium as the carrier gas at a flow rate of 1.0 ml min^{-1}. 2 μl of each sample directly injected with a split flow ratio of 30:1 and an over profile of 40 °C for 5 min, followed by a ramp of 3 °C to 200 °C, and then a second ramp at 25 °C min^{-1} to 220 °C. Injector and detector temperatures were set at 260 °C and 250 °C, respectively. Monoterpene enantiomers were identified by comparing retention times of known standards and mass spectra using the NIST library and MSD Chemstation software. Concentrations for each

compound were calculated using 4-point calibration curves with injections of known amounts of pure standards and the internal standard, fenchone. All standards were purchased from Sigma Aldrich (Saint Louis, MO) with the exception of β-phellandrene (Glidco Organics, Jacksonville, FL). The lab then dried all phloem samples to a constant weight at 60 °C, and used dry weight values to calculate concentration.

Data analysis

Dr. Trowbridge and Dr. Seipel assessed potential differences among the various classes of terpenes that might exist between fast and slow growing families using SAS software version 9.4 (SAS Institute 2013). For total concentration data in each class, they conducted single-factor analysis of variance tests (ANOVA; PROC MIXED function) after square-root transforming the data to meet assumptions of normality. They used growth or family as the factors and tree as a random effect and significant omnibus tests were followed with a Tukey *post hoc* test using a Bonferroni correction to determine differences among the five families. To assess any potential trade-offs in investment in constitutive and induced defenses, they performed correlation analyses for the total concentration of each compound class and for each individual compound. They used the cor.test command in R (R version 2.4-5) to calculate a P-value and the Pearson's moment coefficient and used the lm function to solve for the best-fit linear model. To evaluate potential differences in composition between constitutive and induced mono- and sesquiterpene chemistry for fast and slow growing families, a permutational analysis of variance in R was used (R package version 2.4-5 vegan: Community Ecology Package). To evaluate potential differences in composition between constitutive and induced mono- and sesquiterpene chemistry for fast and slow growing families, they used a permutational analysis of variance in R (R package version 2.4-5 vegan: Community Ecology Package). To visualize dissimilarity in

chemical composition among families, and then they performed non-metric multidimensional scaling analyses (NMDS).

RESULTS

Total concentrations

Neither constitutive nor induced average total monoterpene concentrations differed between fast- and slow-growing families of ponderosa pine (Fig. 1A and B) and there was no trade-off observed between investment in constitutive and induced total monoterpene concentrations across samples (P=0.21 r=0.20). Average total sesquiterpene concentrations followed similar trends with no difference observed for constitutive or induced concentrations between fast-and slow growing families (Fig. 1C and D) and no trade-off observed between the two defense strategies (P=0.56 r=0.09). The lab did fail to observe any significant correlations between constitutive and induced concentrations of total mono- and sesquiterpenes across all growth rates and families (P=0.21 r=0.20 and P=0.56 r=0.09, respectively) or within fast and slow families.

Variation in total constitutive and induced monoterpene concentrations were significant among slow-growing families (Fig. 2A and B), but not among fast-growing families (P=0.56 for constitutive and P=0.74 for induced). The same pattern held for constitutive and induced defense with Family 115 exhibiting significantly higher levels of total monoterpenes relative to the other four families. In other words, the family with the highest constitutive levels of monoterpenes also exhibited the highest induced concentration. Similar to the monoterpenes, there was an observed significance variation in total constitutive and induced sesquiterpene concentrations (Fig. 2C and D) but only in the slow-growing families (P=0.25 for constitutive and P=0.65 for induced in the fast-growing families). However, the relationship between constitutive and induced levels of total sesquiterpenes were quite variable among families. For example, Families 115 and 147

contained higher constitutive sesquiterpene levels relative to Family 526 (Fig. 2C); however, only Family 115 maintained elevated induced sesquiterpene concentrations in response to inoculation while Family 147 actually exhibited a reduction in concentration that was as low as that of Family 526 (Fig. 2D).

