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THE IMPACTS OF REMOVING WILDFIRE ON PINUS PONDEROSA RESISTANCE AGAINST DENDROCTONUS PONDEROSAE

Sharon Metzger Hood
The University of Montana

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DENDROCTONUS PONDEROSAE

By

SHARON METZGER HOOD

B.S., Mississippi State University, Starkville, Mississippi, 1998

M.S., Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 2001

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Approved by:

Sandy Ross, Dean of The Graduate School

Graduate School

Anna Sala, Chair

Organismal Biology and Ecology

Barbara Bentz

USDA Forest Service, Rocky Mountain Research Station

Cara Nelson

College of Forestry and Conservation

John Maron

Organismal Biology and Ecology

Ragan Callaway

Organismal Biology and Ecology

The Impacts of Removing Wildfire on *Pinus ponderosa* resistance against *Dendroctonus ponderosae*

Chairperson: Anna Sala

Disturbance and disturbance interactions drive multiple ecosystem processes over multiple spatial and temporal scales. I studied the role of frequent, low-severity fire in *Pinus ponderosa* (ponderosa pine) forests in altering the success of the irruptive insect *Dendroctonus ponderosae* (mountain pine beetle (MPB)). I had three overarching research questions: (1) What are the best predictors of ponderosa pine resin flow, a trait related to MPB attack success, and how do these predictors relate to tree growth?; (2) Are predictors of resin flow also predictors of MPB attack success and are they affected by changes to wildfire disturbance regimes?; (3) How do forest management treatments affect individual tree defense and forest resistance to a MPB outbreak? I first show that the best predictors of resin flow are resin duct size and total resin duct area, both of which increase with tree growth rate. Next, I show that resin ducts are good predictors of MPB attack success. Based on field data in natural stands subjected to wildfires, I then show low-severity fire induces resin duct production, and that resin ducts decline when fire is excluded. Therefore, low-severity fire can trigger a long-term induced resistance that may increase tree survival from subsequent herbivory. Lastly, I investigated the consequences of removing fire from fire-dependent ponderosa pine forests on resistance to a MPB outbreak 10 years after experimental density and prescribed fire treatments. Annual growth and resin ducts increased after thinning, but contrary to results from natural fires, prescribed burning did not affect resin duct production, although slight changes in resin quality that may reduce attack success were observed in the burn-only treatment. MPB caused ~50% tree mortality in the control compared to ~20% in the burn-only, and almost no morality in the thin-only and thin-burn. These results suggest that thinning in fire-excluded stands is the most effective management to reduce MPB. However, the positive effect of natural fire on ponderosa pine defense combined with anticipated changes in regeneration and stand dynamics after fire suggest that fire treatments should not be ruled out as effective means to increase long-term forest resilience to bark beetles.

Acknowledgments

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INTRODUCTION

Plants and insects drive a huge array of ecological interactions in virtually every community on Earth (Strong et al. 1984, Schoonhoven et al. 2005). Ecologists have been fiercely debating the regulation mechanisms of plant and insect populations for the past 50 years when Hairston et al. (1960) first proposed their "Green World Hypothesis" of the importance of top-down regulation and Ehrlich and Raven (1964) countered with the idea that bottom-up regulation via plant defenses drives plant-insect interactions. These ideas spring from the dominant paradigm of equilibrium theory in ecology, in which biotic interactions govern patterns of community diversity and structure (MacArthur and Wilson 1967). The alternative theory of non-equilibrium dynamics and the role of stochastic processes and disturbance regimes in shaping community diversity and structure did not begin to emerge until the late 1970s and 1980s (Connell 1978, Pickett 1980, Sousa 1984, Pickett and White 1985). We now recognize that disturbance is an importance mechanism in the maintenance of species diversity, as it can alter the competitive balance of species in time and space and from fine-to-landscape scales (Turner 2010).

The role of disturbance in driving plant-insect interactions is the focus of this dissertation. I use the definition of Pickett and White (1985) in which a disturbance is "any relatively discrete event that disrupts the structure of an ecosystem, community, or population, and changes resource availability of the physical environment." Wildfire and bark beetles (Coleoptera: Curculionidae, Scolytinae) are two of the largest disturbance agents in temperate coniferous forests worldwide and have interacted for millennia to drive forest composition and structure (McCullough et al. 1998, Parker et al. 2006). I studied the impact of fire on plant defense and bark beetle attack success. Wildfire is a dominant abiotic disturbance (Bowman et al. 2009) that has shaped the evolution of plant adaptions to survive a given wildfire disturbance regime (Keeley et al. 2011, Keeley 2012). Anthropogenic changes to historical fire regimes have altered flammability, changing the intensity, extent, and effects of subsequent fire in regions around the world (Bond and Keeley 2005, Flannigan et al. 2009, Ryan et al. 2013). In many North American

forests, factors such as domestic livestock grazing, road building, cessation of Native American burning, and organized fire suppression since the late 1800s have reduced fire frequency and thus greatly impacted fire-dependent forests reliant on frequent, low-severity fires for persistence on the landscape (Pyne 1982, Keeley et al. 2009). Bark beetles in the genus *Dendroctonus* are dominant biotic disturbance agents, capable of causing massive tree mortality in temperate coniferous forests in North America and Eurasia (Wood 1982, Raffa et al. 2008). These aggressive bark beetles typically occur at endemic population levels, causing limited mortality. However, periodically widespread regional climatic triggers can allow populations to irrupt to outbreak levels during which beetles kill large extents of coniferous forests (Raffa et al. 2008). Relative to previously recorded outbreaks, recent bark beetle outbreaks are more synchronous and are causing more tree mortality over larger areas (Bentz et al. 2009). These higher severity outbreaks have been attributed to direct and indirect effects of climate change and, in some cases, past land management practices (Bentz et al. 2009, Bentz et al. 2010).

My study system was ponderosa pine (*Pinus ponderosa* (Lawson & C. Lawson) var *ponderosa* (C. Lawson). As one of the most broadly distributed conifers in North America, ponderosa pine is of huge ecological and economic importance (Oliver and Ryker 1990). Ponderosa pine is adapted to survive frequent, low-intensity to mixed-severity fire (Brown and Sieg 1999, Sherriff and Veblen 2007). Fire exclusion in ponderosa pine dominated forests during the last century has resulted in increased density and forest composition conversions to shade-tolerant species in many areas (Allen et al. 2002, Keeling et al. 2006, Kolb et al. 2007). Ponderosa pine is host to several phytophagous bark beetle species, including the native mountain pine beetle (*Dendroctonus ponderosae* Hopkins), an aggressive bark beetle that has recently killed trees over 8 million hectares of *Pinus* spp. in the western U.S. and Canada (Meddens et al. 2012). Conifers, particularly species in the Pinaceae, have specialized physical and chemical defenses to impede and kill attacking bark beetles (Franceschi et al. 2005, Seybold et al. 2006). Constitutive resin consists of a complex mixture of terpenoid compounds, many of which are toxic to beetle metabolism and can affect beetle communication (Raffa 2001, Keeling and Bohlmann 2006). Resin is viscous and first

acts as a physical deterrent against attacking beetles by flowing to a wound site to slow entry into the tree and causing suffocation (Seybold et al. 2006). Resin is produced in specialized cells surrounding axial and radial resin ducts in the secondary xylem, phloem, and bark, and secreted into the ducts to form an interconnected storage network that enables resin to flow to an attack or wound site (Lewinsohn et al. 1991). Upon attack, many conifers rapidly alter the chemical composition of resin to become more toxic to bark beetles and to compartmentalize symbiotic fungi introduced during attack (Paine et al. 1997, Six and Wingfield 2011), increase *de novo* resin production, and form axial traumatic resin ducts in secondary xylem to increase connectivity within the resin duct system (Lewinsohn et al. 1991, Hudgins and Franceschi 2004). Beetles can overcome host defenses through pheromone-mediated cooperative behavior, in which beetles mass attack trees to overwhelm defenses by boring through the bark into the phloem (Berryman et al. 1989). Once defenses are depleted, they mate, deposit eggs and inoculate the tree with fungal symbionts (Six and Wingfield 2011). Larvae then feed on the phloem during development into adults. The combination of phloem girdling during larval feeding and spread of associated symbiotic fungi results in tree death (Paine et al. 1997, Six and Wingfield 2011).

