### DISSERTATION

# POST THINNING INTERACTIONS BETWEEN LODGEPOLE PINE HOST PHYSIOLOGY, GROWTH, CHEMISTRY, AND SURVIVAL WITH MOUNTAIN PINE BEETLE

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### ABSTRACT

# POST THINNING INTERACTIONS BETWEEN LODGEPOLE PINE HOST PHYSIOLOGY, GROWTH, CHEMISTRY, AND SURVIVAL WITH MOUNTAIN PINE BEETLE

Bark beetle outbreaks have caused adverse economic and lumber deficiencies impacting 10.3 million ha since 2000 (Cochran and Barrett 1993; Fettig 2021; Lindgren and Raffa 2013). A common bark beetle management method is to reduce basal area. Reducing within-stand competition may improve individual tree vigor but also can alter stand-level resistance to beetles. Some studies have also shown that density reduction treatments can have deleterious effects on tree resistance. Lodgepole pine trees (*Pinus contorta* Douglas ex Loudon var. latifolia Engelman) across the northern Rocky Mountains have been killed in increasingly severe mountain pine beetle (*Dendroctonus ponderosae*, Hopkins, MPB) outbreaks.

Accordingly, there is a need to fine-tune cultural control methods at a regional scale in order to determine how to best manage forest stands for reduced bark beetle damage. Here, we exploit an outbreak of the mountain pine beetle (*Dendroctonus ponderosa*) in northern Colorado to evaluate basal area reduction treatments (BART) of 21, 42, 63, and 95 m<sup>2</sup>/ha, (hereafter referred to as BART21, BART42, BART63, and UBA95) of lodgepole pine (*Pinus contorta* Douglas ex Loudon var. latifolia Engelman) over three studies.

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In study one, we examined BART impacts on microsite conditions. Conclusions that can be drawn from this research are (1) the positive and negative consequences of thinning treatments in a resource allocation system; response from thinning can spur RAI and height, increase phloem thickness, increase water and photosynthate maintenance demands, reduce allocation to defense and future foliar biomass, increase preference for beetle attack, increase temperature and humidity, reduce localized terpene and kairomone concentrations, (2) natural, non-baited studies are more variable than baited, closed chamber, and lab studies, (3) single parameter beetle studies fail to capture the complex interwoven nature of beetle-tree-stand ecosystems, (4) RAI & height respond best to thinning treatments, (5) when beetle numbers are high enough, management method may not matter, and (6) tree and soil water, while important for growth and increased resin exudation pressure, are less important on a stand level but show an effect on individual tree survival and insect damage rating.

In study two, we examined two measures of water stress with BART. Conclusions that can be drawn from this research are (1) beetle attack and tree water stress interactions within complex interwoven beetle-tree-stand ecosystems are better understood using multiple physiological, environmental, and biotic parameters (2) tree and soil water, while important for growth and MPB defense, are less impactful on a stand level, but show an effect on individual tree survival, (3) RAI & height respond best to thinning treatments, (4) thinning treatments have both positive and negative consequences: they can improve tree vigor and defenses, but they can also spur RAI and escalate DBH into preferred size for MPB attack.

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Managers should be careful about how they prescribe treatments because it is possible that BARTs can have outcomes that are not desirable. Both daily and seasonal measures of needle water stress, in a more comprehensive study, need to be examined to pinpoint the effects of tree level water, physiological parameters, and the interface with MPB.

In study three, we concurrently measured the effects of BART on tree needle monoterpene profiles, tree water stress, and MPB attack behavior over two years during a MPB outbreak to make a small contribution of understanding more about the interaction between lodgepole pine physiology, anthropogenic forest management, and MPB. Conclusions that can be drawn from this research are (1) BART is an effective management method to alter terpene proportions, (2) BART will not impact each terpene proportion the same, (3) Day-of-year is a key factor in terpene proportions, (4) year is only a factor in 10% of terpene proportions, (5) water stress may impact only select terpene proportions (6) the proportion of  $\alpha$ -pinene does not have a bearing on attack status, whereas the proportions of  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, and limonene do. While not tested here, these results may also have some significance for defoliating insects and wildfires.

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### DEDICATION

In memory of my God, my Savior, my religion, my freedom, my peace, my family,

and my children,

whom all I love.

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## CHAPTER 1: Thinning Lodgepole Pine Stands Does Not Alter Microsite Environmental Conditions Rather Migrates Trees into Size Classes Suitable for Mountain Pine Beetle Colonization

### **1.1 Introduction**

Bark beetle outbreaks have substantial effects on forest structure, function, and composition (Amman and Logan 1998; Amoroso, Coates and Astrup 2013; Hindmarch and Reid 2001); accordingly, forest ecology research has focused on methods to control beetle damage for many years. Many of the approaches to managing forest bark beetle resistance rely on cultural control, including radial thinning and density reduction treatments. Density reduction of forest stands can benefit trees in several ways that include increased growth, improving tree access to water, and decreasing stress from competition (Kolb, Holmberg, Wagner and Stone 1998; Negron, Allen, Ambourn, Cook and Marchand 2017; Waring and Pitman 1985). Density reduction can have other important benefits, including an increase in merchantable timber volumes in the decades following treatment (e.g., Cochran and Barrett 1993, Cochran et al. 1998, Oester et al. 2005). However, density reduction (or 'thinning') does not always provide improved resistance to bark beetles, and in some cases may even increase tree susceptibility. For example, thinning treatments can be associated with increased beetle attacks in individual trees (Cerezke 1994), or higher abundances of beetles within stands (Hindmarch and Reid 2001). Consequently, determining the appropriate stand densities and spatial arrangements that will result in improved tree growth, while also reducing susceptibility to bark beetles, has been a complicated problem to solve; but by examining environmental and biological variables simultaneously, management

methods that can resolve both will be developed (Errico and Rr 1989; Fettig, Klepzig, Billings, Munson, Nebeker, Negrón and Nowak 2007; Long and Shaw 2005).

In western North America, the mountain pine beetle (Dendroctonus ponderosae Hopkins; Coleoptera: Curculionidae) has undergone a series of outbreaks which have over the past several decades caused extensive tree mortality across expansive landscapes ranging from Canada to the southwestern United States (Berner, Law, Meddens and Hicke 2017; Erbilgin, Ma, Whitehouse, Shan, Najar and Evenden 2014; Roth, Hussain, Cale and Erbilgin 2017). Across this region, lodgepole pine, *Pinus* contorta (Douglas ex Loudon var. latifolia Engelman) is a primary host of *D. ponderosae* and is a valuable timber species (Farjon 2017). In Colorado, USA, a significant outbreak of *D. ponderosae* affected lodgepole pine forests from approximately 2000-2010 (Klutsch, Negron, Costello, Rhoades, West, Popp and Caissie 2009). At the outbreak's peak, 38 million trees were dying annually (Thompson 2016) which created significant management and safety concerns among land owners, federal and local governments, watersheds, businesses, and tourism entities. Studies to examine the correlation of forest stand factors with *D. ponderosae* damage have concentrated on manipulating multiple metrics including quadratic mean diameter (QMD), stand basal area, and wood volumes (Olsen, Schmid and Mata 1996). There are very few studies that examine physiological responses of trees to thinning treatments, therefore the link between thinning, tree response, and beetle resistance are unclear (Amoroso, Coates and Astrup 2013; Kolb, Holmberg, Wagner and Stone 1998).

Here, we used the regional outbreak of *D. ponderosae* in a Colorado lodgepole pine forest as a natural experiment to ask the question: does thinning impact the site

conditions that are important to bark beetle success, such as larger trees, overly dense stands? To address this question, the following specific hypotheses were tested: (1) the basal area reduction treatments (BARTs) will drive a shift in microsite conditions that are relevant to beetle behaviors such that trees within the thinned plots will exhibit less stressed water potentials, higher tree-soil volumetric content, increases relative humidity, moderate temperatures, increased radial annual increment (RAI), and increased tree height, (2) variability in these microsite changes will be associated with lower beetle abundances, lower attacks, and increased tree survival during a natural *D ponderosae* outbreak. Our results have implications for management and conservation by identifying how BARTs impact microsite conditions related to tree vigor, tree survival of outbreak, and bark beetle behaviors.

### 1.2 Materials & Methods

### 1.2.1 Study System

The study was conducted in pure lodgepole pine (*Pinus contorta* Douglas ex Loudon var. latifolia Engelm) stands within the Gould Quadrangle of the Colorado State Forest Service State Park, Routt National Forest, Jackson County, Colorado, USA. Research plots were established and tagged with identification numbers in 1967 (Hawksworth 1967; Hawksworth and Bailey 1967). Plots had been monitored with stand growth inventoried in 1985, 1997, and 2005, similar to Cochran and Barrett (1993) and Hood et al. (2018).

In the spring of 2008, four 0.20 hectare plots were selected for this 2008 - 2009 study using a combination of GIS and ground inventory information. Three of the plots had basal area reduction treatments (BARTs) in 1985 with residual basal areas of

BART 20.82 m<sup>2</sup>/ha (BART21), BART 42.45 m<sup>2</sup>/ha (BART42), and BART 62.99 m<sup>2</sup>/ha (BART63). The fourth plot remained an unthinned basal area (UBA) as a control with a basal area of 94.59 m<sup>2</sup>/ha (UBA95). Hereafter, unless specifically noted, all four plots are collectively referred to as BART(s) and have a latitude and longitude of 40.590910 N and -106.006300 W, respectively. Trees were 113 years old, grew at 2,719 m elevation in a Cowdrey loam soil texture which transitioned to a clay loam texture from 7.62 to 30.5 cm deep (USDA-NRCS 2020). In addition, the plots had similar environmental and physiographical stand, slope, and mean annual precipitation with the only differentiation being their basal area.

In 2008, all four plots were measured according to Colorado State Forest Service (CSFS) cruising manual guidelines to obtain detailed information on species composition, volume, crown class, DBH, and stand conditions. In June 2008, similar to selection by Klutsch et al. (2009) and Cochran and Barrett (1993), six green and apparently healthy, not-attacked lodgepole pine trees were randomly selected for closer examination through the duration of the study (N=24 trees total). Tree selection criteria were a full green healthy crown, no visible *D ponderosae* entrance holes or pitched-out resin along the trunk, no frass, as well as no other observable biotic organisms or abiotic stressors. For these 24 closely examined trees, additional measurements were taken of total height, condition class, and age using increment cores. Radial annual increment (RAI) was calculated using inventory DBH.

# 1.2.2 Tree Bole and Canopy Evaluation: Insect Damage Rating and Tree Attack Status. Non-Baiting.

Each of the 24 study trees had its bole and canopy evaluated during solar-noon tree branchlet collection on June 27, July 11, 25, August 7, 20, September 5 of 2008 and June 24, July 8, 22, August 5, 19, and September 1 of 2009 by starting examination at the soil surface continuing upward to 6.10 m in height. *D ponderosae* severity was classified by use of a 0 to 10 numerical insect damage rating (IR) system that was based on number of hits, frass, canopy needle color, and exit holes similar to the standardized categorical tree classifications used by Hood et al. (2018) and Klutsch (2009) (Table 1.2). The IR was used to determine a summary attack status of notattacked, attacked, or attacked-killed. Trees with a final value of zero were classified as not-attacked, values 1-8 were classified as attacked, and values 9-10 were classified as attacked-killed. Trees were considered as attacked when the first *D* ponderosae attack was documented, which began to be observed on tree boles starting July 25, 2008, and continued to be recorded throughout the remainder of the study which ended on September 1, 2009. The canopy of each tree was classified as either green, palefading, red, or dead. All 24 experiment trees were classified having green canopies at the start of research on June 27, 2008.

Due to high pressure from the MPB outbreak, the study was conducted without semiochemical baiting.

Each of the 24 selected individual study trees were sampled across twelve dates in two years (2008: June 27, July 11, July 25, August 7, August 20, and September 5; 2009: June 24, July 8, July 22, August 5, August 19, and September 1 to quantify water

potentials, evaluate tree canopy and trunk status and assign an insect damage rating, and make counts of beetle attacks on trunks and beetle abundances in stands. Plotlevel temperature and humidity were also measured at each sample period, and soil volumetric water content means was recorded for each tree in 2009.

### **1.2.3 Tree Branchlet Collection and Water Potentials**

For six days during the summer of 2008 (June 27, July 11, July 25, August 7, August 20, and September 5, 2008) and September 1, 2009, branchlets were collected both for pre-dawn and solar-noon water potential analysis. For five days during the summer of 2009 (June 24, July 8, July 22, August 5, and August 19) branchlet water potentials were only collected at solar noon due to 2008 pre-dawn readings showing no variation. At each study tree, a sun-exposed tree branchlet, between 17.8 – 25.4 cm in length, was clipped at a canopy position, approximately 7.62 – 10.67 m off the ground, depending on the tree, using a pruning pole. Each freshly cut branch tip was immediately bagged in a pre-labeled gallon plastic bag for transport to the mobile lab ~200 m away. Each individual fresh branchlet was then removed from its labeled plastic bag, stripped of approximately 2" (5.1 cm) of its clipped-end needles and exterior epidermis, starting from its clipped-end running laterally towards the branch's terminal bud, clipped-end recut with a fresh razor blade, and placed in the pressure bomb with the clipped-end sticking outward through the rubber membrane. The Scholander pressure chamber (Model 1000, PMS Instruments, Corvallis, OR) was pressurized using nitrogen gas and the bar reading recorded once sap was forced out of the stem. Readings were recorded in bar units and later converted to MPa.

Upon completion of the water potential reading, needles, that had been removed while stripping the epidermis, were collected, immediately placed, and sealed in 40 ml glass vials with silicone PTFE lined septa (Millipore Sigma, St. Louis, MO) and sealed. Each sealed vial was then frozen and stored at 0° C until processed at a Colorado State University Plant Sciences Lab, E211, Fort Collins, Colorado, USA.

### 1.2.4 Insect Damage Ratings and Attack Counts of *D. ponderosa* on Tree Boles

Each tree's bole and canopy were evaluated during each solar-noon tree branchlet collection on June 27, July 11, 25, August 7, 20, and September 5 of 2008 and June 24, July 8, 22, August 5, 19, and September 1 of 2009 by starting examination at the soil surface continuing upward to 6.10 m in height using methods similar to those described in Klutsch (2009) to classify experimental trees based on evidence of insect damage. *Dendroctonus ponderosae* damage was classified by use of a 0 to 10 numerical insect damage rating (IR) system; criteria for assigning damage rating scores are provided in Table 2. All 24 study trees had green canopies at the start of research on June 27, 2008 (i.e., IR=0). *Dendroctonus ponderosae* attacks were initially observed on tree boles starting July 25, 2008; progression of attacks and insect damage rating were recorded on August 7, 20, & September 5 of 2008 and June 24, July 8, 22, August 5, 19, and September 1 of 2009.

### 1.2.5 Soil Water Content, Relative Humidity, and Temperature

Soil bulk volumetric water content was measured around each individual study tree on September 1, 2009. Three subsamples were taken per tree to a depth of 57.15 mm with a Theta ( $\theta$ ) probe type ML2 (Delta-T Devices, Cambridge, England) and used to compute a mean tree-level volumetric water content value. Relative humidity and

temperature were measured and recorded on a plot level (as opposed to tree-level values), in the center of each plot, using a Springfield 91551 Digital Thermometer with Hygrometer (Taylor Precision Products, Oak Brook, IL 60523, USA).

### 1.2.6 Beetle Abundances within Stands

Within each treatment area, a single, unbaited, 12 funnel, Lindgren funnel trap (Synergy Semiochemicals, 7572 Progress Way, Delta, BC, Canada) was placed at the center of each BART treatment on June 27, 2008. Mountain pine beetles were removed and counted at each data collection date for the remainder of 2008.

### **1.2.7 Statistical Analysis**

All statistical analyses were performed in JMP (SAS Institute, Cary, NC) and use a Type I error rate of  $\alpha$ =0.10 for assigning statistical significance. One-way ANOVA that incorporated year as a random effect was utilized to analyze the effects of basal area reduction treatments on the responses of pre-dawn water potentials, solar-noon water potentials, volumetric soil water content, height, and radial annual increment (RAI) microsite responses. A post-hoc test (Tukey's HSD) was applied to make all pairwise comparisons among means. Effects of basal area reduction treatment on relative humidity were compared qualitatively since there was no replication (i.e., a single standlevel recording), but treatment effects on temperature were analyzed using linear regression. Effects of basal area reduction on the response of cumulative Lindgren trap captures were analyzed using a three-parameter logistic model, treating year as a random effect.

A linear model framework was used to predict the effects of pre-dawn water potentials, solar-noon water potentials, volumetric soil water, height, RAI, relative

humidity, and temperature effects on the responses of maximum insect damage rating and cumulative attacks by *D. ponderosae*. Logistic models were used to analyze the effects of pre-dawn water potentials, solar-noon water potentials, volumetric soil water content, height, RAI, relative humidity, and temperature on the probability of tree survival of the outbreak.