Individual compounds

Of the 13 monoterpene hydrocarbons measured, only two—both enantiomers of α-pinene—showed significant correlations between constitutive and induced levels. There was an observed significant positive correlation between constitutive and induced levels of (-)-α-pinene (Fig. 3A; P=0.04 r=0.32) and (+)-α-pinene (Fig. 3B; P=0.03 r=0.35) across all growth rates and families. Interestingly, the correlation for (-)-α-pinene was primarily driven by responses within slow-growing families (P=0.04 r=0.46) while that of (+)-α-pinene was largely the result of fast-growing families (P=0.0001 r=0.78). Constitutive levels of the phenylpropanoid, estragole, were also positively correlated with induced concentrations across all growth rates and families (Fig. 3C; P=0.003 r=0.46), but was largely determined by fast-growing families (P=0.0009 r=0.68) while the oxygenated monoterpene, (-)-linalool, exhibited a positive correlation between constitutive and induced levels across all growth rates and families (Fig. 3D; P=0.05 r=0.30), but was determined largely in part by slow-growing families (Fig. 3D; P=0.03 r=0.40).

Composition

Chemical composition varied significantly in response to the inoculation (e.g. treatment), family, and their interaction (Table 1) with the treatment explaining most of the variation (53%). The interaction between treatment, family and growth was marginally significant (P=0.078); however, growth rate (fast versus slow) was not a significant predictor of composition and

explained less than 1% of the variation in mono- and sesquiterpene composition.

Table 1. *F*-ratios, R^2-values, and *P*-values from a PERMANOVA test comparing compositional differences among treatment (constitutive and induced concentrations), growth rate (fast versus slow), and families.

Factor	***F*-ratio**	**R^2-values**	***P*-values**
Growth	0.92	0.0056	0.43
Treatment	86.21	0.5257	**0.001**
Family	2.63	0.016	**0.048**
Growth x Treatment	0.7	0.0043	0.561
Growth x Family	2.13	0.013	0.102
Treatment x Family	3.12	0.019	**0.041**
Growth x Treatment x Family	2.3	0.014	0.078