I had three overarching research questions, each of which is a chapter presented here. (1) What are the best predictors of ponderosa pine resin flow and how do these predictors relate to tree growth? (2) Are predictors of resin flow also predictors of mountain pine beetle attack success and are they sensitive to fire and changes to wildfire disturbance regimes? (3) How do management practices affect individual tree defense and scale up to affect forest resistance to a mountain pine beetle outbreak? Together these chapters provide new information on the physiological relationship between tree growth and defense, and on how fire impacts these defenses to ultimately impact forest resistance to bark beetle attacks.

What are the best predictors of ponderosa pine resin flow and how do these predictors relate to tree growth?

Most research on defense responses in conifers focuses on resin flow, an important defense component that is extremely difficult to predict due to high variability. Few have investigated the role of

resin ducts, which produce and store resin, as a measure of defense against bark beetles. We investigated the seasonal relationships between resin flow and axial resin duct characteristics, tree growth, and physiological variables and whether mechanical wounding induces *Pinus ponderosa* (Lawson & C. Lawson) defenses in the absence of bark beetles. Resin flow increased later in the growing season under moderate water stress, and was overall higher in a lower density, more productive stand. The best predictors of resin flow were the resin ducts measures not standardized to annual tree growth rates, resin duct size and total resin duct area, both of which increased with tree growth rates. However, while faster growing trees tended to produce more resin, models of resin flow using tree growth were not statistically significant. Further, standardized measures of resin ducts (duct density and duct area relative to xylem area), decreased with tree growth, indicating that slower growing trees invested more in resin duct defenses per unit area of radial growth, despite a tendency to produce less resin. Mechanical wounding induced ponderosa pine defenses, but this response was slow. Resin flow increased after 28 days and resin duct production did not increase until the following year. These slow induced responses may allow trees to resist future bark beetle attacks. These results indicate that forest management that encourages healthy, vigorously growing trees with larger resin ducts should increase resistance to bark beetles.

Are predictors of resin flow also predictors of mountain pine beetle attack success and are they sensitive to fire and changes to wildfire disturbance regimes?

We hypothesized that non-lethal, low-severity wildfire induces defenses in ponderosa pine to better resist bark beetle attack, and that lack of low-severity fire relaxes tree defense in forests dependent on frequent, low-severity fire. We first compared axial resin duct traits between trees that either survived or died from bark beetle attacks to determine whether resin ducts are a good surrogate for tree defense from bark beetles. Next, we studied axial ducts using tree cores with crossdated chronologies in several natural ponderosa pine stands before and after an individual wildfire and also before and after an abrupt change in fire frequency in the 20th century. We show that trees killed by bark beetles invested less in resin ducts relative to trees that survived attack, suggesting that resin duct-related traits provide resistance

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against bark beetles. We then show low-severity fire induces resin duct production, and finally, that resin duct production declines when fire is excluded. Our results demonstrate that low-severity fire can trigger a long-term induced resistance that may increase tree survival from subsequent herbivory.

How do management practices affect individual tree defense and scale up to affect forest resistance to a mountain pine beetle outbreak?

We investigated susceptibility to mountain pine beetle attack and forest resilience in fire-dependent ponderosa pine forests as a function of stand structure resulting from the absence of frequent, low-severity fire during the 20th century, and subsequent management scenarios to mitigate the negative effects of lack of fire. We capitalized on an existing study of fire and stand density treatments implemented approximately 5 years prior a naturally occurring MPB outbreak to explore how tree-level defense and stand structure contribute to bark beetle attack success and ultimately forest resilience from a natural disturbance. While the effects of forest management on bark beetle attack patterns have been widely studied, virtually no studies with replicated density and fire treatments exist that were subsequently subject to a widespread bark beetle outbreak. This offered a unique opportunity to explore the ecological effects of disturbance interactions with far-reaching management implications for the resiliency of fire-dependent coniferous forests. We hypothesized that the control, fire-excluded treatments would have the least resistance and resilience, followed, in order, by the burn-only, thin-only, and thin-burn treatments. By the end of the outbreak, when the study was conducted, whole-tree physiological measures of water stress and gas exchange did not differ among treatments.. Annual tree growth and resin duct size and production increased after the thinning, with and without burning, and remained higher than the control and burn-only throughout the length of the study, 11 years post-treatment. Resin flow and total monoterpene concentration, including relative concentration of (-)-α-pinene, was lower in the burn-only compared to the control, while the thinned treatments did not differ from any treatments. Mortality from MPB was markedly different between treatments: in the control approximately 50% of the ponderosa pine > 10 cm diameter was killed in the outbreak compared to 20% in the burn-only, and almost no mortality

in the thin-only and thin-burn. The high mortality in the control caused a shift in species dominance to

Douglas-fir (*Pseudotsuga menziesii* [Mirbel] Franco var. *glauca* [Beissn.] Franco). The large Douglas-fir

component in both the control and burn-only due to fire exclusion, coupled with the high pine mortality

from MPB has likely reduced resilience of this forest beyond the ability to return to a ponderosa pine

dominated system in the absence of further disturbance. These results suggest that excluding frequent fire

from this system has greatly decreased resistance from bark beetle outbreaks, but management treatments

that reduce tree density and remove shade-tolerant species can increase resistance to bark beetles.

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CHAPTER 1: *PINUS PONDEROSA* RESIN DEFENSES: CONTROLS ON CONSTITUTIVE RESIN FLOW AND INDUCED RESPONSES

ABSTRACT

Bark beetles in the genus *Dendroctonus* cause widespread tree mortality in coniferous forests worldwide. Constitutive and induced host defenses are important factors in an individual tree's ability to survive an attack and in bottom-up regulation of bark beetle population dynamics, yet actually quantifying defense levels is often difficult. One such example is *Pinus* spp. defense, of which resin flow plays an important role in resistance to bark beetles, but is extremely variable and likely related to specialized resin storage structures. This complicates efforts to predict resin flow and tree resistance based on tree morphology alone. We investigated the relationships between seasonal resin flow and axial resin duct characteristics, tree growth, and physiological variables and whether mechanical wounding induces *Pinus ponderosa* (Lawson & C. Lawson) defenses in the absence of bark beetles Resin flow increased later in the growing season under moderate water stress, and was highest in a lower density, faster growing stand. The best predictors of resin flow were non-standardized measures of resin ducts- resin duct size and total resin duct area, both of which increased with tree growth. However, while faster growing trees tended to produce more resin, models of resin flow using only tree growth were not statistically significant. Further, the standardized measures of resin ducts- ducts, density and duct area relative to xylem area, decreased with tree growth rate, indicating that slower growing trees invested more in resin duct defenses per unit area of radial growth, despite a tendency to produce less resin. Mechanical wounding induced ponderosa pine defenses, but this response was slow. Resin flow increased after 28 days and resin duct production did not increase until the following year. These slow induced responses may allow trees to resist future bark beetle attacks, thus forest management that encourages healthy, vigorously growing trees with larger resin ducts should increase resistance to bark beetle attacks.