### 1.3 Results

### **1.3.1 BART Effects on Microsite Conditions and Tree-Level Parameters**

There was no evidence that mean tree pre-dawn water potentials differed across basal area reduction treatments ( $F_{3,23}$ =1.477, P=0.251) with the highest pre-dawn water potentials recorded in BART42 and lowest (most negative) in BART21 treatment. Similarly, there was no evidence that mean tree solar-noon water potentials differed between basal area reduction treatments ( $F_{3,23}=0.351$ , P=0.789). There was also no evidence that mean tree volumetric water content differed among treatment groups  $(F_{3,23}=1.579, P=0.226)$ . However, tree height differed significantly among treatment groups ( $F_{3,23}=2.908$ , P=0.060) and was highest in BART63 treatment at 20.1 m and lowest in non-treated stands (residual basal area of UBA95) at 16.2 m. There was also evidence that radial annual growth increment (RAI) differed significantly among the treatment groups ( $F_{3,23}$ =30.051, P<0.001); the highest mean RAI was observed in the BART21 treatment group, RAI in the BART42 and BART63 treatment groups were intermediate and not statistically different from one another, and the lowest RAI was observed non-treated control stands (Table 3). Relative humidity was highly variable among basal area reduction treatments and differed between years (Figure 1). Variation in patterns of mean temperature over the course of the growing season were similar for

each treatment group, and temperature was generally highest in the BART21 treatment group, which had the lowest basal area and most open canopy structure (Figure 2).

# 1.3.2 Effects of Microsite Conditions and Tree-Level Parameters on Beetle Trap Captures, Insect Damage Rating, Beetle Attacks, and Probability of Tree Survival

Rates of beetle accumulation in traps were similar in all treatment groups early in the growing season, but by mid-July rates of beetle accumulation were significantly higher in the non-treated control stand and remained that way until no further beetles were captured (residual basal area UBA95; Figure 3).

A linear model incorporating site- and tree-level factors explained a substantial portion of variance ( $R^2$ =0.647) in insect damage rating and was statistically significant ( $F_{7,16}$ =4.191, P=0.008). Specifically, volumetric soil water content had the largest overall effect size and explained 62% of the modeled variance. In addition, pre-dawn water potentials had a large effect size and explained 12% of the overall variance. No other individual parameters were statistically significant predictors of insect damage rating (Table 4). When the same model framework was applied to predicting variation in total beetle attacks on trees, the linear model explained only a small portion of the variability in attacks ( $R^2$ =0.245), and was not statistically significant ( $F_{7,16}$ =0.743, P=0.641).

The probability of tree survival did not vary across the basal area reduction treatments ( $\chi^2$ =1.55, *P*=0.670). Neither pre-dawn water potential ( $\chi^2$ =0.007, *P*=0.933, Figure 4a) nor solar-noon water potential were associated with the probability of tree survival ( $\chi^2$ =1.582, *P*=0.209, Figure 4b). However, there was evidence that volumetric soil water was associated with the probability of tree survival ( $\chi^2$ =13.159, *P*<0.001, Figure 4c), and as soil volumetric water content exceeded 20%  $\Theta_{sw}$ , the probability of

tree survival decreased rapidly, indicating a potential threshold. There was no evidence that height was associated with the probability of tree survival ( $\chi^2$ =1.539, *P*=0.215, Figure 4d). There was evidence that RAI was associated with the probability of tree survival ( $\chi^2$ =3.450, P=0.063, Figure 4e), and trees that grew faster were less likely to survive the outbreak. There was no evidence that variation relative humidity ( $\chi^2$ =1.233, *P*=0.267, Figure 4f) nor temperature ( $\chi^2$ =0.932, *P*=0.334, Figure 4g) were associated with probability of tree survival.

### **1.4 Discussion**

### 1.4.1 BART Effects on Microsite Conditions and Tree-Level Parameters

In this study we show that microsite environmental conditions did not differ between BART. Tree water potentials and tree soil volumetric water content did not support the hypothesis for lowered tree water stress due to BART. Similarly, not supported, soil water content was not more abundant in the lower BART densities. Relative humidity did not increase, and understory temperatures did not strongly differ as a result of basal area reduction treatments. It was not expected that relative humidity would be so inconclusive due to extreme variability with no perceivable pattern within each year, stand, BART, and date. The variability is possibly due to a host of factors including wind, sun exposure, water volume within each tree, road airborne particulate matter, and sporadic rainfall events; all of which are not within the scope of this research. It was most surprising that both solar-noon and pre-dawn water potentials, as well as volumetric soil water were not improved with BART and were not consistent with other research (Alavi 1996; Baldwin and Barney 1976).

The responses of RAI and height to BART were as expected since lower densities had more resources available for increased growth, trees in thinned stands grew more rapidly. However, faster-growing trees were more susceptible to mortality from mountain pine beetle, potentially indicating that pre-outbreak density reduction treatments rapidly migrated trees into size classes suitable for mountain pine beetle.

Despite no evidence of thinning treatments impacting microsite conditions, predawn water potentials and volumetric soil water content were both associated with observations of tree damage from mountain pine beetle—as pre-dawn tree water potentials increased, maximum insect damage rating declined, indicating that water stress was associated with higher damage ratings from mountain pine beetle. Conversely, soil volumetric water content was related to both insect damage rating and probability of tree mortality. Accordingly, we conclude that stand level thinning treatments influenced tree growth parameters, but not microsite conditions. Namely, faster growing trees had grown into the preferred size class attacked by MPB and were more likely to succumb to mountain pine beetle (Negron, Allen, Cook and Withrow 2008; Smith, Rizzo and North 2005).

## 1.4.2 Effects of Microsite Conditions and Tree-Level Parameters on Beetle Trap Captures, Insect Damage Rating, Beetle Attacks, and Probability of Tree Survival

Variation in microsite conditions had consequences for patterns of cumulative trap captures over the course of the growing season, insect ratings, bark beetle attacks, and probability of tree survival during a *D. ponderosae* outbreak. We found that cumulative trap captures were higher in UBA95 and indistinguishable among BART21, BART42, and BART63, similar to the report of Zausen et al. (2005). One possible

explanation for this pattern is related to differences in physical properties of airflow through forest stands. Prolonged beetle attacks have been documented in dense stands (Lorio 1980), whereas thinned stands may have better air mixing that can disrupt terpene plumes (Fettig, Klepzig, Billings, Munson, Nebeker, Negrón and Nowak 2007). Factors affecting volatile organic compound production before, during, and after outbreaks include foliage density, light, temperature, and differences in emission profiles across species and ontogenies (Guenther 1997). Presumably the non-treated control stand has higher foliage density and less light penetrating into the understory than the corresponding thinned stands, but we show that the control site had similar temperature patterns. Higher foliage density does not allow for the production of carbohydrates, which can leave trees at a deficit Reduced photosynthates, due to reduced photosynthesis, can leave trees in a deficit in maintaining tree defenses (Waring and Pitman 1985). Other studies have reported delayed insect development in unthinned stands with development and emergence slowed by 7-10 days in comparison with lower density stands (Ross 1995). Our results indicate that this might not be the case in our system, but further tests replicating temperature measurements across a larger gradient of stand densities would be needed to test this hypothesis convincingly.

Insect damage rating was predicted by pre-dawn water potential and volumetric soil water content microsite parameters. Values, from the insect damage rating scale, increased as trees were attacked more, frass increased, green canopy needles fading in color, increasingly negative water potential readings at pre-dawn, and higher volumetric soil water content. Larger trees could also be a legacy of the BART conducted 23 years prior. BART63, BART42, and BART21 were managed to improve

the resistance of the stand to mountain pine beetles. Some of the trees became more vigorous and faster growing possibly migrating trees into the right bark beetle size class that is optimal for colonization.

All seven microsite parameters had no influence on beetle attacks. The water parameters and temperature were extremely not significant, whereas relative humidity, height, and RAI were the nearest to becoming significant. Research has shown variable results with increased or decreased attacks in thinned and unthinned stands (Fettig, Klepzig, Billings, Munson, Nebeker, Negrón and Nowak 2007; Negron, Allen, Ambourn, Cook and Marchand 2017). Whether due to the extreme beetle pressure present during the outbreak and statistically similar beetle trap captures during the flight period, attacks were likely more dependent on tree level factors such as phloem thickness and less on microsite conditions.

The present study has several limitations that should be considered when interpreting the results including a small overall sample size of trees, no replication of plots or passive beetle traps, individual tree temperatures, individual tree humidity readings in both sunlight and shade, clear designation of old and new beetle attacks, and limited geography of plots. Water potentials also need to be coordinated with monitored foliar gas exchange rates that track respiration and stomatal  $CO_2/H_2O$  release. Despite these, having field test data from an actual MPB outbreak provides useful conclusions about BART, water potentials, DBH, RAI, and tree height.

Conclusions that can be drawn from this research are (1) the positive and negative consequences of thinning treatments in a resource allocation system; response from thinning can spur RAI and height, increase phloem thickness, increase

water and photosynthate maintenance demands, reduce allocation to defense and future foliar biomass, increase preference for beetle attack, increase temperature and humidity, reduce localized terpene and kairomone concentrations, (2) natural, nonbaited studies are more variable than baited, closed chamber, and lab studies, (3) single parameter beetle studies fail to capture the complex interwoven nature of beetletree-stand ecosystems, (4) RAI & height respond best to thinning treatments, (5) when beetle numbers are high enough, management method may not matter, and (6) tree and soil water, while important for growth and increased resin exudation pressure, are less important on a stand level but show an effect on individual tree survival and insect rating. Considerations for forest managers are to let the desired outcome of a stand dictate the thinning treatment implemented whether rotation, selection thins of select groups to provide a varied range of age classes, or other (Gillette, Wood, Hines, Runyon and Negron 2014). Objectives will differ based on multiple ecosystem factors that include watersheds, wildlife, industry, climate, and anthropogenic influence. A more comprehensive study is needed to pinpoint the effects of tree level physiological parameters in their interface with mountain pine beetle.

### **1.5 Conclusion**

In this study lodgepole pine trees were subjected to four different BART, BART21, BART42, BART63, and UBA95, which were then examined for their effects on microsite conditions. Microsite conditions of pre-dawn water potentials, solar-noon water potentials, volumetric soil water content, height, RAI, relative humidity, and temperature were further analyzed for their effect on tree survival, IR, beetle trap captures, and sum of beetle attacks. Stand level thinning treatments had an effect on RAI and height, no

effect on beetle trap captures, and microsite water variables had an effect on tree survival and maximum insect rating. The interactions between lodgepole pine, microsite conditions, and then with mountain pine beetle are more complex than previously thought; positive growth factors can become negative tree survival factors as well as to suggest water potentials in lodgepole pine may not be the best measure of water stress. These findings have implications for the management of lodgepole pine stands under pressure from bark beetle outbreaks.

Treatment	Year			
group	1985	1997	2005	2008
BART63	62.99±0.003 a	76.75±0.004 b	85.65±0.0046 b	89.69±0.004 b
BART42	42.45±0.003 a	57.55±0.004 a	65.36±0.005 a	69.23±0.005 a
BART21	20.82±0.004 b	30.53±0.005 ab	37.96±0.005 a	40.83±0.006 a
UBA95	94.59±0.003 b	105.64±0.004 c	113.07±0.004 c	118.38±0.004 c

**Table 1.** Change in basal area over time following thinning to residual basal area in1985, 1997, 2005, and 2008. Lettering shows Tukey's HSD test, means within a columnnot connected by the same letter differ significantly.

Insect damage rating (IR)	Description
0	No hits, green needle canopy
1	1 hit, green needle canopy
2	2 hits, green needle canopy
3	4 hits, green needle canopy
4	12+ hits, frass, green needle canopy
5	18+ hits, frass, green needle canopy
6	Strip attack, frass, green needle canopy
7	24+ hits, frass, green needle canopy
8	36+ hits, frass, pale needles
9	48+hits, boring frass, 2° needles red, current year needles green
10	Exit holes, likely dead, red needles

**Table 2**. Insect damage rating (IR) and description of criteria used to assign rating.

**Table 3.** A summary of thinning treatment effects on means of multiple microsite conditions. Lettering shows Tukey's HSDtest, means within a column not connected by the same letter differ significantly.

Treatment group	Ψ <sub>PD</sub> (MPa)	Ψ <sub>SN</sub> (MPa)	Vol. soil water content θ <sub>sw</sub>	Tree height (m)	RAI m2/ha	Relative humidity (%)	Temperature (°C)
UBA95	-0.43±0.05	-0.52±0.03	10.926±1.314	16.205±1.954 b	0.0013±0.00032 c	26.35±2.716	25.292±0.941
BART63	-0.49±0.02	-0.50±0.01	17.012±2.613	18.694±0.745 ab	0.0078±0.00076 a	30.00±5.018	27.317±1.087
BART42	-0.37±0.03	-0.54±0.03	19.413±3.821	20.015±0.470 a	0.0048±0.00050 b	29.30±2.906	25.708±0.980
BART21	-0.45±0.05	-0.52±0.04	17.986±3.497	20.168±0.183 a	0.0034±0.00027 b	28.20±2.624	25.358±0.802
Response Variable	Parameter	Estimate	Std Error	DF	SS	t Ratio	P-value
-------------------------------------	---	--	---	----------------------------	---	--	--
Max insect damage rating (IR)	Intercept ΨPD ΨSN Vol soil water content (θv) Tree height RAI Humidity (%)	-42.564 -15.562 19.021 0.492 0.054 -141.163 -0.265	42.709 8.141 13.253 0.116 0.076 573.444 0.837	1 1 1 1 1 1	0.000 32.479 18.309 160.411 4.450 0.539 0.889	-1.00 -1.91 1.44 4.25 0.71 -0.25 -0.32	0.334 0.074 0.171 <0.001 0.489 0.809 0.756
	Temperature (°C) Residual variance	1.601 -	1.430 -	1 16	11.145 174.738	1.12 -	0.279
Sum of attacks	Intercept ΨPD ΨSN Vol soil water content (θv)	1075.130 -220.222 -350.416 2.855	2518.757 480.105 781.596 6.831	1 1 1	0.000 6504.151 6213.633 5399.468	0.43 -0.46 -0.45 0.42	0.675 0.653 0.660 0.682
	Tree height RAI Humidity (%) Temperature (°C) Residual variance	4.213 28652.555 -53.450 16.973 -	4.501 33818.670 49.334 84.321 -	1 1 1 1 16	27079.398 22189.903 36286.038 1252.560 550380.649	0.94 0.85 -1.08 0.20 -	0.363 0.409 0.295 0.843 -

**Table 4.** A summary of a generalized linear model describing effects of microsite conditions on variation in insect damage rating (IR) and *D. ponderosae* attack counts on study trees. Significant effects (*P*<0.10) are highlighted in bold text.



Figure 1. Relative humidity for 2008 and 2009 over time within each BART treatment group.



Figure 2. Pooled 2008 and 2009 temperatures over time within each BART treatment group.



**Figure 3.** Cumulative captures of *D. ponderosae* in pheromone-baited Lindgren funnel traps in each basal area treatment group, modeled as a logistic function. Shaded areas show 95% confidence intervals.



**Figure 4.** Logistic models showing effects of microsite conditions including (a) volumetric soil water content  $\theta_{sw}$ , (b)  $\Psi$  solar-noon, (c) RAI, and (d) tree height on probability of tree survival during a *D. ponderosae* outbreak. In each panel, green symbols represent surviving trees and black symbols represent trees that died during the outbreak.

# 1.6 References

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# CHAPTER 2: Needle Water Content%, $\delta$ <sup>13</sup>C‰ Isotope Discrimination, and Survival of Lodgepole Pine Thinning Treatments during Mountain Pine Beetle Outbreak in the Rocky Mountains, USA

#### 2.1 Introduction

Bark beetles are a major forest disturbance agent worldwide and seen in the southern Rocky Mountains of Colorado with 750,000 ha impacted (Briggs, Hawbaker and Vandendriesche 2015; Chapman, Veblen and Schoennagel 2012; Vorster, Evangelista, Stohlgren, Kumar, Rhoades, Hubbard, Cheng and Elder 2017). By 2012, mountain pine beetle (Dendroctonus ponderosae, Hopkins, MPB) outbreaks in British Columbia had damaged 53% of timber resources causing shortages and economic hardships (Corbett, Withey, Lantz and Ochuodho 2015; Institute 2021; Lindgren and Raffa 2013). The preferred host tree of MPB are lodgepole pine trees (*Pinus contorta* Douglas ex Loudon var. latifolia Engelman), which compete for water resources in dense forests. Water stress has been frequently attributed as the reason bark beetles are able to overcome tree defenses and successfully colonize them (Lorio, Hodges and So 1977; McDowell, Pockman, Allen, Breshears, Cobb, Kolb, Plaut, Sperry, West, Williams and Yepez 2008). Droughts and warming forest temperatures further exacerbate tree water stress (Gaylord, Kolb, Wallin and Wagner 2007; Meineke and Frank 2018). Accordingly, approaches to manage climate changes and manage tree water stress are important for increased forest resistance and resilience to MPB disturbance.