FIGURES

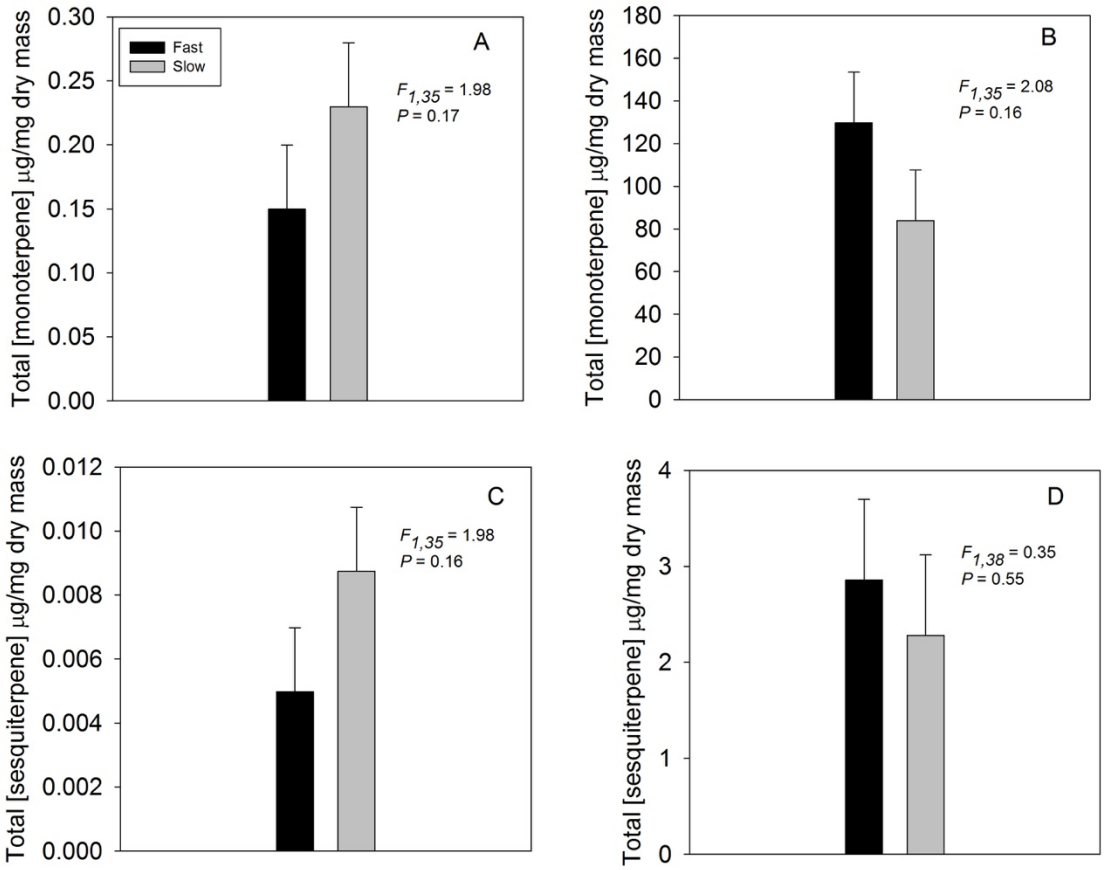

Figure 1. Total monoterepene (A,B) and sesquiterpene (C,D) concentrations (µg/mg dry mass) for fast- (black bars) and slow-growing (gray bars) families of ponderosa pine. Constitutive (A,C) and induced (B,D) values are shown where the bars represent the back-square root transformed least squares mean and the bars represent the standard error of the mean (SEM).

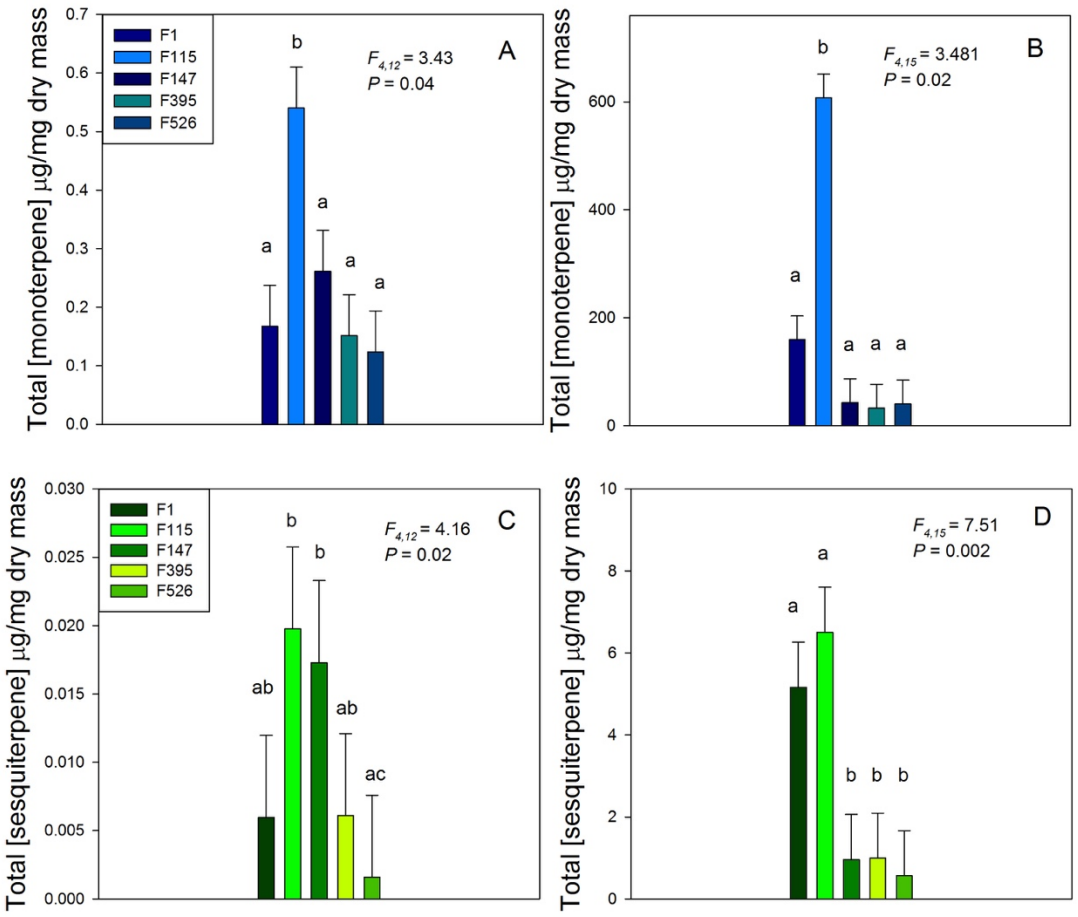

Figure 2. Total monoterepene (blue: A,B) and sesquiterpene (green: C,D) concentrations (µg/mg dry mass) for the five slow-growing families of ponderosa pine. Constitutive (A,C) and induced (B,D) values are shown where the bars represent the back-square root transformed least squares mean and the bars represent the standard error of the mean (SEM). Differences among families for each chemcial class (mono- and sesquiterpene) and time point (constitutive and induced) after applying a Bonferroni correction are represented by lower case letters.