INTRODUCTION

Bark beetles in the genus *Dendroctonus* are a major biotic disturbance agent in temperate coniferous forests and cause widespread tree mortality worldwide (Berryman 1972; Raffa et al. 2008; Wood 1982). Tree species susceptible to bark beetles have evolved an extensive system of constitutive and induced defenses in order to resist attacks (Berryman 1972; Franceschi et al. 2005). Although conifer defenses are under genetic control (Keeling and Bohlmann 2006; Roberds et al. 2003; Rosner and Hannrup 2004), they are also affected by climatic and environmental factors, including water availability and physiological status, as well as morphological traits that are responsible for the production and delivery of resin (Rigling et al. 2003). Most research on defense responses in conifers focuses on resin flow, an important defense component that is extremely difficult to predict due to high variability. Few have investigated the role of resin ducts, which produce and store resin, as a measure of defense against bark beetles.

Dendroctonus beetles attack species within the Pinaceae primarily in the genus *Pinus*, but also in *Picea, Larix, and Pseudotsuga*. Beetles attack the main stem of living trees, boring through the bark to mate and deposit eggs in the phloem. Larvae then feed on the phloem during development into adults. The combination of phloem girdling during larval feeding and spread of associated symbiotic fungi results in tree death (Paine et al. 1997; Six and Wingfield 2011). Upon attack, trees use preformed (i.e., constitutive) resin as an important first line of defense to physically flush the beetle from the wound. Additional resin is then induced to prevent the growth of fungal symbionts introduced by the beetle (Paine et al. 1997; Raffa and Berryman 1983; Wallin et al. 2008). Resin consists of a complex mixture of terpenoid compounds, many of which are toxic to beetle metabolism and can affect beetle communication (Keeling and Bohlmann 2006; Raffa 2001). During the endemic stage, when beetle populations are low, bark beetles typically attack smaller diameter, less defended trees. These smaller trees limit beetle reproductive output and restrain population growth due to lower food quality and quantity. However, environmental factors that favor increased beetle reproductive capacity can allow beetles to attack larger trees resulting in rapid increases in population size and subsequent outbreaks (Raffa et al. 2008).

Resin is synthesized and stored in an interconnected network of axial and radial resin ducts in the secondary phloem and xylem (Bannan 1936; Wu and Hu 1997). Because resin is a complex mixture of carbon-rich mono and tri-terpenes, and duct production requires a large number of several different cell types, both resin and the ducts are costly to produce (Gershenzon 1994). As in other plant defenses, these costs could lead to tradeoffs between growth and defense (Strauss et al. 2002). A tree's ability to produce copious amounts of resin upon attack is likely related to the degree of stress imposed by environmental factors and/or competition and the limits imposed by physiological tradeoffs associated with resin production. The growth-differentiation balance hypothesis (GDB) (Herms and Mattson 1992; Loomis 1932; Loomis 1953) is thought to best explain seasonal allocation to growth and resin flow in conifers. This hypothesis provides a physiological explanation for how trees can reduce the cost of defense and exhibit positive correlations between growth and defense. It predicts that any resource limitation that reduces growth more than photosynthesis (e.g. water, nutrients) will increase carbohydrates available for differentiation-related processes such as defense with little-to-no tradeoff to growth (Herms and Mattson 1992; Stamp 2003). Following this logic, resin flow is predicted to increase under moderate water stress, when growth is more limited than photosynthesis, but to decline under severe water stress when carbon assimilation by photosynthesis is insufficient to meet carbon demands, including resin production.

Several studies in pines have found support for the GDB hypothesis (Gaylord et al. 2013; Gaylord et al. 2007; Lombardero et al. 2000; Lorio 1986; Novick et al. 2012; Wilkens et al. 1998). If resin production does not overlap in time with growth demands, defense costs decrease and no trade-off may occur. In this case, vigorous trees with higher photosynthetic potential may be better defended, as net carbon gains during periods of moderate water stress when growth is limited are expected to be higher. This may explain why suppressed trees are less defended and preferentially attacked by beetles during endemic stages, despite their lower growth rates (Boone et al. 2011). It may also explain why resin production tends to be higher in more productive sites where trees experience higher growth rates (McDowell et al. 2007).

Characterizing the defensive potential of trees is complicated by the fact that resin flow is extremely variable (Gaylord et al. 2011; Gaylord et al. 2007). This variability is due to a variety of factors, including season, temperature, site fertility, age, tree growth, stand dominance, wounding, and disturbance (Blanche et al. 1992; Knebel et al. 2008; Lombardero et al. 2000; Novick et al. 2012; Perrakis and Agee 2006). Because of the variability, there is interest in determining if resin ducts are surrogates for defense potential against bark beetles because duct measurements are repeatable and can be measured in the annual rings of trees to determine annual changes in defense. Recent work in several *Pinus* species has shown that trees with higher resin duct production and/or larger ducts are more likely to survive beetle attacks (Ferrenberg et al. 2014; Gaylord et al. 2013; Hood et al. In Press; Kane and Kolb 2010), indicating that resin ducts are related to defense. Resin ducts are an important component of the *Pinus* defense system, as they represent the production and storage capacity of resin in the tree. However, the variable of interest is the actual constitutive resin flow that occurs upon attack. While several studies have shown positive correlations between resin duct production and density and resin flow (Blanche et al. 1992; Lombardero et al. 2000; Rodríguez-García et al. 2014), there are numerous ways to quantify ducts (e.g. duct production, duct size, duct density, total duct area). Inconsistencies in reporting duct variables and lack of information as to how each specific variable relates to resin flow often make it difficult to compare studies and draw broader inferences.

Tradeoffs exist between constitutive and induced defense responses in plants (Kempel et al. 2011), which are thought to be based upon the probability and severity of an insect attack (Karban et al. 1999). Species in the *Pinus* genus have the most developed constitutive defense structures among conifers (Bannan 1936; Wu and Hu 1997) and relatively low induced responses (Lewinsohn et al. (1991). Induced resin production and timing in *Pinus* is variable and species-specific (Cannac et al. 2009; Knebel and Wentworth 2007; Lombardero and Ayres 2011; Lombardero et al. 2006; Perrakis and Agee 2006; Ruel et al. 1998). In ponderosa pine, Gaylord et al. (2011) did not find increases in induced resin shortly after wounding. In addition to *de novo* resin production from existing resin ducts, the production of new resin

ducts in pines has been shown to increase after mechanical (Fahn and Zamski 1970; Rodríguez-García et al. 2014) or fire-induced wounding (Hood et al. (In Press). These results suggest variable induction responses that require additional research to characterize.