Common management approaches for improved growing space and water availability to individual trees is often achieved through the use of cultural control methods such as radial thinning or basal area reduction treatments (BARTs), (Amman and Logan 1998; McDowell, Brooks, Fitzgerald and Bond 2003). BARTs are an important method to improve a tree's ability to defend against pests and pathogens, improve tree vigor, reduce water stress, and provide more water and resources for the remaining trees due to removed competition (Gillette, Wood, Hines, Runyon and Negron 2014; Martin-Benito, Del Rio, Heinrich, Helle and Canellas 2010; Raffa and Berryman 1982). Thinning research has used needle water content percentages (NWC%), and  $\delta^{13}$ C‰ for decades to quantify the water status of trees (Cregg and Zhang 2001; Farquhar, Ehleringer and Hubick 1989; Running 1980). NWC% provides the current dynamic water status of needles by showing the percentage of water weight divided by the needle's full wet weight.  $\delta^{13}$ C‰ gives the exact water stress of when the needle tissue grew, using rubisco's discrimination against heavier <sup>13</sup>CO<sub>2</sub> from lighter <sup>12</sup>CO<sub>2</sub>, of which the former increases as water becomes more and more scarce (Farguhar, Ehleringer and Hubick 1989). Outcomes from thinning to improve tree resistance to bark beetles and improve vigor have been mixed; sometimes treatments are effective at reducing stand damage from MPB and other times they are not as effective (Negron, Allen, Ambourn, Cook and Marchand 2017; Warren, McGrath and Adams 2001). For example, the rapid radial tree growth following BART can also have an unfavorable legacy, where thinned trees ultimately have grown into the preferred DBH size class of MPB or higher density stands have seen lower beetle mortality (Vandygriff, Hansen, Bentz, Allen, Amman and Rasmussen 2015). Frequently used

forest management methods such as BART and radial thinning, with objectives to reduce MPB caused tree mortality, reduce water stress, and improve tree vitality, are regularly successful at their objectives, but sometimes reasons such as the legacy effects of thinning result in the management objective being fulfilled. Knowing water stress status of trees both seasonal and daily, with the use of reliable water measures, help forest researchers assess for the most efficacious forest management methods.

Despite reduced tree bark beetle mortality from use of BART to manage forests, the ability of trees to defend themselves from pests is still inconsistent, namely thinning does not resolve all lodgepole tree-bark beetle associated mortality (Negron, Allen, Ambourn, Cook and Marchand 2017; Reid, Silins and Lieffers 2006; Vandygriff, Hansen, Bentz, Allen, Amman and Rasmussen 2015). Such inconsistent results lead us to the questions of (1) Does reducing basal area improve tree water relationships and is it associated with a reduced proportion of trees in a stand that are attacked? (2) Is there a relationship among measures of tree growth (tree height, DBH, and RAI) and do they correlate with measures of water stress and beetle attack? (3) How do multiple measures of tree water stress relate to one another? (4) Are trees that are taller, faster growing, and more attacked by MPB under more water stress? Using the well-known methods of NWC% and  $\delta^{13}$ C‰ to measure water stress, (McDowell, Allen and Marshall 2010; Running 1980; Wilson and Maguire 2009) we test the following hypotheses: (1) BARTs will be associated with reduced water stress and reduced MPB attacked and attacked-killed means in comparison to the untreated basal area (UBA) stand, (2) larger, faster growing trees will have less evidence of water stress, (3)  $\delta^{13}$ C‰ and NWC% will be correlated with one another, (4) taller, faster growing, and attacked and

attacked-killed trees are under less water stress as shown by NWC% and  $\delta^{13}$ C‰, and (5) larger, taller, or faster growing trees are under more water stress and more likely to be attacked (or attacked-killed).

# 2.2 Materials & Methods

# 2.2.1 Study System

Data collection was conducted in pure lodgepole pine (*Pinus contorta* Douglas ex Loudon var. latifolia Engelman) stands within the Gould Quadrangle of the Colorado State Forest Service State Park, Routt National Forest, Jackson County, Colorado, USA. Latitude and longitude of the research stands were 40.590910 N and -106.006300 W, respectively. Trees were 113 years old growing at 2,719 m elevation. Soil was sandy loam becoming a clay loam texture at 7.62 cm deep (USDA-NRCS 2020).

Within stands of pure lodgepole pine, in the spring of 2008, four 0.20-hectare stands were selected for this 2008- 2009 study using a combination of GIS and ground inventory information. Three of the stands had basal area reduction treatments (BARTs) in 1985 with residual basal areas (BA) of BART 20.82 m<sup>2</sup>/ha (BART21), BART 42.45 m<sup>2</sup>/ha (BART42), and BART 62.99 m<sup>2</sup>/ha (BART63). Basal area in the fourth stand remained an unthinned (UBA) as a control with a basal area of 94.59 m<sup>2</sup>/ha (UBA95). Trees in each stand were tagged with identification numbers (Hawksworth 1967; Hawksworth and Bailey 1967). Since stand establishment in 1967 and basal area reduction treatments in 1985, the USDA-FS Rocky Mountain Forest & Range Experiment Station in Fort Collins, CO USA (Hawksworth and Bailey 1967) monitored and inventoried in 1985, 1997, and 2005 (Cochran and Barrett 1993; Hood, Cluck, Jones and Pinnell 2018). The stands had similar environmental and physiographical

stand, altitude, slope, water, and soil attributes with differentiation being their basal area. In 2008, all four stands were measured according to Colorado State Forest Service (CSFS) cruising manual guidelines to obtain detailed information on species composition, volume, crown class, DBH, and stand conditions. In June 2008, within each of the four stands, six green not-attacked lodgepole pine trees were randomly selected (Cochran and Barrett 1993; Klutsch, Negron, Costello, Rhoades, West, Popp and Caissie 2009) for closer examination through the duration of the study. Tree selection criteria were a full green healthy crown, no visible *D. ponderosae* entrance holes or pitched-out resin along the trunk, no frass, as well as no other observable biotic organisms or abiotic stressors. For these 24 closely examined trees, additional measurements were taken of total height, condition class, and age using increment cores. Radial annual increment (RAI) was calculated using inventoried DBH.

### 2.2.2 Tree Canopy & *D. ponderosae* Bole Evaluation and Non-Baiting

Each of the 24 study trees had its bole and canopy evaluated during solar-noon tree branchlet collection on June 27, July 11, 25, August 7, 20, September 5 of 2008 and June 24, July 8, 22, August 5, 19, and September 1 of 2009 by starting examination at the soil surface continuing upward to 6.10 m in height. *D. ponderosae* severity was classified by use of a 0 to 10 numerical insect damage rating (IR) system that was based on number of attack locations, frass, canopy needle color, and exit holes similar to the standardized categorical tree classifications used by Hood et al. (2018) and Klutsch (2009) (Table 1.2). The IR was used to determine a summary attack status of not-attacked, attacked, or attacked-killed. Trees with a final value of zero were classified as not-attacked, values 1-8 were classified as attacked, and values 9-10 were classified

as attacked-killed. Trees were considered as attacked when the first *D. ponderosae* attack was documented, which began to be observed on tree boles starting July 25, 2008, and continued to be recorded throughout the remainder of the field study, ending on September 1, 2009. The canopy of each tree was classified as either green, pale-fading, red, or dead. All 24 experiment trees were classified having green canopies at the start of research on June 27, 2008 (i.e., IR=0).

Due to high pressure from the MPB outbreak, the study was conducted without semiochemical baiting.

#### 2.2.3 Tree Branchlet Collection and Silicon/Glass Vial Storage

For six days during the summer of 2008 (June 27, July 11, July 25, August 7, August 20, and September 5, 2008) and six days in 2009 (June 24, July 8, July 22, August 5, August 19, and September 1, 2009) branchlets were collected. At each study tree, a sun-exposed tree branchlet, between 17.8 – 25.4 cm in length, was clipped at a canopy position, approximately 7.62 – 10.67 m off the ground, depending on the tree, using a pruning pole. Each freshly cut branch tip was immediately bagged in a pre-labeled gallon plastic bag for transport to the mobile lab ~200 m away. Each individual fresh branchlet was then removed from its labeled plastic bag, and then stripped of approximately 2" (5.1 cm) of its clipped-end needles, starting from its clipped-end running laterally towards the branch's terminal bud. Needles were placed and sealed in 40 ml glass vials with silicone PTFE lined septa (Millipore Sigma, St. Louis, MO). Each sealed vial was then frozen and stored at 0° C until processed at a Colorado State University plant sciences lab, Fort Collins, Colorado, USA.

#### 2.2.4 Needle Water Content%

On September 1, 2009, extra needles from the 24-experiment tree branchlets were collected at two times-of-day: pre-dawn, when needles should be at their most turgid, and solar-noon, when needles should be at their most stressed. Needles were collected into vials for estimation of needle water content (% of water weight in grams/wet needle weight in grams). At the CSU Plant Sciences Lab, E211, a new, empty labeled brown paper bag was weighed, and weight recorded. Fresh needles were removed from each silicone capped glass vial and placed in a separate labeled brown paper bag. Samples were weighed for their bagged wet weight. Thereafter samples were oven dried at 70°C for 72 hours. After drying, bagged samples were weighed for their dry weight. The difference between dry weight and wet weight was calculated; thereafter the dry weight of the empty paper bag was subtracted. The remaining water weight was then divided by the wet weight to determine NWC% for each tree's needles.

# 2.2.5 δ<sup>13</sup>C‰ Method

Extra needles from 24 trees were collected on September 1, 2008 and September 5, 2009, into for  $\delta^{13}$ C‰ analysis. For every tree and year, needles were oven dried at 70°C for 72 hours, then ground to powder using a mortar, a pestle, and liquid nitrogen. 2.0 mg of crushed needle powder was scooped into a tin capsule, that measured 5 mm by 9 mm, and crimped using tweezers. Each filled capsule was analyzed by a Carlo Erba NA 1500 (Milano, IT) elemental analyzer coupled to a VG Isochrom continuous flow IRMS (Isoprime Inc., Manchester, UK) at CSU, Natural Resource Ecology Laboratory, Natural and Environmental Services Building, A244.

Delta carbon isotope signatures ( $\delta^{13}$ C‰) are presented as ‰ of  $^{13}$ C/ $^{12}$ C of each sample relative to the Vienna Pee Dee Belemnite standard.

#### 2.2.6 Statistical Analysis

A one-way ANOVA, with a random effect included for year, was utilized to analyze the fixed effect of the basal area reduction treatments (BART) on the 2008 and 2009  $\delta^{13}$ C‰ responses with a student's t post-hoc multiple comparison of means test. An identical model was used to analyze variation in pre-dawn NWC% and solar-noon NWC%, with time-of-day incorporated into the model as a random effect followed with a student's t post-hoc multiple comparison of means test. All statistical analyses were performed in JMP<sup>®</sup> Pro 15.0.0. (Cary, NC). Effects were interpreted to be statistically significant using a type one error rate of  $\alpha$  <0.05.

A correlation analysis between tree growth predictors was conducted to determine if tree height, DBH, and radial annual increment (RAI) were correlated with each other. The analysis determined that there were significant correlations between tree height and tree DBH ( $F_{1,22}=11.872$ , P=0.002) and between tree DBH and RAI, ( $F_{1,22}=78.811$ , P<.001). There was no relationship between tree height and tree RAI ( $F_{1,22}=2.357$ , P=0.138), therefore a regression model was fit using only tree height and tree RAI as predictors for the responses of  $\delta^{13}$ C‰ and NWC%.

A correlation analysis of NWC% from needles collected at two times-of-day, predawn and solar-noon, found no significant difference ( $F_{1,46}$ =0.049, P=0.826). Eight NWC% extreme low outlier points were then excluded, and analysis rerun, ( $F_{1,34}$ =0.299, P=0.588), also resulting in no significant difference. Finding no significant difference between pre-dawn and solar-noon collected needles whether with or without the

outliers, the two time-of-day values were averaged together as a tree level NWC% mean. Similarly, a correlation analysis with  $\delta^{13}$ C‰ data points from 2008 and 2009 was conducted, (F<sub>1,46</sub>=0.209, P=0.650), finding no significant difference between years. Therefore, both the means of NWC% and  $\delta^{13}$ C‰ were used for statistical analysis on individual tree analyses. A subsequent multiple regression model was used to determine the correlations between growth predictors of height and RAI with tree water stress responses of mean  $\delta^{13}$ C‰ and daily mean NWC%.

A multiple regression model was used to determine the correlations between growth predictors of height and RAI with tree water stress responses of mean  $\delta^{13}$ C‰ and daily mean NWC%.

Means of  $\delta^{13}$ C‰ and NWC% were compared using a linear regression to test the hypothesis that  $\delta^{13}$ C‰ and NWC% will not be correlated with one another. Since there was no relationship between needle water percentile solar-noon or predawn time-of-day with either 2008's or 2009's  $\delta^{13}$ C‰, means of NWC% and  $\delta^{13}$ C‰ were used for statistical analysis.

Chi Square test was conducted to determine the probability of BART63, BART42, and BART21 having reduced bark MPB attacks and mortality compared to UBA95. Each BART and UBA was compared to the three attack statuses: Attacked-killed, attacked, and not-attacked, analyzing the frequencies of attack status (n=3) among the 4 basal area groups.

#### 2.3 Results

# 2.3.1 Variation in Tree Water Relations Across Basal Area Treatments

 $\delta^{13}$ C‰ and NWC% water stress measures were assessed across the stands of UBA95, BART21, BART42, and BART63.  $\delta^{13}$ C‰ differed statistically between the three BART plots and UBA plot (F<sub>4,43</sub>=4.831, P=0.003, Table 1). The model explained 31.00% of the variance showing thinning improved the explained variance over the untreated stand.

The random effect of  $\delta^{13}$ C‰ year was not statistically significant (F<sub>1,46</sub>=0.282, P=0.598). However, BARTs had a statistically significant effect on  $\delta^{13}$ C‰ (F<sub>3,44</sub>=6.347, P=0.001) with 30.55% of the model variance explained. Student's t-test, as a post-hoc comparison of means, revealed the lowest  $\delta^{13}$ C‰ water stress levels were detected in BART63, which had significantly less water stress than all other treatments. BART21 and BART42 were intermediate in their water stress as measured by  $\delta^{13}$ C‰ and did not differ significantly from one another. The highest degree of water stress was found in the UBA95 stand.

NWC% did not differ statistically between each BART and UBA ( $F_{4,43}$ =0.374, P=0.826, Table 1). The model explained 3.36% of the variance. The needle collection time-of-day random effect was not statistically significant ( $F_{1,46}$ =0.047, P=0.829) and comprised 0.11% of the variance. BART also did not have a statistically significant effect ( $F_{3,44}$ =0.483, P=0.696) with 3.26% of the variance explained. Using Student's t-test, as a post-hoc comparison of means, needle water percentiles among basal area reduction treatments were not significantly different from one another. BART21 was the highest, followed by UBA95, then BART42, and with BART63 having the lowest NWC%.

There was no relationship found between BARTs and UBA with attack status (Pearson  $X^2$ =9.00, P=0.174, df=6, n=24, Figure 5).

Although attack status varied, UBA95 had the highest ratio of not-attacked trees at 0.67, along with its ratio of 0.17 for both attacked and attacked-killed trees. Both BART21 and BART42 had attacked-killed tree ratios of 0.33, but thereafter differed. BART21 had ratios of 0.50 and 0.17 for attacked and not-attacked trees, respectively. BART42 had a ratio of 0.67 attacked trees. BART63 had ratios of 0.33, 0.17, and 0.50 for attacked, not-attacked, and attacked-killed, respectively. A consistent relationship, between attack status and stand level BARTs, including UBA, was not established.

#### 2.3.2 Model of Tree Height and RAI Correlation with $\delta^{13}$ C‰ and NWC%

Turning from stand level treatments to tree level effects, a correlation of unrelated growth factors with  $\delta^{13}$ C‰ and NWC% was conducted. Using the uncorrelated growth factors of RAI and height, a multiple regression model composed of mean  $\delta^{13}$ C‰, height, and RAI found the overall model was significant (F<sub>2,21</sub>=6.235, P=0.008, RMSE value=0.571, RSq=0.37, Figure 6), with 37.26% of the variance was explained by our model. The random effect of height was not statistically significant (F<sub>1,22</sub>=0.400, P=0.534, Table 3) and explained 1.19% of the variance. The random effect of RAI was statistically significant (F<sub>1,22</sub>=9.635, P=0.005, Figure 7) and comprised 28.79% of the variance.

Multiple regression found no relationship between mean NWC%, height, and RAI  $(F_{2,21}=0.223, P=0.802)$ .