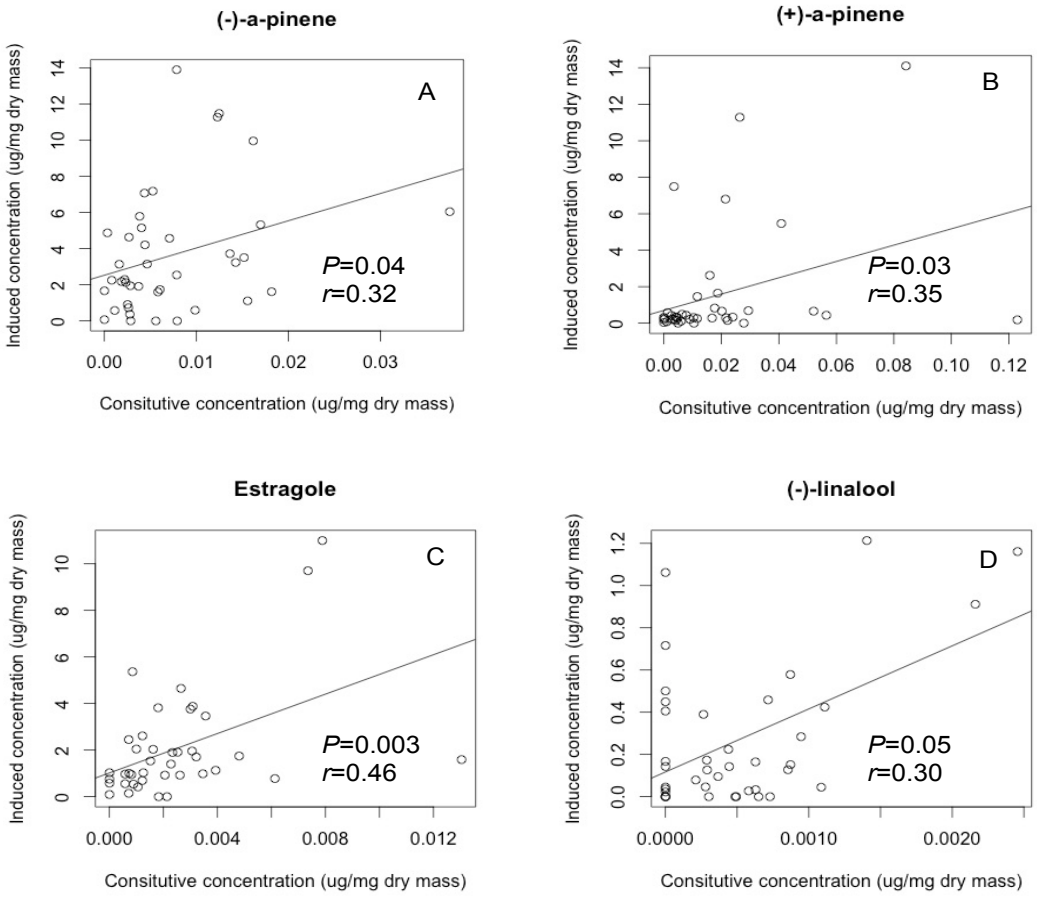

Figure 3. Linear correleations between constitutive and induced total concentrations for (-)-a-pinene (A), (+)-a-pinene (B), estragole (C), and (-)-linalool (D). The line represents the best-fit linear model (lm function) and *P*-values and pearson's correlation coefficients (*r*) are shown.