Here, we investigated seasonal variability of constitutive resin flow in ponderosa pine (*Pinus ponderosa* (Lawson & C. Lawson) var *ponderosa* (C. Lawson) from two stands in western Montana, USA with different stand densities, and explored the relationship between resin flow and growth, water stress, and resin duct variables. Resin ducts can be measured and reported in several different ways, but there has been little research on which variable is most informative as a defense trait. We also examined if mechanical wounding induces ponderosa pine resin flow and the formation of resin ducts. We used ponderosa pine as this species has broad geographic distribution and is susceptible to attack from multiple bark beetle species, including mountain pine beetle (*Dendroctonus ponderosae* Hopkins) and western pine beetle (*D. brevicomis* LeConte) in the northern part of its range. Consistent with previous results, we hypothesized that: 1) constitutive resin flow would be higher later in the growing season when limited water availability reduced tree growth but allowed continued photosynthesis, and that this response would be similar at the two sites. Accordingly, we also hypothesized that 2) regardless of investment to resin ducts relative to wood production, faster growing trees would produce more constitutive resin than slower growing trees because trees with larger crown biomass would have greater carbon surplus to invest in resin, and that 3) trees in the lower density, more productive stand would have higher constitutive resin flow. We also hypothesized that 4) constitutive resin flow would be more dependent on variables related to actual duct area and size than to total number of ducts or duct density, as resin is stored in preformed ducts and flow is related to duct diameter as described by Hagen Poiseuille's law (Schopmeyer et al. 1954). We had no *a priori* hypothesis with respect to induced resin flow, but we hypothesized that 5) wounding would induce the production of resin ducts.

MATERIALS AND METHODS

We conducted two main studies in the summer of 2011. The first study was designed to explore the factors that affect resin flow. Previous work has shown resin ducts and flow are strongly heritable traits, but also influenced by fluctuations in environment (Roberds et al. 2003; Rosner and Hannrup 2004). Therefore, we investigated the relationships between defense and growth characteristics in half-sibling trees in genetic field trials to minimize variation in genetic, age, and growing conditions. To explore inducible responses of ponderosa pine we conducted a second study in a natural stand. Previous work has shown the response time of resin induction is highly variable, and may take several months (Cannac et al. 2009; Klepzig et al. 2005; Knebel et al. 2008; Luchi et al. 2005), but in ponderosa pine induction response time has only been followed for 7 days (Gaylord et al. 2011). Little information exists on level of wounding required to induce resin ducts (but see Hood et al. In Press; Rodríguez-García et al. 2014). Therefore, we examined induced resin flow responses for 60 days after mechanical wounding and the impact of measuring resin flow on formation of resin ducts.

STUDY 1 – FACTORS RELATING TO CONSTITUTIVE RESIN FLOW
Site Description

We used two genetic field trials of *Pinus ponderosa* var. ponderosa in western Montana, USA (Condon and Lubrecht; see Table 1 for site characteristics). Our objective was not to examine heritability of measured traits or family differences but to minimize variation in resin flow, resin ducts, and growth due to differences in genetics, age and growing conditions. Trials were established by the Inland Empire Tree Improvement Cooperative in 1974 in western Montana. Seeds were collected from open-pollinated trees in western Montana and Northern Idaho and reared at the USDA Forest Service tree nursery in Coeur d'Alene, ID. One-year-old bare-root seedlings were planted at each site in 1974 on a 3 x 3 m spacing, and containerized 2-year-old seedlings were planted in 1975 to replace mortality during the first year. Sites were level and cleared prior to planting. The Condon stand was thinned in 2003 to favor trees with higher growth and better form by removing one to two trees around each residual tree. The Lubrecht stand was not thinned.

Data Collection and Preparation

In 2011 at each site, we randomly selected six half-sibling families that had at least nine trees per family and randomly assigned three trees from each family to one of three sampling periods: June 27, July 27, and August 22 (18 trees per sampling period). For trees assigned to the June 27 sampling period, we also measured resin flow during the July and August periods (3 times total). Trees assigned to July and August periods were sampled once. We measured diameter at breast height (DBH; 1.37 m above ground), height (m), and live crown base height (m) for each tree in June. During each sampling period we collected 24-hour constitutive resin flow samples on the north and south aspects of each tree at approximately 1.37 m above ground using the methods of Ruel et al. (1998). Briefly, we removed a 2.5 cm circular section of the bark and phloem using an arch punch and measured phloem thickness to the nearest 0.1 mm. We than created a funnel immediately below the tapping site with silicone and attached a 50 ml vial to the tree below the funnel to collect the resin. After 24 hours, we measured the volume of resin to the nearest 0.25 ml.

We collected additional data at Condon to determine how tree physiology influenced resin flow. We measured AM water potential (0700-0900) with a Model 1000 pressure chamber (PMS Instrument Company, Albany, Oregon, USA), photosynthesis, stomatal conductance to quantify water stress and gas exchange at each 2011 sampling period. Gas exchange measurements were done with a LiCor 6400 (LiCor, Lincoln Nebraska), at 1000 µmol m^{-2} s^{-1} light provided by an LED light source. Temperature and CO_2 concentration were maintained at 25°C and 380 µmol mol^{-1}. Branches from the lower third of the crown were collected using a pruning pole and one-year old needles were used for measurements. We were not able to reach the crown for some trees; therefore, sample size was 15 in June and July and 14 in August.

We extracted one, 4.5 mm wide increment core from each tree in July 2013 at Condon and October 2013 at Lubrecht using a manual increment borer to obtain a cross section of wood containing annual rings from 2013 to the tree pith. We used these cores to quantify annual tree growth and resin duct

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production. Cores were collected at approximately 1.37 m above the ground, below and within 3 to 6 cm

of the resin-flow-tapping site. In addition, we collected cores centered directly in the tapping locations on

a portion of trees sampled three times for resin flow at each site in 2012. Because we removed the

cambium when sampling resin flow, growth ceased on the sampling date. This allowed us to determine

when the trees began transitioning to latewood xylem production.

We prepared cores using standard techniques (mounted and sanded until cellular structure was

visible through a binocular microscope). For the cores used to quantify annual growth and resin duct

production, we assigned the correct calendar year to each tree ring (i.e., crossdated) (Grissino-Mayer

2001). We scanned all cores using an Epson platform scanner at 2400 dpi and measured ring widths to the

nearest 0.001 mm using CooRecorder v7.7 (Cybis Elekronik & Data AB, Saltsjöbaden, Sweden). We

measured resin ducts in ImageJ (version 1.46r, National Institutes of Health, USA) to the nearest 1×10^{-7}

mm^2 using the ellipse tool and assigned the calendar year in which each duct formed. We measured ducts

formed from 2001 until 2013 for Lubrecht and from 2001 until 2012 for Condon, as these cores were

collected while the trees were still growing at this site.

Data Analysis

We calculated five resin duct metrics for each core (Table 2). Three of these metrics are capture

annual absolute investment in resin ducts, unadjusted for ring area: (1) mean duct size, calculated as the

mean size of all ducts per annual ring (mm^2); (2) duct production, calculated as the total number of ducts

per annual ring (no. $year^{-1}$); and (3) total duct area calculated as the sum of duct area per annual ring (mm^2

$year^{-1}$). The other two metrics capture the annual investment in resin ducts standardized to ring area: (4)

duct density, calculated by dividing duct production by ring area (ring width x core diameter; no. mm^{-2}

$year^{-1}$), and (5) relative duct area calculated by dividing total duct area by ring area and multiplying by

100 (% annual ring).

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We used raw ring width values and basal area increment as annual growth metrics. We observed no age-related decline in the ring width for the section of the chronology we used for analyses (the most recent 13 years); therefore we did not detrend raw ring width. We calculated basal area increment (BAI; cross-sectional area of secondary xylem produced per year) from ring widths and the tree diameter inside bark using dplR package v. 1.6.0 in R v. 3.0.1 (Bunn 2008).