The actual NWC% and predicted NWC% with gray low outliers included was not significant (Predicted RMSE=0.208, R2=0.02, P=0.802). The random effect of height

was not statistically significant (F<sub>1,22</sub>=0.099, P=0.757). The random effect of RAI was not statistically significant (F<sub>1,22</sub>=0.213, P=0.649). Six extremely low values were then excluded finding no relationship between NWC%, height, and RAI (F<sub>2,15</sub>=1.213, P=0.325). Actual NWC% and predicted NWC% with gray low outliers included (Predicted RMSE=0.023, R2=0.20, P=0.540). The random effect of height was not statistically significant (F<sub>1,16</sub>=2.129, P=0.165) explaining 12.22% of the variance. The random effect of RAI was not statistically significant (F<sub>1,16</sub>=2.129, P=0.165) explaining 12.22% of the variance. The random effect of RAI was not statistically significant (F<sub>1,16</sub>=2.129, P=0.165) explaining 12.22% of the variance.

# 2.3.3 $\delta^{13}$ C‰ vs. NWC% Correlation

There was no clear relationship found between mean  $\delta^{13}$ C‰ and mean NWC% (F<sub>1,22</sub>=0.022, P=0.884, Figure 8A). The model using  $\delta^{13}$ C‰ as a predictive factor for NWC% only explained <1.0% of the variation. The six extremely low values were then excluded and still no relationship between  $\delta^{13}$ C‰ and NWC% was exhibited (F<sub>1,16</sub>=0.796, P=0.385, Figure 8B) despite  $\delta^{13}$ C‰, as a predictive factor for NWC%, explained 74.67% of the variation. A correlation between water stress measures of  $\delta^{13}$ C‰ and NWC% was not established.

#### 2.3.4 Model of Tree Height, RAI, and Attack Status with δ13C‰ and NWC%

A model of tree water stress, using tree RAI, height, and attack status as predictive factors for  $\delta^{13}$ C‰ and NWC%, was analyzed. The model was statistically significant for  $\delta^{13}$ C‰ and explained 44.9% of the variance in (F<sub>4,19</sub>=3.871, P=0.018, RMSE=0.563, R2=0.45, Figure 9). However, when tree parameter sources were examined, RAI ( $\delta^{13}$ C‰ leverage residuals with tree RAI leverage), was the only statistically significant factor (F<sub>1,22</sub>=11.666, P=0.003, Figure 10) which accounted for

33.83% of variance in the total model (Table 3). Tree height was not significant  $(F_{1,22}=0.666, P=0.425)$ . There were no significant differences found with the attack status of the trees (F<sub>2,20</sub>=01.318, P=0.291), but that did not affect the model of tree water stress relative to growth rate. In contrast, the model for NWC% tree water stress using height, RAI, and attack status as predictive factors, low outliers included, was weak explaining 22.3% of the variance and was not statistically significant (F<sub>4,19</sub>=1.363, P=0.284, Figure 11A). Analysis of the tree parameters showed no statistical significance: attack status ( $F_{2,21}=2.483$ , P=0.111) explained 20.22% of the variance in the total model, height (F<sub>1,22</sub>=0.003, P=0.955), and RAI (F<sub>1,22</sub>=0.147, P=0.706). The distribution was not normal, so the six low outlier values were then excluded (Figure 11B). This resulted in a normal distribution using the same model of tree water stress, with results remaining not statistically significant ( $F_{4,17}=0.810$ , P=0.540) and only explained 19.96% of the variance explained by the model. Examination of the tree parameters showed no statistically significant parameters: attack status ( $F_{2,15}=0.490$ , P=0.623), height (F<sub>1,16</sub>=2.218, P=0.160), and RAI (F<sub>1,16</sub>=0.137, P=0.717). A model for  $\delta^{13}$ C‰ and NWC% tree water stress, as predicted by tree RAI, height, and attack status was significant for  $\delta^{13}$ C‰ and predicted by RAI predicted whereas the model for NWC% tree water stress was not significant nor was predicted by any factors.

# 2.3.5 Tree DBH, Height, and RAI with Attack Status, $\delta^{13}$ C‰, and NWC%

Relationships were examined for  $\delta^{13}$ C‰ with DBH, RAI, height, and attack status, NWC% with DBH, RAI, height, and attack status, and for attack status with tree DBH, RAI, and height. There was a strong positive relationship between mean  $\delta^{13}$ C‰ and tree DBH (F<sub>1,22</sub>=10.866, P=0.003, Figure 12A) which showed as trees were less

water stressed as they became larger. DBH explained 33.06% of the variance. Similarly significant, trees with higher RAI had decreasing  $\delta^{13}$ C‰ water stress (F<sub>1.22</sub>=12.41, P=0.002, Figure 12B) as RAI values increased. RAI explained 36.06% of the variance.  $\delta^{13}$ C‰ did not have a significant relationship with tree height (F<sub>1,22</sub>=2.036, P=0.168). NWC% showed no relationship with DBH whether with six low outliers ( $F_{1,22}=0.666$ .) P=0.423) or outliers excluded (F<sub>1,16</sub>=1.017, P=0.328). DBH only explained 2.94 and 5.97% of the variance, respectively. NWC% had no relationship with RAI ( $F_{1,22}=0.361$ , P=0.554) when six low outliers were included as well as when the six outliers were excluded (F<sub>1,16</sub>=0.277, P=0.606). Similarly, tree height showed no relationship with NWC% with the six low outliers ( $F_{1,22}=0.241$ , P=0.629) or without ( $F_{1,16}=2.585$ , P=0.128). Height only explained 1.08 and 13.91% of the variance, respectively. Water stress, as measured by  $\delta^{13}$ C‰, showed significant differences for DBH and RAI, but not for height. NWC% had no significant relationships with DBH, RAI, or height. An ANOVA with attack status predicting the response of  $\delta^{13}$ C‰, calculated with an outlier, showed no differences between not-attacked, attacked, or attacked-killed trees (F2,21=0.412, P=0.668, Figure 13A), with means of -27.810, -27.995, and -27.664‰ for attackedkilled, not-attacked, and attacked, respectively. The attacked-killed  $\delta^{13}$ C‰ mean was 0.52% lower than the attacked  $\delta^{13}$ C‰ mean while the not-attacked  $\delta^{13}$ C‰ mean was 0.66% lower than the attacked-killed mean. Attack status only explained 3.77% of the variance.  $\delta^{13}$ C‰ analysis was repeated excluding the outlier whereupon results also showed no differences between not-attacked, attacked, or attacked-killed trees (F<sub>2,21</sub>=2.112, P=0.147, Figure 13B), with means of -27.810, -28.330, and -27.664‰ for attacked-killed, not-attacked, and attacked, respectively. The attacked-killed  $\delta^{13}$ C‰

mean was 0.52% lower than the attacked  $\delta^{13}$ C‰ mean while the not-attacked  $\delta^{13}$ C‰ mean was 1.84% lower than the attacked mean. Attack status only explained 17.44% of the variance. An identical ANOVA, with attack status predicting the response of NWC%, showed there was a significant difference between not-attacked, attacked, or attackedkilled trees due to an abnormal distribution ( $F_{2,21}=2.895$ , P=0.078, Figure 14), with means of 0.283, 0.518, and 0.427% for attacked-killed, not-attacked, and attacked, respectively. The attacked NWC% mean was 17.49% lower than the not-attacked NWC% mean while the attacked-killed NWC% mean was 33.72% lower than the attacked mean. Attack status explained 21.61% of the variance. Due to the non-normal distribution, the six low NWC% outliers were excluded to determine if the outliers were artificially influencing the distribution; whereupon results showed no significant difference between not-attacked, attacked, or attacked-killed trees ( $F_{2,15}=0.125$ , P=0.884) with means of 0.510, 0.518, and 0.514% for attacked-killed, not-attacked, and attacked, respectively. The attacked NWC% mean was 0.68% lower than the notattacked NWC% mean while the attacked-killed NWC% mean was 0.83% lower than the attacked mean. Attack status only explained 1.64% of the variance when the distribution was normal.

DBH means, with the low attacked tree outlier included, vs. attack status showed no significant difference, ( $F_{2,21}=2.392$ , P=0.116), with mean values of 26.353, 21.209, and 24.130 cm for attacked-killed, not-attacked, and attacked, respectively (Figure 15A). The attacked mean diameter was 8.44% lower than the attacked-killed mean, while the not-attacked mean diameter was 12.11% lower than the attacked mean diameter. The model of attack status explained 18.56% of the variance.

After the low attacked tree outlier was excluded, there was a significant difference between each attack status, ( $F_{2,20}$ =5.003, P=0.017, Figure 15B), with values of 26.353, 21.209, and 25.598 cm for attacked-killed, not-attacked, and attacked, respectively (Figure 9B). The not-attacked mean of was 19.52% lower than the attacked-killed mean while the attacked mean was 2.86% lower than the attacked-killed mean.

The model of attack status explained 33.35% of the variance. The legacy of BART with 23 years of growth thereafter resulted in trees having grown into a MPB's preferred size of  $\geq$  20.32 cm.

Analysis for RAI vs. attack status showed no significant differences ( $F_{2,21}=2.462$ , P=0.110) with mean values of 0.006, 0.003, and 0.004 cm for attacked-killed, notattacked, and attacked, respectively. The not-attacked mean RAI was 52.70% lower than the attacked-killed RAI mean while the attacked mean RAI was 26.95% lower than the attacked-killed mean. Attack status explained 18.99% of the variance.

Attack status vs. height when a shorter outlying tree was included showed no significant differences ( $F_{2,21}$ =1.002, P=0.384, Figure 16A) with mean values of 19.698, 17.450, and 18.821 m for attacked-killed, not-attacked, and attacked, respectively. The not-attacked mean height of 17.450 m was 11.41% lower than the attacked-killed height mean while the attacked mean height of 18.821 m was 4.45% lower than the attacked-killed height killed mean. Attack status only explained 8.71% of the variance.

However, once the outlier was excluded, height vs. attack status did show a significant difference (F<sub>2,20</sub>=3.744, P=0.042, Figure 16B) with mean values of 19.698, 17.450, and 19.948 m for attacked-killed, not-attacked, and attacked, respectively. The

not-attacked mean height of 17.450 m was 11.41% lower than the attacked-killed height mean while the attacked mean height of 19.948 m was only 1.25% higher than the attacked-killed mean. Attack status explained 27.24% of the variance. Significant relationships were seen for  $\delta^{13}$ C‰ with DBH or RAI, no significant patterns for  $\delta^{13}$ C‰ with attack status or height, no significant patterns for NWC% with DBH, RAI, height, or attack status, no significant relationship between attack status and RAI, and significant relationships were seen for attack status with tree DBH or tree height.

### 2.4 Discussion

#### 2.4.1 Variation in Tree Water Relations Across Basal Area Treatments

Water stress  $\delta^{13}$ C‰ and NWC% values showed unexpected results that differed when examining if BARTs would result in higher  $\delta^{13}$ C‰ values and if BARTs would result in increased NWC%. Supporting the hypothesis for carbon isotopes, there were significant differences in mean  $\delta^{13}$ C‰ due to the fixed effects of the BARTs (Table 1).  $\delta^{13}$ C‰ values in BART stands showed lower water stress, albeit different than the expected linear gradient of lowest density being least water stressed, to highest density having the greatest water stress. Most surprising was BART63 and not BART21 had the highest  $\delta^{13}$ C‰ mean. Such results could be explained by BART63 having tree shade to reduce water evapotranspiration as compared to the direct sunlight from canopy to soil surface as is found in BART21 and BART42, while having lower water demand than the highest density of UBA95 (Zausen, Kolb, Bailey and Wagner 2005).

Our range of results, -27.14‰ down to -28.42‰, are in keeping with the values found by Korner, Farquhar, and Roksandic (1988). However, our  $\delta^{13}$ C‰ values were slightly more stressed than values of -25.9‰ up to -23.4‰ found by Choi et al. (2005).

Fernandez et al. (2005) explained that such variation can come from the soil's parent material and site index. All four stands had a homogenous soil texture but did vary in density, which could explain why our results may not coincide with other research. Other considerations explaining lower  $\delta^{13}$ C‰ values in BART21 and BART42 come from the water demand of larger trees (Alavi 2002; Martinez-Vilalta, Vanderklein and Mencuccini 2007). As a tree gets large, the water balance becomes more precarious; where at a certain point, the water demand of large trees exceeds the water availability in the soil (Martinez-Vilalta, Cochard, Mencuccini, Sterck, Herrero, Korhonen, Llorens, Nikinmaa, Nole, Poyatos, Ripullone, Sass-Klaassen and Zweifel 2009). Another consideration comes from the negative carbon assimilation / stomatal conductance ratio relationship found with more accessible water (Dupouey, Leavitt, Choisnel and Jourdain 1993). With more abundant water comes an increase in stomatal conductance (Meinzer, Goldstein and Grantz 1990). This causes the net carbon assimilation / stomatal conductance ratios to decline (McDowell, Brooks, Fitzgerald and Bond 2003; Running 1980). Both considerations could explain why the basal areas in BART21 and BART42 were able to grow at a higher rate than BART63 yet have lower  $\delta^{13}$ C‰ values in 2008 and 2009. From our results, it's apparent that  $\delta^{13}$ C‰ values are not a standalone measure of water status and that other tree physiologic systems are to be considered.

Switching from the  $\delta^{13}$ C‰ measure of water stress to NWC% water stress measure, the hypothesis was not proven true (Table 1) as NWC% did not discern a statistical difference among each stand's mean NWC%. While BARTs are an important method for improving tree vigor and water use efficiency (Reid, Silins and Lieffers 2006;

Warren, McGrath and Adams 2001), the NWC%s of this research were not improved from BARTs, despite no difference between needles collected at pre-dawn or solarnoon. These results were in agreement with Kainulainen et al. (1992), yet not in agreement with Bengtson (1980). With such discrepancies, NWC% results for BARTs and UBA, it is possible that stomatal regulation of needle conductance played a role but is beyond the scope of this study (Cregg and Zhang 2001; Martinez-Vilalta, Vanderklein and Mencuccini 2007; McDowell, Brooks, Fitzgerald and Bond 2003; Running 1980). These results should be interpreted with caution due to needles being collected at the end of the study and because six of the 24 trees had extremely low NWC% values as they were dead or near death when needles were collected. It goes to reason that dead or dying trees would have more water stress. Such isolated data does not allow for a representative NWC% analysis of the trees prior to being attacked by bark beetles (Martinez-Vilalta, Sala and Pinol 2004). To determine if there was water stress before the trees were attacked, it would require repetitive measures data. While BART21 and UBA95 had the highest NWC%, other than the hypothesis was not supported, no definitive conclusions can be made from these results due to the collection factors listed above. A follow up study could be strengthened by collecting needles at different time periods throughout the season, especially needles prior to attack, to see if there is a correlation of NWC% and beetle attack.

After examining the effect BARTs had on  $\delta^{13}$ C‰ and NWC%, we examined the relationship between BARTs and MPB attack status. Contrary to reasoning, BART did not have a significant difference in reducing MPB attacks and mortality compared toUBA95 (Figure 1). Unlike the reduction of bark beetle attacks due to BARTs found in

other research (Gaylord, Hofstetter and Wagner 2010; Hood, Baker and Sala 2016), we found that the proportion of trees that were not-attacked differed across the BARTs probably as a result of some trees being too small in diameter to be attacked, as the mean DBH for not-attacked trees was 20.0 cm (Calatayud, Ahuya, Wanjoya, Le Ru, Silvain and Frerot 2008) (Figure 11B).

The intense MPB outbreak beetle pressure may have also made our results not agree with other BART studies that lacked outbreak beetle pressure (Progar 2003; Temperli, Hart, Veblen, Kulakowski, Hicks and Andrus 2014) as well as the legacy of BART and 23 years of growth since treatment, had grown the thinned trees into MPB's preferred diameter of >20.32 cm (Negron, Allen, Ambourn, Cook and Marchand 2017; Temperli, Hart, Veblen, Kulakowski, Hicks and Andrus 2014) (Figure 11). Lastly, as reported by JMP analysis, Chi-Square could be suspect due to average cell counts being less than five, lacking sufficient degrees of freedom. BARTs have both positive and negative consequences, such as more resources for defenses and a legacy of growth that may results in tree diameters that MPB prefer.