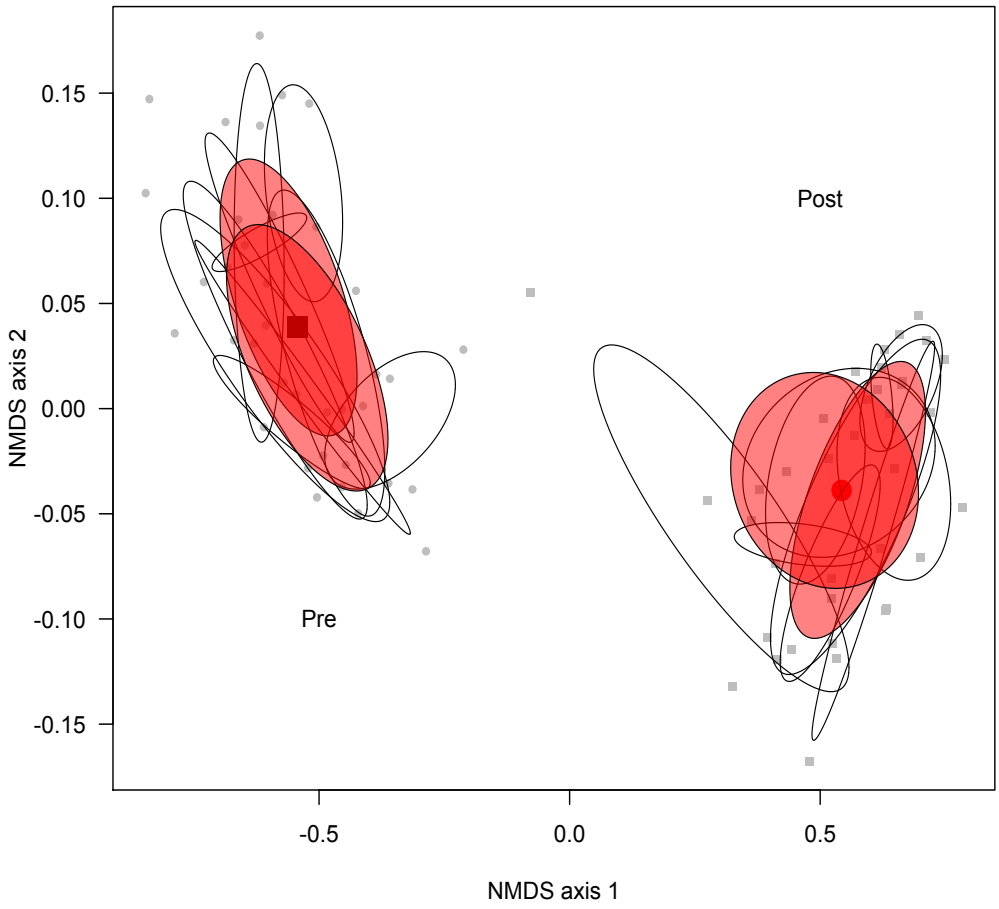

Figure 4. Nonmetric multidimensional scaling (NMDS) plot of mono and sesquiterpenes present in ponderosa pine phloem. Samples closer in space are indicative of having a more similar composition. The composition of constitutive (Pre) and induced (Post) compounds are significantly different with red elipses representing the standard deviation around the centroid of fast and slow growing families and the black elipses representing the standard deviation around the centroild of individual families within each treatment (constitutive versus induced).

DISCUSSION

The overreaching objective of this work was to investigate the potential differences in investment of consittutive and induced mono- and sesquiterpenes that might result from trade-offs with relative growth-rates of fast-and slow-growing families of ponderosa pine. While we failed to observe significant trade-offs between defensive strategies for either growth type (Fig. 1A and B), there was an observed trend that fast-growing families tend to have lower constitutive levels of mono- and sesquiterpenes and higher induced responses relative to slow-growing families that exhibit the opposite trend of higher consittutive investment and a lower induced response. This lack of a clear trade-off is likely due to the high level of vartiation observed between families within each growth type, paticularly the slow-growing families (Fig.2). The high level of variation in the slow-growing families may potentially benefit the tree by making individuals less predictable as a source for bark beetles, resulting in higher mortality rates for fast-growing individuals (de la Mata et al. 2017). The lab also failed to observe trade-offs between constitutive and induced levels for individual terpenes, and in fact, four compounds did exhibit positive correlations (Fig. 3). Furthemore, the differential responses of individual compounds to stimulated bark beetle attack resulted in significatly different compositions between constiutive and induced resin, regardless of growth (Fig.4). Below I discuss each of these points in more detail and describe how the observations relate to previous work investigating growth-defense trade-offs and how these results might explain previous observations of higher beetle attacks in fast-growing families during epidemics.