We performed correlation analyses of resin duct and growth variables for the last 10 years of growth prior to resin flow sampling in 2011 (2001 – 2010) by site and also pooled for both sites. Mean values were calculated for each core (n = 54 at each site) for the analyses.

Resin ducts can remain functional for numerous years (Lewinsohn et al. 1991); thereby potentially contributing to resin flow long after the ducts were formed. To investigate the contribution of previous years' resin ducts to resin flow we created 5- and 10-year independent variables from the resin duct and annual growth variables. We chose 5 and 10 years based on preliminary correlation analysis that showed resin flow was related to total duct area in a given year for 9 of the last 10 years, and also based on previous studies that pooled duct data over numerous years to determine either resin flow or resistance to attack (Blanche et al. 1992; Ferrenberg et al. 2014; Kane and Kolb 2010; Schopmeyer et al. 1954). For duct production, total duct area, ring width, and basal area increment, we summed the values for (1) 5 years (2006-2010) and (2) 10 years (2001-2010) prior to resin flow sampling. We then calculated 5- and 10-year resin duct density and relative duct area from these summed values. For duct size, we calculated the mean duct size for the 5 and 10 year period. We used average resin flow calculated from the north and south aspects of each tree, as aspect did not influence resin flow at either site ($F_{1,193} = 1.18$, $P = 0.2793$) and preliminary analyses showed no improvement in fit if resin flow from only one aspect was used (Supplemental Figure 1).

We used general linear mixed models to examine the relationship of average resin flow to these duct and growth metrics, site, and sampling period, using family as a random effect (SAS Institute v. 9.3,

Cary, NC, USA). One tree at Lubrecht died in July, and resin leaked out of sample vials on other four trees; these were excluded from all analyses of resin flow. Individual trees were the experimental unit. We used a lognormal distribution to stabilize residuals. We applied a Bonferroni correction factor for the 5- and 10-year metrics and considered independent variables with a p-value < 0.025 significant; for all other variables we used a p-value < 0.05 to determine significance.

We then used general linear mixed models to examine the seasonal pattern of resin flow, water stress, and carbon acquisition at Condon. Family nested within month was used as a random effect. Individual trees were the experimental unit. We used a lognormal distribution to stabilize residuals for resin flow. Water potential, photosynthesis, and stomatal conductance were normally distributed. Pairwise differences in sampling period LS means were tested using Tukey's post hoc test (significance level α = 0.05).

STUDY 2 – INDUCIBILITY OF PONDEROSA PINE DEFENSES
Site Description
We investigated the extent to which mechanical wounding induces resin flow and resin duct formation and the timing of induction. We randomly selected 15 dominant or codominant trees between 15 and 23 cm DBH growing in a naturally established ponderosa pine-dominated stand also on the Lubrecht Experimental Forest in June 2011. This second site was necessary because we could not mechanically wound trees tapped for resin flow in the Condon and Lubrecht genetic trials, as these trees are part of a long-term research program.

In addition to the above site, we also used the trees in *Study 1* at both Condon and Lubrecht to investigate whether the minor wounding caused by measuring resin flow induces resin flow later in the season using the trees sampled during all three sampling periods versus only once. We used the cores collected from all trees in *Study 1* to determine if tapping trees to sample resin flow changed resin duct production in the year after tapping.

Treatment Description

We randomly assigned trees to one of two treatments: control (n=6) and wounding (n=9). Trees in the wounding treatment were then randomly assigned an aspect for the wounding (north or south). Wounding was designed to drain the area of constitutive resin in order to measure induced resin production. We generally followed the methods used by Ruel et al. (1998) and Gaylord et al. (2011).We predicted that the unwounded side of the trees could serve as a control to the wounded side due to limited connectivity of the ducts on opposite sides of the tree. Our sampling design allowed us to test this hypothesis by comparing resin flow between wounded and unwounded trees and also between the wounded-side and control-side of wounded trees.

To begin the experiment, we tapped all trees for constitutive resin on 6 July 2011 on the north and south aspect of each tree using the methods described for *Study 1* (Day -1). The following day (7 July 2011; Day 0), 24 hours ± 1 hour after tapping, we measured the amount of constitutive resin to the nearest 0.25 ml. We then wounded the trees in the wounding treatment by using a chisel to remove 1.5 cm tall strips of bark and phloem 20 cm above and below the constitutive resin flow site on trees in the wounding treatment. Strips were 40% of the circumference of the tree bole, centered from the resin flow site (Supplemental Figure 2). After wounding we tapped all trees to measure resin flow approximately 3 cm to one side of the first tapping site. On 8 July 2011 (Day 1), we measured resin flow from the 2nd tapping site and re-wounded trees in the wounding treatment by expanding each strip to equal 3 cm. We tapped all trees to measure 24-hour resin flow three additional times: 15 July 2011 (Day 8), 4 August 2011 (Day 28), and 6 September 2011 (Day 61). Tapping sites were always positioned in a horizontal line within 3 cm of one side of a previous tapping site. We assumed resin flow from trees in the control and wounded-control side (unwounded sides of mechanically wounded trees) treatments was constitutive resin for all dates, and resin collected from trees in the wounded-wound side treatment (the wounded side of mechanically wounded trees) after wounding (Day 1 and after) was induced resin.

Data Analysis

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We used general linear mixed models for all analyses of induced defense (SAS Institute v. 9.3, Cary, NC, USA). We used a random statement identifying tree as the experimental unit to account for repeated measurements on the same tree and a lognormal distribution to stabilize residuals for resin flow. To compare resin flow between control, wounded-control, and wounded trees based on day after wounding we modelled resin flow as the dependent variable with day, treatment, and day*treatment as fixed effects. We compared resin flow between trees sampled once versus multiple times at Condon and Lubrecht, using sampling period, sample class (sampled once vs. sampled multiple times), and their interaction as fixed effects.

Lastly, we compared annual resin duct and growth metrics between the ten years prior to sampling resin flow and 1 year after sampling at Condon and for 2 years after sampling at Lubrecht. We first examined if differences existed in resin duct and growth metrics between trees sampled once versus multiple times. Preliminary analyses showed the number of times sampled (1 vs. 3 times) did not differ for any measured resin duct or growth variable the years following tapping ($P > 0.1247$ for all variables; Supplemental Table 1.) Therefore, all trees were included in the analyses and number of times sampled was not included in the final models. Separate analyses by site and metric were performed, with duct production, total duct area, duct size, and BAI as dependent variables and site, period (Before = 10-year average prior to sampling; After = 2012), and site*period as independent, fixed effects. We used a random statement identifying tree as the experimental unit to account for repeated measurements on the same tree. Duct size was normally distributed, but we used a lognormal distribution for all other variables to stabilize residuals and backtransformed values for reporting means. Pairwise differences in sampling period and site LS means were tested using Tukey's post hoc test (significance level $\alpha = 0.05$).

RESULTS

SEASONAL RESIN FLOW AND TREE GROWTH

Resin flow varied among months ($F_{2,87.42}=19.86$, $p<0.001$). As predicted from the GDB hypothesis, both sites exhibited the same seasonal pattern with highest flow in August when water stress

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presumably began limiting growth (Figures 1 and 2). While resin flow was lowest in July relative to August (Condon: p=0.0092; Lubrecht: p<0.0001), June values did not differ from July or August. At Condon, where we collected additional physiological measurements, water potential, photosynthesis, and conductance were significantly lower in August than either June or July (Figure 2). Based on the cores collected directly from the monthly tapping sites, the trees began transitioning to latewood between the July and August sampling periods, indicating that seasonal water stress increased in August.