# 2.4.2 Model of Tree Height and RAI Correlation with $\delta^{13}$ C‰ and NWC%

Transitioning from stand level to tree level effects, tree height and RAI were combined to examine how well they predicted  $\delta^{13}$ C‰ and NWC%. Supporting the hypothesis as true, taller & faster growing trees had less water stress as shown by higher  $\delta^{13}$ C‰ values (Figure 2). Surprising was that both factors did not bear equal responsibility for that relationship with  $\delta^{13}$ C‰; RAI was the prevalent factor in the model as height was not significant (Figure 3). The results agree with Liu et al. where thinning was found to decrease height growth while radial growth increased (2003). Similarly,

higher growth rates and net assimilation rates were associated with lower  $\delta^{13}$ C‰ values (Virgona and Farquhar 1996) since substantial radial growth can occur with increased water use efficiency (WUE) while other times will have no substantial growth despite increased WUE (Soule and Knapp 2011). Contrary to  $\delta^{13}$ C‰ results, NWC% did not prove the hypothesis. The random effects of RAI and height were also not found to be significant. Concurring with these results, tree growth may not always be linear; radial growth can be more dependent on early rates of expansion (Kaufmann and Watkins 1990) or be influenced by rain showers despite low water potentials (Zweifel et al., 2005). It is also likely that collecting needles for water content at the end of the study, rather than throughout the study, thwarted any possibility of proving or disproving the hypothesis. The model of tree height and RAI with  $\delta^{13}$ C‰ proved taller and faster growing trees had less water stress primarily due to RAI while no correlations of NWC% with height and RAI were able to be proven.

# 2.4.3 $\delta^{13}$ C‰ vs. NWC% Correlation

A correlation analysis between the water stress measures of  $\delta^{13}$ C‰ and NWC% values was examined. Not supporting the hypothesis, NWC% and  $\delta^{13}$ C‰ were not correlated (Figure 4A). This outcome was expected due to the differences between the water stress measures. NWC% is a dynamic, fluctuating measure of water within a needle on any given day throughout a season (Running 1980; Salle, Ye, Yart and Lieutier 2008).  $\delta^{13}$ C‰ values, for the same needles, are determined when each new needle tissue is produced; not changing thereafter (Farquhar, Ehleringer and Hubick 1989). Mean annual precipitation, particularly December to March precipitation, and annual soil moisture are positively correlated with  $\delta^{13}$ C‰ (Anderson, Williams,

Kriedemann, Austin and Farquhar 1996) whereas NWC% may be more sensitive to rain showers, vapor pressure deficit or soil water (McDowell, Bowling, Schauer, Irvine, Bond, Law and Ehleringer 2004). There is no clear relationship that can be concluded because some of the trees were dead and attacked at the time of collection. NWC% is likely not a good measure of tree water stress for this study due to the previously discussed reasons, but more so due to its highly variable nature. Only if NWC% were to be continuously observed across an entire season or many seasons simultaneously with each tree in the study would its data give a better indication of water status. Further, volumetric water content across the same period would provide data to rule out any stomatal conductance influences. As the data stands in this study,  $\delta^{13}$ C‰ and NWC% were found to not be correlated measures of water stress and cannot be used as water stress indicators of the other.

# 2.4.4 Model of Tree Height, RAI, and Attack Status with $\delta^{13}$ C‰ and NWC%

A multiple regression was conducted to learn if taller, faster growing, and more attacked trees are. The hypothesis that RAI was a good predictor of  $\delta^{13}$ C‰ water stress was supported (Figure 6) while height and attack status did not support the hypothesis. Our  $\delta^{13}$ C‰ results agree with Ehleringer et al. who found open stomata C<sup>13</sup> ratios between -28 and -32‰ and closed stomata ratios between -23 and -25‰ (Ehleringer, Hall and Farquhar 1993). Our  $\delta^{13}$ C‰ values were between -27.14 down to -28.42‰. Stomatal conductance can sometimes make C<sup>13</sup> isotope discrimination results seem contrary to water availability; higher C<sup>13</sup> isotope discrimination in wetter sites and lower C<sup>13</sup> discrimination in drier sites (Prasolova, Xu, Farquhar, Saffigna and Dieters 2001; Sun, Livingston, Guy and Ethier 1996; Walcroft, Silvester, Whitehead and Kelliher

1997). The hypothesis for NWC% was not supported. As dead trees would understandably have more water stress than living trees, removal of the dead trees from analysis did not show a significant relationship. Height and attack status were not shown to be good predictors for NWC% as was also seen in their relationship with  $\delta^{13}$ C‰. RAI had been significant for the height, RAI, attack status and  $\delta^{13}$ C‰ model. However, here RAI was not significant, likely due to limited repeated measures data. Repetitive measures showing water stress before the trees were attacked would resolve that deficiency. The height, RAI, attack status and  $\delta^{13}$ C‰ model was found to be significant as well as the RAI parameter. Support for or against the hypothesis that NWC% showed more water stress in taller, faster growing, and more attacked trees was not able to be determined due to the limited data that was collected after trees had already been attacked or attacked-killed.

# 2.4.5 Tree DBH, Height, and RAI with Attack Status, $\delta^{13}$ C‰, and NWC%

The hypothesis that larger trees are under more water stress was not supported for  $\delta^{13}$ C‰ nor for NWC%. NWC% lack of significance could be due to needle collecting at the end of the season, rather than throughout the study, and before water stress would have been detected. It would be difficult to make conclusions because some of the trees were dead and attacked at the time of needle collection. Larger trees showed less water stress as they grew larger. Water, particularly in the form of precipitation, is critical for the outward radial growth of trees (Oberhuber and Gruber 2010). Martin-Benito et al. (2008) found the previous fall's moisture, absence of a harsh winter, and spring moisture promoted growth in diameter. The hypothesis that taller trees are under more water stress was not supported true for both  $\delta^{13}$ C‰ and NWC%. Due to the trees

being 113 years old, it is possible that photosynthate maintenance demands or hydraulic limitation may have been a factor artificially restricting upward growth and true water stress levels (Martinez-Vilalta, Vanderklein, and Mencuccini 2007; McDowell, Licata, and Bond 2005). The hypothesis that faster growing trees are under more water stress was not supported for  $\delta^{13}$ C‰ (Figure 8B), but not for NWC%. Surprising was that faster growing trees showed less water stress and not more stress as expected, which couldn't be explained by  $\delta^{13}$ C‰ as the ranges were overlapping between the three attack statuses. From a nearby Niwot Ridge AmeriFlux site (Colorado, USA), lodgepole pine had  $\delta^{13}$ C‰ values of -25.77, -27.81, and -26.91‰ for years 2003, 2006, and 2007, respectively, showing improved water use efficiency in the years after a 2002 drought (Monson, Prater, Hu, Burns, Sparks, Sparks and Scott-Denton 2010). Our measurements were taken the following two years after was Niwot Ridge's and may explain our slightly higher  $\delta^{13}$ C‰ values. Thereafter, the hypothesis of larger trees are more attacked (or attacked-killed) was supported (Figure 11B).

Means of trees that were not-attacked were smaller in diameter from both attacked and attacked-killed trees. The hypothesis that taller trees are more attacked (or attacked-killed) was supported, after excluding an outlier (Figure 12B). Means of trees that were not-attacked were shorter in height than attacked and attacked-killed trees. The hypothesis that trees that are faster growing are more attacked (or attacked-killed) is unsupported. Beetles in flight look for larger diameters and not likely looking for faster radial growth rates (Nelson, Rocca, Diskin, Aoki and Romme 2014). Among the single growth predictors, attack status, and water stress measurements,  $\delta^{13}C_{\infty}$  was not correlated with MPB attack status, NWC% is likely not a good measure of tree water

stress or is saying something different than what  $\delta^{13}$ C‰ is saying, and DBH was found significant among the three different attack statuses.

Limitations of the study design on inferential power included insufficient repeated needle collection measures across the whole study to determine NWC%, particularly prior to beetle attack, during, and after attack. Additionally, a small sample size of trees within stands, no replication of stands, no clear designation of old MPB attacks with a marker or other visual sign to distinguish from new attacks to obtain a more accurate number of increased attacks and stands that were limited in geography.

## 2.5 Conclusions

Conclusions that can be drawn from this research are (1) beetle attack and tree water stress interactions within complex interwoven beetle-tree-stand ecosystems are better understood using multiple physiological, environmental, and biotic parameters (2) tree and soil water, while important for growth and MPB defense, are less impactful on a stand level, but show an effect on individual tree survival, (3) RAI & height respond best to thinning treatments, (4) thinning treatments have both positive and negative consequences: they can improve tree vigor and defenses, but they can also spur RAI and escalate DBH into preferred size for MPB attack.

Managers should be careful about how they prescribe treatments because it is possible that BARTs can have outcomes that are not desirable. Both daily and seasonal measures of needle water stress in a more comprehensive study need to be examined to pinpoint the effects of tree level water, physiological parameters, and the interface with MPB.

**Table 5**. Means separation of  $\delta^{13}$ C‰ and NWC% by basal area reduction treatment (BART) or untreated basal area (UBA). Lettering shows Student's t test, means within a column not connected by the same letter differ significantly.

BA Treatment	$\delta^{13}$ C‰	Needle Water Content%
BART21	-27.74±0.172 b	44.250±7.482
BART42	-27.88±0.221 bc	37.283±10.123
BART63	-27.14±0.192 a	36.033±9.090
UBA95	-28.42±0.240 c	43.083±7.522

Response	Parameter	Estimate	Std Error	t Ratio	P-value
	Intercept	-28.928	0.770	-37.57	<0.001
δ <sup>13</sup> C‰	Tree Ht	0.027	0.043	0.63	0.534
	Tree RAI	145.327	46.819	3.10	0.005
	Intercept	0.527	0.280	1.88	0.074
Needle Water	Tree Ht	-0.005	0.015	-0.31	0.757
content%	Tree RAI	-7.848	17.003	-0.46	0.649

**Table 6**. Summary of a linear model showing responses of tree  $\delta^{13}$ C‰ and NWC% as predicted by RAI and height.

Parameter	Source	SS	df	F Ratio	P-value	Relative Effect Explained %
	RAI	3.696	1	11.666	0.003	33.83%
8 <sup>13</sup> C0/	Height	0.211	1	0.666	0.425	1.93%
0 0700	Attack Status	0.835	2	1.318	0.291	7.65%
	Error	6.020	19	-	-	55.1%
	RAI	0.006	1	0.147	0.706	0.61%
Needle Water	Height	<0.001	1	0.003	0.955	0.01%
Content%	Attack Status	0.187	2	2.473	0.111	20.22%
	Error	0.717	19	-	-	77.7%

**Table 7**. ANOVA summary of parameters tree  $\delta^{13}$ C‰ and NWC% with RAI, height, and attack status sources of the model error.


**Figure 5**. Stacked bar chart showing variation in frequencies of attack status across treatments. Green represents not-attacked trees, yellow represents attacked trees, and red represents attacked-killed trees.



**Figure 6**. The relationship between actual mean  $\delta^{13}$ C‰ and predicted  $\delta^{13}$ C‰ with height and RAI included in the model. A plot of actual by predicted values from a multivariate model of  $\delta^{13}$ C‰ that includes height and RAI as factors. Green points represent not-attacked trees, yellow points represent attacked trees, and red points represent attacked-killed trees. Shaded area shows 95% confidence interval.



**Figure 7**. The relationship between actual mean  $\delta^{13}$ C‰ leverage residuals and tree RAI leverage with height and RAI included in the model. Green points represent not-attacked trees, yellow points represent attacked trees, and red points represent attacked-killed trees. Shaded area shows 95% confidence interval.



**Figure 8.** Fit of NWC% with  $\delta^{13}$ C‰ with low outliers included (A) and with low outliers excluded (B). Green points represent not-attacked trees, yellow points represent attacked trees, red points represent attacked-killed trees, and gray points are the low outliers.



**Figure 9**. Actual mean  $\delta^{13}$ C‰ by predicted  $\delta^{13}$ C‰ mean with tree height, RAI, and attack status in the model. Green points represent not-attacked trees, yellow points represent attacked trees, and red points represent attacked-killed trees. Shaded area shows 95% confidence interval.



**Figure 10**. The relationship between mean  $\delta^{13}$ C‰ leverage residuals and tree RAI leverage with tree height and attack status included in the model. Green points represent not-attacked trees, yellow points represent attacked trees, and red points represent attacked-killed trees. Shaded area shows 95% confidence interval.



**Figure 11.** The relationship between actual mean NWC% and predicted NWC% with height, RAI, and tree attack status included in the model. Shown are mean NWC% with outliers showing clustered results per attack status (A), mean NWC% with outliers excluded in normal distribution (B), and mean NWC% with outliers excluded but shown (C). Green points represent not-attacked trees, yellow points represent attacked trees, red points represent attacked-killed trees, and gray points are the low outliers. Shaded areas show 95% confidence intervals.



**Figure 12.** Relationship between mean  $\delta^{13}$ C‰ and tree DBH showing no attack status pattern (A) and RAI (B). Green points represent not-attacked trees, yellow points represent attacked trees, and red points represent attacked-killed trees.



**Figure 13.**  $\delta^{13}C_{\infty}$  per tree by attack status showing that attacked trees had the highest mean  $\delta^{13}C_{\infty}$  value (lowest water stress). Not-attacked trees had the lowest mean  $\delta^{13}C_{\infty}$  (highest water stress) with attacked-killed mean  $\delta^{13}C_{\infty}$  intermediate for analysis containing not-attacked outlier (A) and without not-attacked outlier (B). Green points represent not-attacked trees, yellow points represent attacked trees, and red points represent attacked-killed trees.



**Figure 14.** NWC% per tree by attack status showing that not-attacked trees had the highest mean NWC%, attacked-killed trees had the lowest mean NWC%, and attacked trees had the intermediate mean NWC%. However, due to a non-normal distribution, NWC% means are to be used with caution. Green points represent not-attacked trees, yellow points represent attacked trees, and red points represent attacked-killed trees.



**Figure 15**. An ANOVA showing the relationship between attack status and tree DBH shown with low attacked outlier (A) and with outlier excluded (B). Green points represent not-attacked trees, yellow points represent attacked trees, and red points represent attacked-killed trees.



**Figure 16**. An ANOVA showing the relationship between attack status and tree height shown with low attacked outlier (A) and with outlier excluded (B). Green points represent not-attacked trees, yellow points represent attacked trees, and red points represent attacked-killed trees.

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# CHAPTER 3: Water, Basal Area Reduction Treatments, Year, and Day-of-Year Impacts on Lodgepole Pine Terpene Chemistry and its Relationship to Mountain Pine Beetle Signaling and Attack Response

#### **3.1 Introduction**

Throughout the last century, bark beetle outbreaks have caused adverse economic and lumber deficiencies impacting 10.3 million ha since 2000 (Fettig 2021; Institute 2021; Lindgren and Raffa 2013). As beetle killed trees are altered in their chemistry, including their volatile terpene chemistry, the recurrence and severity of wildfires intensify (Institute 2021; Jenkins, Runyon, Fettig, Page and Bentz 2014) burning 4,156,229.6 ha in 2020 alone. The recent trend in the western United States has reached billions in property loss and tragically, dozens of lives lost (Corbett, Withey, Lantz and Ochuodho 2015; Institute 2021; Wayman and Safford 2021). In dense forests, Lodgepole pine trees (*Pinus contorta* Douglas ex Loudon var. latifolia Engelman), compete for water resources which are intensified by droughts and warming forest temperatures (Gaylord, Kolb, Wallin and Wagner 2007; Meineke and Frank 2018). Such pine trees have constitutive defenses throughout their life to defend against bark beetles as well as induced defenses when danger is detected (Arango-Velez, Gonzalez, Meents, El Kayal, Cooke, Linsky, Lusebrink and Cooke 2013; Chiu, Keeling and Bohlmann 2017; Clark, Huber and Carroll 2012; Keeling and Bohlmann 2006). Both defenses need water to be produced, as water stress is frequently attributed as the reason bark beetles are having success colonizing trees (Lorio, Hodges and So 1977; McDowell, Pockman, Allen, Breshears, Cobb, Kolb, Plaut, Sperry, West, Williams and

Yepez 2008). Tree constitutive and induced defenses rely on monoterpenes and sesquiterpenes as part of their protection from bark beetles (Clark, Huber and Carroll 2012; Huber and Bohlmann 2004). However, many of these same terpenes are also used by bark beetles as signals to locate suitable host trees (Chiu, Keeling and Bohlmann 2018). Changes in climate and water stress require effective management methods, more knowledge of tree terpenes, and terpene interactions with bark beetles for the health and resilience of forests and safety of life and property.