Growth rate can have beneficial fitness advantages but can also include intrinsic and extrinsic costs (de la Mata et al. 2017). Faster growth rates can result in larger size and the

potential to provide faster reproductive rates, stronger competitive abilities, and an increased probability of early establisment and survival (de la Mata et al. 2017). However, faster growth rates are also known to be assosciated with a shorter life span and decreased defense rates (de la Mata et al. 2017). Research conducted by de la Mata et al. (2017) discovered that prior to an endemic MPB outbreak fast growing trees survived at higher rates then those that were slow growing, but after the outbreak the opposite was found. Although such evidence does indicate a growth-survival trade-off we failed to observe such interactions in either slow or fast-growing trees (Fig.2). While total concentrations were not different, it is possible that the lower level of variation observed for major chemical defenses within fast- growing trees would be benefcial to beetles searching for more consistent and honest host cues of potential host defences, where the more variable chemistry observed in the slow-growoing families (Fig.3) could make them less reliable indicators for success.

Current research also suggests that trees found to survive subsequent bark beetle attacks invest more carbon into resin defenses then those that are observed to die from these attacks (Kane and Kolb 2010). Carbon allocation to resin ducts is also considered to be more of an important determinant of tree mortality than allocation to radial growth (Kane and Kolb 2010). Compositional changes in induced and consitutive resin is thought to be largely due to additional substrates provided by excess carbon for terpeniod biosynthesis (Manninen et al. 1998). In the absence of costs, there are no constraints on resource allocation, and selection may favor the maintance of multiple defenses if they do enhance the probability of possessing a trait that enables confers to resist such invaders (Koricheva et al. 2004). Although the resource availibility hypothesis (RAH) predicts an inverse relationship between constitutive and inducible defenses (Hood et al. 2015) we could hypothesize that the results here could be due to the lack of live

beetle interactions and the induced nature of the treatement or positive correlations between resin duct and resin flow (Hood et al.2015).

CONCLUSION

I originally hypothesized that fast-growing trees would allocate more resources towards growth than physical defenses resulting in reduced constitutive levels of terpene defenses while slow growing trees would allocate more resources towards constitutive chemical defenses as a part of trade-off with respect to growth. I also hypothesized that there would be an associated trade-off between constitutive and induced chemical defenses with fast-growing trees exhibiting higher levels of induced defenses when compared to that of slow-growing trees. I also aimed to explore how changes in the ratio of different classes of terpenes alters resin composition and how inherent growth rates may shift both chemical toxicity and physical defenses as part of the tree's comprehensive defense syndrome.

The lab did fail to observe any significant trade-offs between defense strategy from either slow growing or fast-growing trees. I originally thought that trade-offs would be found similar to that mentioned within the GDBH, since this hypothesis does allow for such predictions of costs at the intraspecific level based on the degree of the stressor affecting growth when compared to carbon assimilation (Hood et al. 2015). However, predictions surrounding the RAH indicate a more probable cause for the observed results as constitutive defenses are favored in slower growing species due to the higher costs of replacing tissues (Hood et al. 2015). There was an observed a trend among the fast-growing families where there were significantly lower levels of constitutive levels of mono-and sesquiterpenes as well as higher induced responses when compared to that of slow growing families. This evidence however does not indicate a clear trade-off between the allocation of resources with respect to growth rate, but this does suggest

that there is a higher associated level of chemical variation among slow-growing families than that of fast-growing families. Such variation could prove to act as a selective force during eruptive MPB outbreaks, if slow-growing trees possess higher variations of chemical defenses then they may prove to be more successful in beetle defense (de la Mata et al. 2017). Although this study did explore how changes in the ratios of different classes of terpenes alter resin composition and how the inherent growth rate may shift the chemical toxicity and physical defenses, there was no observed association to growth rate. Chemical composition between induced and constitutive resin did significantly vary, although such variations could be due to treatment response, availability of stored resources, or even treatment timing (Hood et al. 2015).

The information found within this study can help determine the associated trade-offs between defense and growth for other conifer systems experiencing eruptive bark beetle outbreaks. This information could also prove to be beneficial for research understanding stand selection and adaptation to current endemic beetle populations. Further research would need to be done to determine if drought stress, increased levels of climatic change, or the just the physical presence of beetles rather than induced treatments would cause variations in these results.

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