Trees at Condon had higher growth rates than at Lubrecht and more crown biomass, but similar phloem thickness and crown ratio (Table 3). Following our predictions that faster growing trees should produce more resin, resin flow was higher at Condon compared to Lubrecht (3.3 ml v. 1.3 ml; $F_{1,9.803}$=15.87, p=0.0027; Figure 1, Table 3).

RESIN DUCT METRICS AND TREE GROWTH
Duct production, duct size, and total duct area were positively correlated with ring width and BAI at both sites (Table 4). Resin duct variables standardized to ring area (resin duct density and relative duct area) were negatively correlated with annual growth variables at Condon. At Lubrecht, only resin duct density, but not relative duct area, was significantly negatively correlated with growth. These standardized measures were much more weakly related to growth (Table 4; r=-0.32 to -0.45) than the non-standardized variables (Table 4; r=0.57 to 0.84). Total duct area had the strongest relationship to both ring width (r=0.84, p<0.0001) and BAI (r=0.84, p<0.0001) of all resin duct variables measured. Duct size and duct production were positively correlated at Lubrecht (r=0.34, p=0.0110), but not at Condon (Table 4). Duct size and duct production were positively correlated with total duct area at both sites, but the relationship was stronger for number of ducts (r=0.91, p<0.0001) than duct size (r=0.79, p<0.0001). Relative duct area was only correlated with duct production and duct size at Lubrecht.

RESIN FLOW RELATIONSHIPS TO SITE, TREE GROWTH, AND RESIN DUCT METRICS
Resin flow was significantly higher at the site with faster growing trees (Condon), but growth metrics (e.g. DBH, height, BAI) did not predict resin flow (Table 5). Phloem thickness was the only non-

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duct variable that was significantly and positively related to resin flow (Table 5). Phloem thickness was also positively correlated with DBH (r=0.55, p<0.0001). Site was a significant factor influencing resin flow, with resin flow higher at Condon than Lubrecht for a given growth value. These patterns were similar when examined over average monthly resin flow for trees sampled three times (Figure 3C-D). However, phloem thickness was only weakly positively related to average monthly flow ($F_{1,10}$=4.75; p=0.0543, Figure 3D) .

Of the resin duct variables, only duct size and total duct area were related to resin flow, and these relationships did not depend on site (Table 5). Site was significant for all other resin duct variables, with higher resin flow at Condon than Lubrecht for a given duct or growth value (Figure 3). Mean duct size for both the previous 5 years and previous 10 years prior to resin flow sampling were positively related to resin flow, but only total duct area during the previous 5 years was significant (Table 5). Month was a significant factor in resin flow in all models. These patterns were similar when examined over average monthly resin flow for trees sampled three times. Mean 5 year duct size and total duct area was positively related to resin flow (Figure 3A-B). Resin flow did not differ as a function of site in any of these models of average monthly resin flow (p > 0.05, Figure 3).

INDUCIBILITY OF RESIN FLOW AND RESIN DUCTS
Induced resin flow changed by day ($F_{4,122}$=16.82, p<0.0001), treatment ($F_{2,30.63}$=4.61, p=0.0178), and by day*treatment ($F_{8,122}$=2.17, p=0.0346). There were no differences in resin flow prior to wounding (Day 0, constitutive flow; Figure 4). After wounding, there was no difference in resin flow between any treatment through day 8. Resin flow in the wounded-wound side treatment, however, was higher on day 28 compared to the wounded-control side treatment (3.0 ml vs. 0.08 ml; p=0.0064), but was not different from the control treatment (3.0 ml vs. 1.4 ml; p=0.7789). On Day 61, resin flow generally increased in the wounded treatment, but this increase was not statistically different from the control and wounded-control treatments due to large variation in flow. Resin flow increased throughout the season in all treatments (Supplemental Table 2).

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Tapping trees once vs. three times had no effect on resin flow within the same year at either site (Sample time: Lubrecht $F_{1,47.69} = 1.78$, $p = 0.1887$; Condon $F_{1,50.02} = 0.25$, $p = 0.6221$; Supplemental Figure 3), but did affect duct production the following year. There was annual variation in all duct and growth variables, but variation was not related to resin flow sampling for any variable but resin duct production (Figure 5, only duct size, duct production, and BAI shown). Sampling resin flow increased duct production the year after tapping at both sites and duct production was higher in 2012 (Condon: 5.7±0.3 s.e.; Lubrecht: 6.0±0.4 s.e.) compared to the previous 10-year average (Condon: 4.0±0.2 s.e.; Lubrecht: 2.9±0.2 s.e.) (Figure 5B, $F_{1,99} = 111.51$, $p<0.0001$). The magnitude of increase differed by site ($F_{1,99} = 13.90$, $p=0.0003$). Prior to tapping, resin duct production was higher at Condon than Lubrecht ($p=0.0003$), but there was no difference one year after tapping ($p=0.9265$). At the slower growing Lubrecht site, duct production increased an average of 3.15 ducts per 4.5 mm wide core ($p<0.0001$), while Condon duct production increased 1.7 ducts per 4.5 mm wide core ($p<0.0001$).

DISCUSSION

Constitutive resin flow in pines is the first line of defense against bark beetle attacks, and induced resin flow is significant for minimizing attack effects by compartmentalizing fungal growth and the attacking beetles. However, predicting resin flow as a function of tree size, growth and site characteristics is difficult due to large variability, and has resulted in inconsistency among previous studies (see below). Here we show that the best predictor of resin flow is average resin duct size and total duct area. We also show that mechanical wounding induced ponderosa pine defenses, although this effect was slow (i.e., 28 days).

Perhaps the most consistent pattern reported in the literature is an increase of resin flow later in the growing season, as predicted by the GDB hypothesis (Blanche et al. 1992; Gaylord et al. 2007; Lombardero et al. 2000). Our results are consistent with this pattern. Moderate water stress at the end of summer when carbon demand for growth decreases but photosynthesis continues, presumably causes a shift of carbohydrate allocation from growth to defense (resin production and resin duct formation). Most

ducts occurred in the latewood portion of the annual ring, which began forming in August at both sites, which is concurrent with other studies of resin duct formation (Rigling et al. 2003; Wimmer and Grabner 1997). At both sites resin flow was significantly greater in August than July. Overall, these results suggest that a tradeoff between growth and defense may only occur during periods of active growth, but also that this tradeoff is relaxed as water stress restricts growth more than photosynthesis. However, severe drought should restrict both growth and defense (Dunn and Lorio 1993; Gaylord et al. 2013).

As predicted, we found higher resin flow at Condon, the thinned and more productive (i.e., greater BAI) site, where higher precipitation and lower competition likely resulted in more resources for both growth and defense. Across stands, McDowell et al. (2007) also found the best predictor of ponderosa pine resin flow was basal area increment. However, stand-level effects on resin flow remain unclear, with some studies reporting reduced resin flow after thinning (Lombardero et al. 2000; Zausen et al. 2005) and mixed effects in response to fertilization (see Novick et al. 2012 for summary).