Frequently used cultural control methods such as basal area reduction treatments (BARTs) and radial thinning are two management approaches where improved individual tree growing space and water availability are often achieved (Amman and Logan 1998; McDowell, Brooks, Fitzgerald and Bond 2003). BARTs have been shown to be a valuable method to increase tree vigor, improve tree terpene defense against pests and pathogens, and lower tree water stress while increasing water resources for the remaining trees (Gillette, Wood, Hines, Runyon and Negron 2014; Martin-Benito, Del Rio, Heinrich, Helle and Canellas 2010; Raffa and Berryman 1982). Thinning research has used tree water potentials ( $\Psi$ MPa), and  $\delta^{13}$ C‰ for decades to quantify the water status of trees (Cregg and Zhang 2001; Farquhar, Ehleringer and Hubick 1989; Running 1980). Tree water potentials give immediate water tension status for the current day and time while  $\delta^{13}$ C‰ gives the water stress of when the needle tissue was produced, which increases when water is scarce (Farguhar, Ehleringer and Hubick 1989). Other methods come from constitutive and induced defensive terpenes within a tree, the interaction between bark beetles and tree terpenes upon attack, and tree terpenes signaling suitable hosts to bark beetles (Alfaro, King

and vanAkker 2013; Borden, Pureswaran and Lafontaine 2008; Erbilgin, Krokene, Kvamme and Christiansen 2007). Terpenes have been measured above, within, and below the tree canopy which can give seemingly conflicting terpene data (Schade and Goldstein 2003). Thinning and terpene management are sometimes effective and other times not as effective against mountain pine beetle, (*Dendroctonus ponderosae*, Hopkins, MPB) (Borden, Chong, Earle and Huber 2003; Negron, Allen, Ambourn, Cook and Marchand 2017; Warren, McGrath and Adams 2001).  $\Psi$ MPa and  $\delta^{13}$ C‰ are common measures of tree water stress. Host monoterpenes act as signals of suitable hosts to bark beetles and thinning has been shown to change tree terpene chemistry (Erbilgin, Mori, Sun, Stein, Owen, Merrill, Bolanos, Raffa, Montiel, Wood and Gillette 2007; Seybold, Huber, Lee, Graves and Bohlmann 2006; Wood 1982).

Despite encouraging outcomes from thinning and terpene management methods, these methods don't prevent all lodgepole tree-bark beetle associated mortality; the ability of trees to defend themselves from pests is inconsistent and host chemical communication is not fully understood (Borden, Birmingham and Burleigh 2006; Borden, Pureswaran and Lafontaine 2008; Reid, Silins and Lieffers 2006). Such inconsistent results and need for more understanding of tree communication to bark beetles lead us to the questions of (1) How does relative terpene composition change over BART and year? (2) How does relative terpene composition change over time, BART, and over day-of-year nested within year? (3) How is water stress impacting monoterpene relative compositions? (4) Are terpene relative compositions different among trees that are attacked, not-attacked, or attacked-killed? Using the well-known methods of  $\Psi$ MPa and  $\delta^{13}$ C‰ isotopes to measure water stress and solid-phase micro-extraction (SPME) with

GC/MS to analyze terpenes, (McDowell, Allen and Marshall 2010; Running 1980; Wilson and Maguire 2009) we test the following hypotheses: (1) Terpene relative proportions will not differ across BART and year, (2) Terpene relative proportions will not significantly differ across day-of-year, year, and BART, (3) The predictor, waterstressed, will significantly predict each terpene's proportion significance, other variables of year and day-of-year will remain constant, and water-stressed\*year will have no effect, and (4) Terpene compositions will not significantly differ between attack statuses of attacked, not-attacked, or attacked-killed.

#### 3.2 Materials and Methods

#### 3.2.1 Study System

Field analysis was conducted in pure lodgepole pine (*Pinus contorta* Douglas ex Loudon var. latifolia Engelman) stands within the Gould Quadrangle of the Colorado State Forest Service State Park, Routt National Forest, Jackson County, Colorado, USA. Latitude and longitude of the research plots were 40.590910, -106.006300, respectively. Trees were 113 years old growing at 2,719 m elevation. Soil was sandy loam becoming a clay loam texture at 7.62 cm deep (USDA-NRCS 2020).

Within stands of pure Lodgepole pine, in the spring of 2008 four 0.20 hectare plots were selected for this 2008- 2009 study using a combination of GIS and ground inventory information. Three of the plots had basal area reduction treatments (BARTs) in 1985 with residual basal areas of BART 20.82 m<sup>2</sup>/ha (BART21), BART 42.45 m<sup>2</sup>/ha (BART42), and BART 62.99 m<sup>2</sup>/ha (BART63). The fourth plot remained an unthinned basal area (UBA) as a control with a basal area of 94.59 m<sup>2</sup>/ha (UBA95). Hereafter, unless specifically notated, all four plots are collectively referred to as BART(s). Trees in

each plot had previously been tagged with identification numbers (Hawksworth 1967; Hawksworth and Bailey 1967). Since plot establishment in 1967 and basal area reduction treatments in 1985, the USDA-FS Rocky Mountain Forest & Range Experiment Station in Fort Collins, CO USA (Hawksworth and Bailey 1967) had regularly monitored and taken plot inventories in the years 1985, 1997, and 2005 (Cochran and Barrett 1993). The plots had similar environmental and physiographical stand, altitude, slope, water, and soil attributes with differentiation being their basal area. In 2008, all four plots were measured according to Colorado State Forest Service (CSFS) cruising manual guidelines to obtain detailed information on species composition, volume, crown class, DBH, and stand conditions. In June 2008, within each of the four plots, six green unattacked lodgepole pine trees were randomly selected (Cochran and Barrett 1993; Klutsch, Negron, Costello, Rhoades, West, Popp and Caissie 2009) for closer examination through the duration of the study. Tree selection criteria were a full green healthy crown, no visible D ponderosae entrance holes or pitched-out resin along the trunk, no frass, as well as no other observable biotic organisms or abiotic stressors. For these 24 closely examined trees, additional measurements were taken of total height, condition class, and age using increment cores. Radial annual increment (RAI) was calculated using inventories' DBH.

# 3.2.2 Tree Bole and Canopy evaluation- Insect Rating, Tree Attack Status and Non-Baiting

Each of the 24 study trees had its bole and canopy evaluated during solar-noon tree branchlet collection on June 27, July 11, 25, August 7, 20, September 5 of 2008 and June 24, July 8, 22, August 5, 19, and September 1 of 2009 by starting examination

at the soil surface continuing upward to 6.10 m in height. *D ponderosae* severity was classified by use of a 0 to 10 numerical insect rating (IR) system that was based on number of hits, frass, canopy needle color, and exit holes similar to the standardized categorical tree classifications used by Hood et al. (2018) and Klutsch (2009) (Table 1.2). The IR was used to determine a summary attack status of not-attacked, attacked, or attacked-killed. Trees with a final value of zero were classified as not-attacked, values 1-8 were classified as attacked, and values 9-10 were classified as attacked-killed. Trees were considered as attacked when the first *D ponderosae* hit was documented, which began to be observed on tree boles starting July 25, 2008, and continued to be recorded throughout the remainder of the study which ended on September 1, 2009. The canopy of each tree was classified as either green, palefading, red, or dead. All 24 experiment trees were classified having green canopies at the start of research on June 27, 2008.

Due to high pressure from the MPB outbreak, the study was conducted without semiochemical baiting.

#### 3.2.3 Tree Branchlet Collection

Sun exposed tree branchlets were collected at a lower canopy position in each  $\sim$ 22.25 m tall tree based on proximity to the bole. For six days during the summer of 2008 (June 27, July 11, July 25, August 7, August 20, and September 5, 2008) and 2009 (June 24, July 8, July 22, August 5, and August 19) branchlets were collected. At each tree, a branchlet between 17.8 – 25.4 cm in length was clipped at a lower canopy position, approximately 7.62 – 10.67 m off the ground, using a rope pruning pole with 6-foot extensions. Each freshly cut branch tip was immediately bagged in a pre-

labeled gallon plastic bag, walked  $\leq$  400 ft (121.9m) back to the mobile vehicle lab for water potential analysis.

#### 3.2.4 Water Potential & Needle Storage

Each individual fresh branchlet was removed from its labeled plastic bag, stripped of its exterior epidermis approximately 2" (5.1 cm) from their clipped point laterally towards the branch's terminal tip, placed in the pressure bomb with the clipped stripped-of-epidermis end sticking outward through the rubber membrane. The pressure bomb was then pressurized using nitrogen gas and the bar reading recorded once sap was forced out of the stem. Readings were recorded in bar units and later converted to MPa. Branchlet Water Potentials (MPa  $\Psi$ ) using nitrogen gas and were analyzed using a Model 1000 Pressure Chamber (PMS Instrument Co.), Albany, Oregon, USA. Upon completion of the water potential reading, needles were collected from each branch tip, immediately placed, and sealed in 40 ml glass vials with silicone PTFE lined septa (Millipore Sigma, St. Louis, MO). Each sealed vial was then frozen and stored at 0° C until processed at Colorado State University lab, Fort Collins, Colorado, USA.

### 3.2.5 δ<sup>13</sup>C‰ Method

Needles from 24 trees were collected at the end of 2008 and 2009, in early September, for  $\delta^{13}$ C‰ analysis. For every tree and year, needles were oven dried at 70°C for 72 hours, then ground to powder using a mortar, a pestle, and liquid nitrogen. An average of 2.01 mg of crushed needle powder was scooped into a tin capsule, that measured 5 mm by 9 mm, and crimped using tweezers. Each filled capsule was analyzed by a Carlo Erba NA 1500 (Milano, IT) elemental analyzer coupled to a VG Isochrom continuous flow IRMS (Isoprime Inc., Manchester, UK) at Colorado State

University, Fort Collins, CO, USA). Delta carbon isotope signatures ( $\delta^{13}$ C‰) are presented as ‰ of  $^{13}$ C/ $^{12}$ C of each sample relative to the Vienna Pee Dee Belemnite standard.

#### 3.2.6 SPME-GC and Terpene Analysis

Although many compounds were detected, we chose to focus on four compounds that were found by Chiu et al. (2017) to be highly toxic to MPB and one compound not known as toxic for comparison. The four toxic compounds were  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, and limonene with  $\alpha$ -phellandrene as the non-toxic comparison. The 5 compounds represent 58.63% of the total monoterpene profile composition. While recognizing the five terpenes don't capture the entire range of biological possibilities, they do capture most of the variation. The one not toxic and four toxic selected compounds are known to function in terms of defense chemistry and beetle signaling.

Foliage (whole needle) samples, that had been previously collected into the glass vials with silicone PTFE lined septa and frozen, were later analyzed using headspace solid phase micro-extraction (HS-SPME) gas chromatography with mass spectrometry (GC/MS). Frozen vials were allowed to come to room temperature before analysis. Thereupon, a 100µm polydimethylsiloxane coated fiber (Supelco-Sigma-Aldrich, Bellefonte, PA, USA) was separately lowered through each vial's septa and exposed to the headspace within the vial for 60 seconds. The exposed fiber was then desorbed into a Hewlett Packard Series II 5980 gas chromatograph with FID detection using a Famewax column (30 m, 0.25mm interior diameter, Cat. No. 12497, Restek U.S., Bellefonte, PA, USA) to analyze volatiles. Inlet temperature was 220°C. The column

temperature started at 60°C (1 min hold) and went to 230°C (12 min hold) at 10°C min<sup>-1</sup>. The resulting chromatogram data of each peak's retention time, center x, height, width, and area of each volatile peak was then compiled in a spreadsheet. Areas were subsequently standardized by dividing total area by needle sample weight to obtain their relative percentage of the full volatile profile. Synthetic standards were identified on a HP Series II 5980 gas chromatograph connected to a HP 5971 mass selective detector and checked with a probability-based computer search of the NIST/EPA/NIH 75K spectral library (Hewlett Packard, Palo Alto, CA). The program had a starting temperature of 60°C (1 min hold) and went to 230°C (17 min hold) at 10°C min<sup>-1</sup>. Injection temperature was 280°C. Using each synthetic standard and calibrations, the quantity of each terpene was calculated.

#### 3.2.7 Statistical Analysis

A multiple regression model was used to determine the relationships between  $\alpha$ pinene,  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, or limonene relative areas, with predictors of BART and with year incorporated into the model as a random effect; followed with a student's t post-hoc multiple comparison of means test (Table 1). All statistical analyses were performed in JMP<sup>®</sup> Pro 15.0.0. (Cary, NC). Effects were interpreted to be statistically significant using a type one error rate of  $\alpha$  <0.05.

Similarly, a multiple regression model was used to determine the correlations between  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, or limonene relative areas with predictors of day-of-year, BART, and with year incorporated into the model as a random effect followed with a student's t post-hoc multiple comparison of means test (Table 2).

A linear regression analyzed  $\delta^{13}$ C‰ and  $\Psi$ MPa values against each terpene. No relationships between MPa and terpene proportions were found for  $\alpha$ -pinene (F1,260=0.004, P=0.953),  $\beta$ -pinene (F1,260=2.077, P=0.151), 3-carene (F1,260=1.752, P=0.187),  $\alpha$ -phellandrene (F1,260=0.696, P=0.405), or limonene (F1, 260=0.028, P=0.868). Further analysis using  $\Psi$ MPa did not occur, opting to solely use  $\delta^{13}$ C‰. We analyzed the distribution of the  $\delta^{13}$ C‰ mean responses, which was found to be normal. The median value of -27.795‰ was used as the boundary between water-stressed responses; values above were categorized as "no" and values below categorized as "yes". A two-way ANOVA model was then used to determine relationships between  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, or limonene relative areas with predictors of  $\delta^{13}$ C‰ character values, day-of-year, year, and  $\delta^{13}$ C‰ values crossed with year (Table 3) followed with a Tukey HSD post-hoc multiple comparison of means test (Table 4).

A multiple regression model, with a random effect included for year, was utilized to compare the fixed effect of the three attack statuses, not-attacked, attacked, and attacked-killed, with year incorporated into the model as a random effect, in the relative area responses of  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, and limonene from 2008 and 2009 followed by a student's t post-hoc multiple comparison of means test (Table 5).

#### 3.3 Results

#### 3.3.1 Terpene Relationships with BART Across Two Years

Terpene relative areas from 2008 and 2009 were examined for their relationship with BART, which showed significant differences most prominently with  $\beta$ -pinene and

more subtly with  $\alpha$ -pinene, 3-carene,  $\alpha$ -phellandrene, and limonene profiles (Table 1).  $\alpha$ pinene relative areas were not significantly different (F7, 280=1.555, P=0.149). The random effect of year was not a statistically significant effect (F1,286=1.528, P=0.218). BART also did not have a statistically significant effect (F6,281=1.560, P=0.159). Using student's t-test, as a post-hoc comparison of means, relative area compositions among basal area reduction treatments and years were significantly different from one another. Relative areas ranged from the highest of 21.5% in 2008 BART42, which had significantly higher relative area than 2008 and 2009 BART63 with the lowest areas of 11.28 and 10.50%, respectively. 2008 and 2009 BART 21, 2009 BART42, as well as 2008 and 2009 UBA95 were intermediate in their  $\alpha$ -pinene relative areas and did not differ significantly from one another.  $\beta$ -pinene relative areas were significantly different (F7, 280=4.394, P=<0.001) with the model explaining 9.90% of the variance. The random effect of year had a statistically significant effect (F1,286=11.093, P=0.001), with only 3.57% of the variance. BART also had a statistically significant effect (F6,281=3.278, P=0.004), with 6.33% of the variance explained. Using Student's t-test, as a post-hoc comparison of means, relative area compositions among basal area reduction treatments and years were significantly different from one another. Relative areas were the highest of 25.9% in 2008 BART63, which was significantly higher than all but the intermediates of 2008 BART42 and 2009 BART63. The lowest relative areas were 2009 BART21 and 2009 UBA95 of 16.08 and 15.50%, respectively, and were significantly different than all other areas, with the exceptions of intermediates 2008 BART21, 2008 UBA, and 2009 BART42. 2008 BART42 was significantly different than its 2009 counterpart, differing by 5.16%.  $\beta$  -pinene intermediates of 2009 BART63, 2008

BART21, 2008 UBA95 and 2009 BART42 relative areas did not differ significantly from one another. 3-carene relative areas were not significantly different (F7, 280=1.620, P=0.130) with the model explaining 3.89% of the variance. The random effect of year was a statistically significant effect (F1,286=14.245, P=0.040) yet only explained 1.46% of the variance. BART did not have a statistically significant effect (F6,281=1.182, P=0.316) with 2.44% of the variance explained. Using Student's t-test, as a post-hoc comparison of means, there was found some significantly different relative areas among basal area reduction treatments and years. The highest relative areas were 24.14, 23.65, and 23.55% in 2009 UBA95, 2008 UBA95, and 2009 BART42, respectively, which had significantly higher relative areas than the lowest area of 14.62% from 2008 BART21. 2009 BART63, 2009 BART21, 2008 BART63, and 2008 BART42 were intermediate in their 3-carene relative areas and did not differ significantly from one another.  $\alpha$ -phellandrene relative areas were significantly different (F7,280=2.080, P=0.048) with the model explaining 4.94% of the variance. The random effect of year did not have a statistically significant effect (F1,286=2.879, P=0.091), with only 0.98% of the variance explained. BART similarly did not have a statistically significant effect (F6,281=1.947, P=0.073), with 3.97% of the variance explained. Using Student's t-test, as a post-hoc comparison of means, relative area compositions among basal area reduction treatments and years had some significant differences from one another. Other than the intermediates of 2008 UBA95 and 2009 BART42, the highest relative area of 3.97% in 2008 BART21 was significantly higher than all other BART and their years for  $\alpha$ -phellandrene. The lowest relative area was 2009 UBA95 with only 0.36%. There was no significant difference between 2008 UBA95, 2009 BART42, 2008

BART42, 2009 BART21, 2009 BART63, and 2008 BART63. Limonene relative areas were significantly different (F7, 280=2.50, P=0.017) with the model explaining 5.88% of the variance. The random effect of year did not have a statistically significant effect (F1,286=.100, P=0.752), with only 0.00% of the variance explained. BART did have a statistically significant effect (F6,281=2.896, P=0.009), with 5.84% of the variance explained. The student's t-test post-hoc comparison of means showed one significantly different relative area, 2009 UBA95 at 3.14%, as the highest. 2009 UBA95 was significantly different from 2008 UBA95, 2008 BART63, 2009 BART42, 2009 BART63, 2008 BART21, and lastly, the lowest relative area of 0.03% from 2009 BART21. 2008 BART42 was intermediate and did not differ significantly from any limonene relative area.

#### 3.3.2 Day-of-Year, BART, and Year Predictions of Terpene Relative Areas

Analysis was conducted of the relationships between the five terpenes and how well they were predicted by day-of-year, BART, and year parameter sources. The overall model for  $\alpha$ -pinene with random effects was significant (F6,281=5.975, P=<0.001) and explained 11.31% of the variance.  $\alpha$ -pinene was also found to have a relationship with day-of-year (F2,285=12.906, P<0.001) explaining 8.15% of the variance (Table 2). BART was significant (F3, 284=2.791, P=0.041) while explaining only 2.64% variance. Year was not significant (F1,286=2.795, P=0.096).  $\beta$ -pinene's overall model was significant (F6,281=5.759, P<0.001) explaining 10.95% of the variance. Day-of-year showed no relationship with  $\beta$ -pinene (F2,285=1.826, P=0.163). BART was significant (F3,284=6.545, P<0.001) accounting for 6.22% in variance. Year was also significant (F1,286=10.794, P=0.001) which explained 3.42% of the variance. The whole model for 3-carene was significant (F6,281=3.288, P=0.004) but explained only 6.56% of the variance. BART was the only source that was not significant (F3,284=1.702, P=0.167). The remaining two parameter sources were found significant. The significant day-of-year (F2, 285=5.122, P=0.007) explained 3.41% of the variation. Year was also significant (F1,286=5.436, P=0.020) with 1.81% variance explained. The whole model for  $\alpha$ -phellandrene was not statistically significant (F6,281=1.843, P=0.091). All three random effects had no statistical significance with day-of-year (F2,285=0.743, P=0.477). BART showed no relationship with  $\alpha$  - phellandrene (F3,284=2.239, P=0.084). Year responded without significance (F1,286=2.898, P=0.090). Limonene's whole model was not significant (F6,281=1.710, P=0.119). Day-of-year was also not significant (F2,285=0.271, P=0.763). Year showed a strong lack of significance with limonene (F1,286=0.0705, 0.791). However, as seen with some previous terpenes, limonene did have a relationship with BART (F3,284=3.206, P=0.024).

#### 3.3.3 Water-stressed, Year, and Day-of-year with Terpene Proportions

The relative proportions of tree foliage  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, and limonene were examined for relationships with year, water-stressed, day-of-year, and water-stressed\*year yielding significant models for  $\alpha$ -pinene,  $\beta$ -pinene, and 3-carene, but not for  $\alpha$ -phellandrene, and limonene. The model for  $\alpha$ -pinene's relative area was significant (F4,283=6.879, P=<0.001, Table 3) and explained 8.86% of the variance. Year was marginally not statistically significant (F1,286=2.741, P=0.099). No significance was found for water-stressed (F1,286=1.189, P=0.276). Day-of-year was significant (F1,286=24.392, P=<.001) and accounted for most, 7.86%, of the

variation in the model. Lastly, water-stressed \*year was not significant (F1,286=0.304, P=0.582). b-pinene's model was statistically significant (F4,283=9.595, P=<0.001) and explained 11.94% of the variation. Year was also significant (F1,286=10.996, P=0.001) with 3.42% variance explained. Explaining 8.17% of the model variation, water-stressed was statistically significant (F1,286=26.253, P=<0.001). Day-of-year was found not to be significant (F1,286=0.618, P=0.433) as well as water-stressed\*year (F1,286=0.037, P=0.847). The last whole model to be statistically significant was 3-carene (F4,283=1.126, P=<0.001) with 8.89% of variance explained. Year was a significant source of variation (F1,286=5.615, P=0.019) with 1.81% explained. Water-stressed was statistically significant (F1,286=12.692, P=<0.001) with the majority, 4.09%, of variance explained. Day-of-year was similarly significant (F1,286=10.330, P=0.002) explaining a large portion, 3.33%, of the variation. Water-stressed\*year was not statistically significant (F1,286=0.051, P=0.821). The whole model for  $\alpha$ -phellandrene's relative area was not significant (F4,283=6.900, P=0.344). As was found with  $\alpha$ -pinene, year was marginally not significant (F1,286=2.854, P=0.092). No significance was found for water-stressed (F1,286=0.004, P=0.949). Day-of-year was also not significant (F1,286=0.055, P=0.814). Lastly, water-stressed\*year was not significant for  $\alpha$ phellandrene (F1,286=1.635, P=0.202). Limonene's relative proportion whole model was not significant (F4,283=1.506, P=0.201). The variation source of year was not significant (F1,286=0.070, P=0.792). The only significant source of variance was waterstressed (F1,286=4.681, P=0.031) with 1.62% being explained. Day-of-year was not significant (F1,286=0.363, P=0.548) as was the cross of water-stressed\*year (F1,286=0.884, P=0.348).

After the above ANOVA results, comparisons between relative proportion means of tree foliage  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, and limonene per year with water-stressed showed some statistical differences within  $\beta$ -pinene and 3-carene, but not for  $\alpha$ -pinene,  $\alpha$ -phellandrene, and limonene.  $\alpha$ -pinene had a pattern of having a higher proportion in water-stressed trees over both years, without being statistically different (Table 4). In both years, there was a significant pattern of trees that were not water-stressed having a much higher proportion of b-pinene. 3-carene showed a significant difference in 2009 of a much higher proportion in water-stressed trees than did both years of the not water-stressed trees. There was no pattern of either water-stressed trees or not water-stressed trees with a higher proportion of  $\alpha$ -phellandrene. Although both years of the not water-stressed trees had 300% higher proportion of limonene than both years of the not water-stressed trees, there was no statistical significance between the proportions of both groups.

#### 3.3.4 Terpene Relative Profiles for Attack Status in 2008 and 2009

Terpene relative area profiles were evaluated against the three attack statuses of not-attacked, attacked, and attacked-killed with the nested year 2008 or 2009. The whole model for  $\alpha$ -pinene was not significant (F5,282=0.679, P=0.640). There was no statistical significance found between  $\alpha$ -pinene and attack status (F4,283=0.474, P=0.755). Similarly, the random effect of year was not significant (F1,286=1.221, P=0.270). Relative area means ranged from the highest relative area of  $\alpha$ -pinene of 16.8% in 2008 attacked to the lowest of 12.4% in 2009 attacked. Using student's t-test, as a post-hoc comparison of means, relative area compositions of  $\alpha$ -pinene showed no significant difference with any attack status for either year (Table 5).  $\alpha$ -pinene was

relatively constant among all attack statuses and across years.  $\beta$ -pinene's whole model concluded there was a significant difference (F5,282=9.035, P<0.001) with the model explaining 13.81% of the variance. Attack status had a significant relationship with  $\beta$ pinene (F4,283=8.374, P<0.001) with 10.24% variance explained. Likewise, year was shown to be significantly different (F1,286=9.952, P=0.002) with a meager 3.04% explained variance. Student's t-test showed a host of significantly different means ranging from the highest relative area of  $\beta$ -pinene in 2008 attacked-killed of 25.5% to the lowest of 13.4% with 2009 not-attacked. 2008 attacked-killed and 2008 attacked were statistically different from 2009 attacked, 2008 not-attacked, and 2009 notattacked. Attacked 2009 was statistically different from both years of not-attacked. Lastly, 2009 attacked was significantly different from 2009 not-attacked. Noteworthy was attacked-killed occupied two of the three highest percentages of b-pinene. Next, 3carene's whole model was not statistically significant (F5,282=2.098, P=0.066). Attack status also showed no significance (F4,283=1.558, P=0.186). The random effect of year was significant (F1,286=3.937, P=0.048), but only explained 1.35% of variation. Posthoc means comparisons showed the 2009 not-attacked with the highest relative area of 26.2% was significantly different from the low 18.0% of 2008 attacked. 2009 notattacked and 2009 attacked were statistically different than both 2008 and 2009 attacked-killed, 2008 not attacked, and 2008 attacked. Last of all, 2009 not-attacked was significantly different than 2008 and 2009 attacked-killed. Not-attacked trees had two of the three highest percentages of 3-carene. Despite being a small percentage of the relative area profile,  $\alpha$ -phellandrene's whole model was significantly different (F5,282=2.606, P=0.025) with 4.42% of variance explained by the model. Both attack

status and year were significant; attack status (F4,283=2.537, P=0.040) and explained 3.44% of the variance. Year was significant (F1,286=4.701, P=0.031), but only explained 1.59% of variation. Student's t-test showed 2008 not-attacked mean significantly different than all other means. However, as the highest and lowest  $\alpha$ phellandrene relative areas of 4.30 and 0.72%, respectively, were both not-attacked, there is no pattern of attack status found with these primarily constant means. Finally, limonene's whole model showed there was no significant difference (F5,282=2.191, P=0.056). Year effects were not significant (F1,286=0.013, P=0.911). Attack status was remarkable with 37.05% of the model variance explained and was significantly different (F4,283=2.713, P=0.030). Relative areas of limonene ranged from a high of 2.3% in 2009 not-attacked to a low 0.2% in 2008 attacked. Post-hoc student's t-test mean comparisons showed 2009 not-attacked was significantly different from 2009 attackedkilled and both years of attacked. 2008 attacked-killed was significantly different from 2008 and 2009 attacked. Limonene had both 2008 and 2009 not-attacked in the top three relative area means while having 2008 and 2009 attacked as the two lowest relative areas.  $\beta$ -pinene had the most means significant differences and decreasingly differences with 3-carene, limonene  $\alpha$ -phellandrene, and no significant differences among  $\alpha$ -pinene relative area means.

#### 3.4 Discussion

#### 3.4.1 Terpene Relationships with BART Across Two Years

Terpene proportions tended to differ across BARTs, however less commonly across years. BART effects did not support the hypothesis for all terpenes, as differences were seen in each terpene's profile, most prominently with β-pinene

proportions (Table 1). Supporting the hypothesis, mean terpene proportions between the years were the same in all but two comparisons:  $\alpha$ -phellandrene in BART21 and limonene in UBA95. α-pinene 2008 BART42 was double the relative area of the lowest relative areas, both 2008 and 2009 BART63. It was expected, if there were a significant difference between means, the highest defensive terpene relative areas would be from the lowest densities, since denser stands have been found to be more attacked (Lorio 1980). Surprising was both 2008 and 2009 UBA95 did not have the lowest terpene proportions and 2008 and 2009 BART21 did not have the highest relative areas, as they had the least and most resources, respectively. In Finland, a 48 year old uniform stand of Scots pine had two distinct monoterpene profiles, which traced back to if they were artificially or naturally restored, with the latter having more 3-carene (Back, Aalto, Henriksson, Hakola, He and Boy 2012). Dormont et al. found differences in cone, foliage, and oleoresin of same species, and different profiles from same organ likely due to rearrangements from heat during steam distillation and solvent extraction isolating non-volatile compounds (Dormont, Roques and Malosse 1998). With β-pinene, surprising was 2008 BART63 had the highest relative area. Also remarkable was two BART opposites, 2009 BART21 and 2009 UBA95, had the two lowest relative areas of  $\beta$ -pinene. 3-carene mean separation showed the opposite of expectations with the densest plots having more 3-carene than did BART21. However, Erbilgin et al. found (+)-3-carene very appealing (2007). As expected, the pattern of higher  $\alpha$ -phellandrene in the lower density BARTs and a pattern of diminishing terpene proportions, due to less resources per tree, was found as tree density increased. Limonene also was surprising with 2009 UBA95 having a proportion that was greater than 200-300% of the lowest
densities. The same tree species, *Pinus cembra*, from two different locations in France, had two different profiles of  $\alpha$ -pinene,  $\beta$ -pinene,  $\alpha$ -phellandrene, and limonene/ $\beta$ -phellandrene of 38.9, 20.7, -, and 34.0% for the Tueda, northern French Alps location and 26.4,10.1, -, and 57.9% for the Chevreloup, north central France location, respectively (Dormont, Roques and Malosse 1998). Terpene proportions are not consistent across BARTs whereas terpene proportions are primarily the same across years within each BART.

#### 3.4.2 Day-of-Year, BART, and Year Predictions of Terpene Relative Areas

The relative proportions of  $\alpha$ -pinene and 3-carene fluctuated across the year, which did not support the hypothesis; while  $\beta$ -pinene,  $\alpha$ -phellandrene and limonene did support the hypothesis by not fluctuating over the year (Table 2). In other research,  $\alpha$ pinene and 3-carene have been shown to fluctuate across the year, but  $\beta$ -pinene and limonene have been shown to fluctuate too, especially in induced defensive responses (Roth, Hussain, Cale and Erbilgin 2017). It has been noted, environmental factors, such as temperature and humidity, drive monoterpene emissions (Schade, Goldstein and Lamanna 1999; Tingey, Turner and Weber 1991). The density of UBA95 did not allow for much sun penetration whereas BART21 was exceptionally sunny and warmer. Temperatures fluctuate across the growing season, often culminating in late July to early August, while likely coevolutionary, is also the peak of MPB flight. Having the fullest cacophony of monoterpenes emitted for a beetle with finite lipid reserves, can positively influence efficient host locating leaving more reserves for successful brood production. Although not in agreement with the hypothesis, BART predicted the proportions of  $\alpha$ -pinene,  $\beta$ -pinene, and limonene; by so doing supported the use of

BART to manage forests against MPB tree mortality. As found by Amman and Logan (1998),  $\alpha$ -pinene,  $\beta$ -pinene, and limonene are produced in higher proportions when resources are abundant. Year only predicted  $\beta$ -pinene and 3-carene. This can be explained due to the majority of trees being attacked in 2008 and due to  $\beta$  -pinene and 3-carene being highly reactive to initial attack, their difference across years is explained (Roth, Hussain, Cale and Erbilgin 2017). The examined terpenes involved with MPB showed individual responses by day-of-year, BART, and year.

#### 3.4.3 Water-stressed, Year, and Day-of-year with Terpene Proportions

Water deficit does not automatically translate to a negative or consistent terpene response from a tree. While resin flows are reduced by drought, individual needle terpene amounts or concentrations may increase, decrease, or fluctuate by weather, day-of-year, and even by year (Blanch, Penuelas, Sardans and Llusia 2009; Kainulainen, Oksanen, Palomaki, Holopainen and Holopainen 1992; Lorio, Hodges and So 1977). Further, the total monoterpene response of lodgepole pine may not even change with water deficit (Erbilgin, Cale, Lusebrink, Najar, Klutsch, Sherwood, Bonello and Evenden 2016). Here, the five terpenes had mixed results, with only limonene fully supporting the whole hypothesis. Specifically, relative areas of  $\beta$ -pinene, 3-carene, and limonene were predicted by water-stressed, year predicted  $\beta$ -pinene and 3-carene, dayof-year predicted  $\alpha$ -pinene and 3-carene, while water-stressed\*year did not predict any terpene proportions. For example, Kainulainen et al. found drought treated Norway spruce trees had a significant increase in  $\alpha$ -pinene amounts, a small increase in  $\beta$ pinene,  $\alpha$ -phellandrene, and limonene, and a slight decrease in 3-carene (1992). Confounding tree needle drought responses further, our results showed a significant

relative area increase in 3-carene, a significant decrease in  $\beta$ -pinene, a slight decrease in  $\alpha$ -phellandrene, and small increases in both  $\alpha$ -pinene and limonene in drought stressed trees. Perhaps day-of-year explains the varying needle terpene responses as Blanch et al. (2009) found  $\alpha$ -pinene and 3-carene concentrations decreased while  $\beta$ pinene increased in drought treated *Pinus halapensis* needles in mid-June, all three increased by late June, and the largest increase between control to drought needles was in late July, including limonene's only recorded increase all season. In early August, limonene decreased while the other three had increases, albeit reduced in comparison to late July (Blanch, Penuelas, Sardans and Llusia 2009). Mean separation showed  $\beta$ -pinene and 3-carene supported the hypothesis while  $\alpha$ -pinene,  $\alpha$ phellandrene, and limonene did not support the hypothesis (Table 4). The varied terpene responses to sources of variance illustrates the dynamic impact abiotic stresses, such as water stress, seasonal weather, and annual weather, may have on tree terpene proportions (Manninen, Tarhanen, Vuorinen and Kainulainen 2002). While not tested here, these results may also have some significance for defoliating insects and wildfire risks (Alessio, Penuelas, De Lillis and Llusia 2008).