At the individual tree level, however, neither tree size, radial growth, nor crown ratio (an indicator of tree vigor) explained resin flow (Table 5, Figure 3c). Results reported in the literature on the relationship between tree size or growth and constitutive resin flow are also mixed, with some studies finding no relationship (Blanche et al. 1992; Kyto et al. 1998) and others reporting a positive relationship (Gaylord et al. 2007; Kolb et al. 1998; Kyto et al. 1999; Rodríguez-García et al. 2014). Similarly, and in contrast to our results, Rodríguez-García et al. (2014) and Davis et al. (2012), reported a positive relationship between crown ratio and resin flow.

The only growth-related variable with a positive effect on resin flow was phloem thickness (Table 5). Phloem, as the main food source for bark beetles, impacts host suitability and brood production (Amman 1972). Consistent with this and other studies (Davis and Hofstetter 2014; Shrimpton and Thomson 1985; Zausen et al. 2005) we found that phloem thickness was positively related to growth. Again, results in the literature on the relationship between resin flow and phloem thickness are mixed,

with both positive (Davis and Hofstetter 2014) and negative (Zausen et al. 2005) relationships reported. Overall, the conflicting results on the relationship between resin flow and tree and stand level characteristics suggest that resin production is the integrated effect of many factors, which likely vary depending on the species, growing conditions, and site.

In our study the best predictors of resin flow were duct size and total duct area over the past five years. We note that, based on previous studies (Blanche et al. 1992; Hodges et al. 1981), we focused only on axial ducts, and future studies may reveal that radial resin duct area also contributes to resin flow in ponderosa pine as has been shown in other pines (Rodríguez-García et al. 2014). Although resin duct size and area predicted resin flow, resin duct production, density, and relative duct area did not (Table 5). Similar results were reported in *P. sylvestris* (Kyto et al. 1999). However, in *P. taeda*, Lombardero et al. (2000) found a positive relationship between induced, but not constitutive, resin flow and duct production, although the relationship with duct density was negative.

Our results suggest that growth *per se* is not a good predictor of resin flow despite the fact that resin duct production, size and total duct area increased with tree growth (Table 4). Although faster growing trees with more and larger resin ducts tended to produce more resin as expected, this relationship was not statistically significant (Figure 3). The influence of duct size on resin flow in our study is clear in our models on the effect of morphological traits, site and time of season, where the site effect (higher resin flow at Condon, the more productive site) becomes no longer significant when resin duct size is accounted for. That is, resin flow at Condon is greater (Figure 1) because duct size is greater at this site (Figure 3a). The strong effect of resin duct size on resin flow is consistent with theory: flow is proportional to the fourth power of the duct diameter following Hagen-Poiseuille's law for liquid laminar flow within a tube (Schopmeyer et al. 1954). Therefore, for a given total duct area, contribution by fewer larger ducts relative to more, smaller ducts should result in higher resin flow. Many studies of pines have reported increased resin duct production with tree growth as we found (Blanche et al. 1992; Ferrenberg et

al. 2014; Kane and Kolb 2010; Rigling et al. 2003; Rodríguez-García et al. 2014). Fewer studies have quantified resin duct size, but Ferrenberg et al. (2014) also found a strong positive relationship between duct size and radial growth. The negative correlation between growth variables and resin duct density or relative duct area (Table 4) is consistent with other studies (Blanche et al. 1992; Kane and Kolb 2010; Rigling et al. 2003) and indicates that while faster growing trees produce more and bigger ducts relative to slower growing trees overall, proportionally they invest less in resin ducts relative to xylem production.

The mixed responses of resin production as a function of specific resin-duct traits and individual tree- and stand-level factors may be explained by how these factors affect resin duct size and total area. Because ducts synthesize, store, and deliver resin, resin duct size and total duct area are likely better predictors than the investment in resin ducts relative to xylem area. Thus, as our results and those of others suggest (see above), a small tree with a high relative resin duct investment (i.e., duct density or relative duct area) is likely to produce less resin than a larger tree with lower relative resin duct investment because the absolute number of ducts, size and area is larger due to strong correlations with growth. In addition to the existing duct infrastructure to produce resin, producing resin requires resources and the mixed resin flow responses may also reflect the degree to which resources can be allocated to resin production (or the formation of new resin ducts) rather than to growth. This, in turn, depends on the site, species and, as predicted by the GDB hypothesis, the degree to which environmental variables influences growth versus photosynthesis.

The length of time resin ducts remain functional and contribute to resin flow is hard to measure, but our results suggest that it could be for at least 10 years, as indicated by the models predicting resin flow from the 10-year total duct area and size (Table 5). Previous studies of resin ducts and flow have used a wide variety of methods to collect and quantity resin ducts, and some have not reported the number of annual rings used to relate to resin flow, making generalizations difficult. However, numerous studies have shown that resin duct production from the previous 3 to 30 years is associated with tree resistance to

bark beetle attacks (Ferrenberg et al. 2014; Gaylord et al. 2013; Hood et al. In Press; Kane and Kolb 2010). This body of work investigating the role of resin in resistance to bark beetles supports our findings that resin ducts are long-term investments in a tree's defense system.

We found both resin flow and resin ducts are inducible in ponderosa pine, although the response time was slow (Figures 4 and 5). The slow response in induced resin flow (28 days after wounding) is consistent with that in *P. nigra* (Luchi et al. 2005) and with the lack of induced resin flow shortly after wounding (7 days) in ponderosa pine (Gaylord et al. (2011). Both the degree and timing of resin flow induction in pines appears to be very variable, even within a single species as is the case for *P. taeda* (Klepzig et al. 2005; Knebel et al. 2008; Lombardero et al. 2000; Ruel et al. 1998). Wounding by low-severity fire has also been shown to induce resin flow, but again the response time was slow, taking months to increase above unburned tree levels (Cannac et al. 2009; Lombardero et al. 2006; Perrakis et al. 2011). These slow responses are perhaps to be expected, as pines generally have high levels of constitutive defenses and a general tradeoff exists between constitutive and induced defense (Kempel et al. 2011; Lewinsohn et al. 1991; Moreira et al. 2014).

In addition to increasing induced resin flow, we also found that wounding increased resin duct production the following year (Figure 5). This is consistent with mechanical wounding responses by *P. pinaster* (Rodríguez-García et al. 2014), and by ponderosa pine after fire (Hood et al. In Press). Interestingly, the increase in duct production after resin flow sampling was greater at the site with slower growing trees (Lubrecht), which is also consistent with studies showing tradeoffs between growth and induced defense (Kempel et al. 2011). Resin flow and resin duct induction is slow, but these long-lasting induced defenses could affect resistance of surviving trees in the event of an attack in subsequent years.

Constitutive and induced host defenses are important in the ability of an individual tree to survive an insect attack and in bottom-up regulation of bark beetle population dynamics (Raffa et al. 2008). We show here that direct measures of total resin duct area and average duct size, but not tree growth variables

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or relative measures of resin ducts, were predictive of resin flow. Faster growing trees had larger ducts and higher total duct area, which generally led to higher resin flow than slower growing trees. Therefore, although slower growing trees invested more in resin duct defenses per unit area of radial growth (i.e., duct density and relative duct area), faster growing trees had larger ducts and tended to produce more resin. Forest management that encourages healthy, vigorously growing trees should therefore also increase resistance to bark beetles via an increase in resin duct size and duct area.

Table 1. Site description and mean (range) of tree characteristics sampled at Condon and Lubrecht, Inland Empire Tree Improvement Cooperative, Montana, USA.