### 3.4.4 Terpene Relative Profiles for Attack Status in 2008 and 2009

Each monoterpene had a unique response to attack status. The profile of  $\alpha$ pinene did not change among attack statuses, even with trees being attacked and killed over both years (Table 5).  $\alpha$ -pinene needs to have a continual presence as it has many roles: its common presence in most MPB hosts, is utilized in production of aggregation pheromones, and can act in synergy with other compounds to attract MPB beetles (Blomquist, Figueroa-Teran, Aw, Song, Gorzalski, Abbott, Chang and Tittiger 2010;

Miller 2006; Smith and Psw 2000). Dormont et al. found  $\alpha$ -pinene to be the dominant monoterpene in foliage (Dormont, Roques and Malosse 1998). Manninen found  $\alpha$ pinene increased in Scots pine as latitude increased (Manninen, Tarhanen, Vuorinen and Kainulainen 2002). β-pinene's four highest proportions were with attacked and attacked-killed statuses for both years. Attacks were more lethal when there were lower proportions of β-pinene compared to higher proportions in non-lethal attacks as it is found in induced defensive responses (Bentz, Boone and Raffa 2015; Roth, Hussain, Cale and Erbilgin 2017). Not-attacked trees had two of the three highest proportions of 3-carene. Although often found in conjunction with defensive  $\beta$ -pinene due to its toxicity being second to limonene, 3-carene can also be a kairomone for host location, synergize beetle sex pheromones, and invite predators (Chiu, Keeling and Bohlmann 2017; Erbilgin, Mori, Sun, Stein, Owen, Merrill, Bolanos, Raffa, Montiel, Wood and Gillette 2007; Miller and Borden 2003). However, as the highest and lowest  $\alpha$ phellandrene relative areas were both not-attacked, it appears as attacked and attacked-killed trees were found with intermediate values. a-phellandrene has been seen in both attacked and not-attacked branches in other research. For example, concentrations of  $\alpha$ -phellandrene remained constant in *Pinus sylvestris* subspecies nevadensis whether an attacked branch, not-attacked branch, or not-attacked tree while *Pinus sylvestris* subspecies *iberica* had no difference between attacked and notattacked branches but was almost 50% less in the not-attacked tree (Achotegui-Castells, Llusia, Hodar and Penuelas 2013). Such a parabolic response is not a new phenomenon; Erbilgin et al. (2003) also found *lps pini* to have a similar parabolic response from α-pinene concentrations. Limonene had both 2008 and 2009 not-

attacked in the top three relative areas while attacked and attacked-killed had 3 of the lowest relative areas suggesting its toxicity to MPB. Chiu et al. found (-)-Limonene to be the most toxic monoterpene to MPB followed closely by (+)-Limonene (2017). Roth et al. also found limonene in induced defensive responses (Roth, Hussain, Cale and Erbilgin 2017). It was expected, if there were a significant difference between means, the highest defensive terpene proportions would be from the lowest densities, since trees in denser stands have been found to be more attacked (Lorio 1980). The hypothesis was true for  $\alpha$ -phellandrene. The monoterpenes  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, and limonene played a role in attack status, while  $\alpha$ -phellandrene's impact remains unclear.

Limitations of the study design on inferential power include a small sample size of trees within stands, replication of stands, clear designation of old and new MPB attacks, limited geography of stands, and not having mass-spec for all samples.

#### 3.5 Conclusions

Monoterpenes from host trees are known to operate as beacons to bark beetles across vast distances without the additive effect of bark beetle generated pheromones (Dormont, Roques and Malosse 1998; Gijzen, Lewinsohn, Savage and Croteau 1993; Seybold, Huber, Lee, Graves and Bohlmann 2006). MPB have been documented flying a distance commonly between 2.12 and 5.95 km in less than a day with a record of more than 24 km flown in the same amount of time (Evenden, Whitehouse and Sykes 2014). Many studies utilize atmospheric data from towers above the canopy or surface instruments. However, like Crawford et al. (2014), we opted to measure primary biological aerosol concentrations where they are found at their utmost, in the forest

canopy. We concurrently measured the effects of BART on tree needle monoterpene profiles, tree water stress, and MPB attack behavior over two years during a MPB outbreak to make a small contribution of understanding more about the interaction between lodgepole pine physiology, anthropogenic forest management, and MPB. Conclusions that can be drawn from this research are (1) BART is an effective management method to alter terpene proportions, (2) BART will not impact each terpene proportion the same, (3) Day-of-year is a key factor in terpene proportions, (4) year is only a factor in 10% of terpene proportions, (5) water stress may impact only select terpene proportions (6) the proportion of  $\alpha$ -pinene does not have a bearing on attack status, whereas the proportions of  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, and limonene do. While not tested here, these results may also have some significance for defoliating insects and wildfires (Alessio, Penuelas, De Lillis and Llusia 2008). Caution is advised against transferring these findings from needle monoterpenes to phloem monoterpenes, as they are different organs in a tree (Dormont, Roques and Malosse 1998). As found by Manninen et al., monoterpenes needles were found to have 500% higher monoterpene concentration than wood which had 400% greater concentration of tri-cyclic resin acid (Manninen, Tarhanen, Vuorinen and Kainulainen 2002).

BART	Year	α-pinene	β-pinene	3-carene	$\alpha$ -phellandrene	limonene
BART21	2008	0.174±0.028	0.203±0.020	0.146±0.018	0.040±0.014	0.001±0.001
		ab*	bcd	b	а	b
	2009	0.145±0.027	0.161±0.014	0.215±0.020	0.015±0.004	0.000±0.000
		ab	d	ab	b	b
BART42	2008	0.215±0.028	0.237±0.017	0.174±0.023	0.015±0.006	0.018±0.009
		а	ab	ab	b	ab
	2009	0.152±0.027	0.185±0.014	0.235±0.026	0.018±0.005	0.001±0.000
		ab	cd	а	ab	b
BART63	2008	0.113±0.013	0.259±0.013	0.198±0.015	0.004±0.001	0.011±0.006
		b	а	ab	b	b
	2009	0.105±0.018	0.227±0.016	0.216±0.022	0.008±0.002	0.001±0.000
		b	abc	ab	b	b
UBA95	2008	0.150±0.037	0.193±0.023	0.237±0.041	0.024±0.017	0.012±0.009
		ab	bcd	а	ab	b
	2009	0.153±0.035	0.155±0.019	0.241±0.035	0.004±0.001	0.031±0.014
		ab	d	а	b	а

**Table 8.** Means separation with standard error of  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene,  $\alpha$  - phellandrene, and limonene relative areas per year, as a random variable, and by basal area reduction treatment (BART). Lettering shows Student's t test, means within a column not connected by the same letter differ significantly.

**Table 9**. Day-of-year, year, and BART summary of parameters tree  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, and limonene relative areas with random effects of day-of-year, year, and BART the sources of model error.

Parameter	Source	SS	df	F Ratio	P-value
	Day-of-year	0.652	2	12.906	<0.001
a ninono	BART	0.212	3	2.791	0.041
d-pinene	Year	0.071	1	2.795	0.096
	Error	7.099	281	-	-
	Day-of-year	0.039	2	1.826	0.163
ßningng	BART	0.211	3	6.545	<0.001
p-pinene	Year	0.116	1	10.794	0.001
	Error	3.013	281	-	-
	Day-of-year	0.248	2	5.122	0.007
2 corono	BART	0.124	3	1.702	0.167
3-carene	Year	0.132	1	5.436	0.020
	Error	6.809	281	-	-
	Day-of-year	0.004	2	0.743	0.477
α-	BART	0.017	3	2.239	0.084
phellandrene	Year	0.007	1	2.898	0.090
	Error	0.707	281	-	-
	Day-of-year	0.001	2	0.271	0.763
limonono	BART	0.017	3	3.206	0.024
innonene	Year	0.000	1	0.071	0.791
	Error	0.504	281	-	-

Parameter	Source	SS	df	F Ratio	P-Value
	Year	0.071	1	2.741	0.099
	Water-stressed	0.031	1	1.189	0.276
α-pinene	Day-of-year	0.629	1	24.392	<.001
	Water-stressed*Year	0.008	1	0.304	0.582
	Residual	7.295	283	-	-
β-pinene	Year	0.116	1	10.996	0.001
	Water-stressed	0.276	1	26.253	<.001
	Day-of-year	0.007	1	0.618	0.433
	Water-stressed*Year	0.000	1	0.037	0.847
	Residual	2 979	283	_	-

0.132

0.298

0.242

0.001

6.640

0.007

0.000

0.000

0.004

0.723

0.000

0.008

0.001

0.002

0.511

1

1

1

1

1

1

1

1

1

1

1

1

283

283

283

5.615

12.692

10.330

0.051

2.854

0.004

0.055

1.635

0.070

4.681

0.363

0.884

-

-

-

0.019

<.001

0.002

0.821

0.092

0.949

0.814

0.202

0.792

0.031

0.548

0.348

-

-

-

Year

3-carene

α-

phellandrene

limonene

Water-stressed

Water-stressed

Water-stressed

Day-of-year

Residual

Day-of-year

Residual

Year

Water-stressed\*Year

Water-stressed\*Year

Water-stressed\*Year

Day-of-year

Residual

Year

**Table 10.** Relationships between  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene,  $\alpha$ -phellandrene, or ç

**Table 11.** Means separation with standard error of  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene,  $\alpha$  - phellandrene, and limonene relative areas per year and by  $\delta^{13}C$ ‰ water-stressed or not-water-stressed characterization.

δ <sup>13</sup> C‰ Water- stressed	Year	α-pinene Mean Relative Area	β-pinene Mean Relative Area	3-carene Mean Relative Area	α- phellandrene Mean Relative Area	limonene Mean Relative Area
No	2008	0.158±0.017	0.253±0.012a	0.158±0.011b	0.017±0.006	0.007±0.003
No	2009	0.123±0.016	0.214±0.011ab	0.193±0.012b	0.015±0.003	0.001±0.000
Yes	2008	0.168±0.023	0.193±0.014bc	0.219±0.024ab	0.024±0.010	0.013±0.006
Yes	2009	0.154±0.022	0.150±0.011c	0.261±0.022a	0.007±0.002	0.016±0.007

\*Levels not connected by the same letter are significantly different.

Not- attacked, Attacked, or Attacked- killed	Year	α-pinene Mean Relative Area	β-pinene Mean Relative Area	3-carene Mean Relative Area	α- phellandren e Mean Relative Area	Limonene Mean Relative Area
Not-	2008	0.151±0.036	0.160±0.020	0.211±0.041	0.043±0.019	0.010±0.009
attacked			cd	abc	а	abc
Not-	2009	0.130±0.031	0.134±0.017	0.262±0.034	$0.007 \pm 0.003$	0.023±0.013
attacked			d	ab	b	а
Attacked	2008	0.168±0.019	0.235±0.013	0.180±0.015	$0.011 \pm 0.003$	0.002±0.002
			а	С	b	С
Attacked	2009	0.124±0.019	0.181±0.011	0.238±0.018	0.012±0.003	0.004±0.002
			bc	ab	b	С
Attacked	2008	0.166±0.023	0.255±0.016	0.182±0.018	0.017±0.008	0.020±0.007
-killed			а	bc	b	ab
Attacked	2009	0.164±0.024	0.219±0.014	0.187±0.019	0.013±0.003	0.004±0.003
-killed			ab	bc	b	bc

**Table 12.** Means with standard error separation of  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -carene,  $\alpha$  - phellandrene, and limonene relative area units per year and attack status of not-attacked, attacked, or attacked-killed in.

\*Levels not connected by the same letter are significantly different.

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## **CHAPTER 4: Conclusions and Future Research**

Bark beetle outbreaks have caused adverse economic and lumber deficiencies impacting 10.3 million ha since 2000 (Cochran and Barrett 1993; Fettig 2021; Lindgren and Raffa 2013). A common bark beetle management method is to reduce basal area. Reducing within-stand competition may improve individual tree vigor but also can alter stand-level resistance to beetles. Some studies have also shown that density reduction treatments can have deleterious effects on tree resistance. Lodgepole pine trees (*Pinus contorta* Douglas ex Loudon var. latifolia Engelman) across the northern Rocky Mountains have been killed in increasingly severe mountain pine beetle (*Dendroctonus ponderosae*, Hopkins, MPB) outbreaks.

Accordingly, there is a need to fine-tune cultural control methods at a regional scale in order to determine how to best manage forest stands for reduced bark beetle damage. Here, we exploit an outbreak of the mountain pine beetle (*Dendroctonus ponderosa*) in northern Colorado to evaluate basal area reduction treatments (BART) of 21, 42, 63, and 95 m<sup>2</sup>/ha, (hereafter referred to as BART21, BART42, BART63, and UBA95) of lodgepole pine (*Pinus contorta* Douglas ex Loudon var. latifolia Engelman) over three studies.

The first study examined BART to examine basal area reduction treatments) impacts microsite conditions important to tree vigor. Specifically, we analyzed pre-dawn and solar-noon water potentials, volumetric soil water content, tree height and radial annual increment (RAI), relative humidity, and temperatures among density treatments. Variation in tree-level factors were further analyzed for their effects on tree survival during, insect damage rating, beetle trap captures within stands, and total beetle attacks

on trees. When basal areas were reduced, RAI and height increased. BART did not have an effect on beetle trap captures, and microsite water variables had an effect on tree survival and maximum insect rating. These findings have implications for the management of lodgepole pine stands under pressure from bark beetle outbreaks. The interactions between lodgepole pine, microsite conditions, and then with mountain pine beetle are more complex than previously thought; positive growth factors can become negative tree survival factors as well as to suggest water potentials in lodgepole pine may not be the best measure of water stress.

The second study examined BART with tree water stress as basal area reduction treatments (BARTs) have been implemented as a method to reduce tree competition for water. Beetle attacks were measured, as well as tree mortality, diameter at breast height (DBH), radial annual increment (RAI), height, needle water content (NWC%), and annual water-drought history ( $\delta^{13}$ C‰) as a benchmark. These measurements were taken prior to being attacked by the MPB outbreak moving through the four basal area reduction treatments of decreasing density, located within the Colorado State Forest Service State Park near Gould, Colorado, USA. The measurements were compared against the same criteria, after the MPB had infested these same trees to determine what factors were responsible in determining the underlying cause of mortality and why specific trees were at greater risk. The same BARTs had a significant relationship in reducing  $\delta^{13}$ C‰ tree water stress compared to the control plot. However, reduced water stress was negatively correlated with MPB attacked and attacked-killed trees. RAI was significant for the BART stands whose legacy was likely what caused DBH to be positively correlated with MPB attacked and attacked-killed trees despite lower water

stress. BART moves trees into a susceptible size class. This research will help to shed light on tree physiological parameters and BARTs that influence MPB caused mortality with which forest managers can use to reach desired stand outcomes.

The third study examined BART impacts on tree foliage terpene chemistry while also quantifying terpene proportions over the day-of-year and across two years since beetle killed trees are altered in their chemistry (Jenkins, Runyon, Fettig, Page and Bentz 2014). In dense forests, Lodgepole pine trees (*Pinus contorta* Douglas ex Loudon var. latifolia Engelman), compete for water resources which are intensified by droughts and warming forest temperatures (Gaylord, Kolb, Wallin and Wagner 2007; Meineke and Frank 2018). Pine trees have constitutive defenses and induced defenses which rely on monoterpenes and sesquiterpenes as part of their protection from bark beetles (Chiu, Keeling and Bohlmann 2017; Clark, Huber and Carroll 2012; Huber and Bohlmann 2004). Terpenes are also used by bark beetles as signals to locate suitable host trees (Chiu, Keeling and Bohlmann 2018). Within each stand, six healthy green trees' monoterpene profiles of five monoterpenes were examined prior to and over two years for correlations to water stress, BART, and attack status interactions with Mountain Pine beetle (Dendroctonus ponderosae, Hopkins, MPB). All five monoterpenes' proportions rarely differed across years, but differed across BARTs, with  $\beta$ -pinene most impacted. The relative proportions of  $\alpha$ -pinene and 3-carene fluctuated across the year, while  $\beta$ -pinene,  $\alpha$ -phellandrene and limonene did not fluctuate over the year. Tree needle drought responses showed a significant relative area increase in 3carene, a significant decrease in  $\beta$ -pinene, a slight decrease in  $\alpha$ -phellandrene, and small increases in both  $\alpha$ -pinene and limonene in drought stressed trees.  $\beta$ -pinene, 3-

carene, and limonene had a relationship with attack status,  $\alpha$ -pinene was similar whether tree was not-attacked, attacked, or attacked-killed, while  $\alpha$ -phellandrene's relationship remains unclear. Changes in climate and water stress require effective management methods, more knowledge of tree terpenes from all tree foliage, and terpene interactions with bark beetles for the health and resilience of forests and safety of life and property.