Variable	Condon n = 54	Lubrecht n = 54
Longitude	-113.7182	-113.4753
Latitude	47.5437	46.8874
Elevation (m)	1123	1260
Year Planted[1]	1974, 1975	1974, 1975
Year Thinned[2]	2003	NA
Original Spacing (m)	3 x 3	3 x 3
DBH (cm)	26.1 (17.3 – 37.1)	19.0 (10.9 – 33.0)
Tree Height (m)	17.4 (12.5 – 21.3)	11.7 (8.2 – 18.9)
Live Crown Ratio (0-1)	0.6 (0.3 – 0.9)	0.6 (0.4 – 0.7)

[1]Bare root seedlings were planted in 1974 and 1-year old seedlings from containers were planted in 1975, making all trees in the trial the same age.

[2]Thinning removed at least one tree from around each residual tree.

Table 2. Resin duct variable name and description.

Variable	Type[1]	Description
Duct size (mm^2)	Unstandardized	mean size of all ducts per annual ring
Duct production (no. $year^{-1}$)	Unstandardized	total number of ducts per annual ring
Total duct area (mm^2 $year^{-1}$)	Unstandardized	sum of duct area per annual ring
Duct density (no. mm^{-2} $year^{-1}$)	Standardized	total number of ducts per annual ring divided by ring area
Relative duct area (% annual ring)	Standardized	Total duct area divided by ring area

[1]Standardized = adjusted for growth rate; unstandardized = unadjusted for growth rate.

Table 3. Mean (standard error) tree growth and resin flow of trees samples at Condon and Lubrecht, Inland Empire Tree Improvement Cooperative, Montana, USA. Different letters within rows indicate significant site differences (α=0.05).

Variable	Condon n = 54	Lubrecht n = 54
DBH (cm)	26.1 (0.71)a	19.0 (0.71)b
Tree Height (m)	17.4 (0.31)a	11.7 (0.31)b
Live Crown Length (m)	9.6 (0.27)a	6.7 (0.27)b
Live Crown Ratio (0-1)	0.55 (0.01)a	0.57 (0.01)a
Phloem Thickness (mm)	2.58 (0.11)a	2.28 (0.10)a
BAI (cm^2)*	11.2 (0.84)a	6.9 (0.51)b
Resin Flow (ml)	3.3 (0.54)a	1.4 (0.22)b

*Average basal area increment (BAI) for most recent 20 years of growth.

Table 4. Correlation matrices of mean annual resin duct and growth variables by site and pooled based previous ten years (2001 - 2010) and 4.5 mm core diameter.

	Duct Size (mm^2)	Total Duct Area (mm^2)	Duct Density (no. ducts year^{-1})	Relative Duct Area (%)	Ring Width (mm)	Basal Area Increment (mm^2)
Condon (n= 54)						
Duct Production	0.26605	0.89180***	0.10658	0.22709	0.68323***	0.62638***
Duct Size		0.65540***	-0.44792**	-0.16711	0.58193***	0.57755***
Total Duct Area			-0.15886	0.06211	0.80941***	0.76663***
Duct Density				0.94442***	-0.50944***	-0.45250**
Relative Duct Area					-0.40080*	-0.34693*
Ring Width						0.93365***
Lubrecht (n= 54)						
Duct Production	0.34366*	0.90517***	0.17030	0.35415**	0.70814***	0.66721***
Duct Size		0.68839***	-0.29660*	0.06777	0.65953***	0.69827***
Total Duct Area			-0.02036	0.26951*	0.84724***	0.84296***
Duct Density				0.92138***	-0.41274**	-0.32389*
Relative Duct Area					-0.18641	-0.08668
Ring Width						0.95426***
Both Sites Pooled (n=108)						
Duct Production	0.50168***	0.91242***	0.14020	0.38736***	0.74304***	0.71345***
Duct Size		0.79107***	-0.27511**	0.14762	0.68108***	0.71611***
Total Duct Area			-0.05619	0.30351**	0.83702***	0.83846***
Duct Density				0.89674***	-0.41797***	-0.33820**
Relative Duct Area (%)					-0.15458	-0.06457
Ring Width						0.94438***

Significant correlations indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$. Duct production = mean number of resin ducts per ring; Duct size = mean duct size (mm^2); Total duct area (mm^2) = sum of annual resin duct area; Duct density (no. ducts mm^{-2}) = number of ducts per ring/ring area; Relative duct area (%) = total duct area per ring/ring area * 100; Ring width (mm) = annual ring width.

Table 5. Relationship of ponderosa pine resin flow with morphological variable, site, and month as indicated by F-values (p-values) and Akaike Information Criterion (AIC) from analyses of covariance by morphological variable. Bolded F-values indicate significant relationship between variable and resin flow ($P < 0.05$ and a Bonferroni correction factor $P < 0.025$ for variables based on 5- and 10-year averages) (n=103).

Morphological Variable[1]		Site	Month	AIC
Duct Production 5	3.60 (0.0610)	**6.74** (0.0111)	**20.88** (<0.0001)	279.80
Duct Production 10	2.99 (0.0874)	**9.15** (0.0033)	**20.66** (<0.0001)	281.68
Duct Size 5	**11.48** (0.0011)	2.46 (0.1196)	**23.63** (<0.0001)	258.84
Duct Size 10	**6.92** (0.0101)	4.42 (0.0384)	**21.74** (<0.0001)	262.91
Total Duct Area 5	**6.51** (0.0125)	3.64 (0.0597)	**21.91** (<0.0001)	270.78
Total Duct Area 10	5.17 (0.0255)	**5.49** (0.0214)	**21.13** (<0.0001)	273.37
Duct Density 5	0.02 (0.8946)	**15.49** (0.0002)	**19.93** (<0.0001)	276.79
Duct Density 10	0.05 (0.8264)	**15.41** (0.0001)	**19.98** (<0.0001)	275.66
Relative Duct Area 5	1.87 (0.1748)	**11.44** (0.0011)	**20.31** (<0.0001)	276.76
Relative Duct Area 10	2.73 (0.1021)	**8.36** (0.0048)	**20.06** (<0.0001)	274.96
Ring Width 5	1.35 (0.2491)	**9.22** (0.0032)	**20.60** (<0.0001)	281.22
Ring Width 10	1.27 (0.2624)	**11.38** (0.0011)	**20.48** (<0.0001)	282.58
Basal Area Increment 5	1.87 (0.1747)	**8.40** (0.0047)	**20.56** (<0.0001)	294.19
Basal Area Increment 10	1.67 (0.2002)	**9.91** (0.0022)	**20.35** (<0.0001)	295.66
DBH	2.78 (0.0987)	**7.58** (0.0072)	**20.51** (<0.0001)	280.48
Height	0.62 (0.4328)	**5.48** (0.0215)	**20.11** (<0.0001)	281.01
Live Crown Ratio	0.74 (0.3923)	**14.82** (0.0002)	**19.88** (<0.0001)	274.35
Phloem Thickness	**5.15** (0.0257)	**11.06** (0.0013)	**22.27** (<0.0001)	273.91

[1]Morphological variables: 5 indicates variable is based on most recent 5 years (2006-2010) and 10 the most recent 10 years (2001-2010) based on a 4.5 mm core diameter. Duct production = sum of resin ducts; Duct size = mean duct size (mm^2); Total duct area (mm^2) = sum of resin duct area; Duct density (no. ducts mm^{-2}) = number of ducts/ring area; Relative duct area (%) = total duct area/ring area * 100; Ring width (mm) = sum of ring width; Basal area increment (mm^2) = sum of cross-sectional area of secondary xylem produced; DBH (cm) = diameter at breast height, 1.37 m above ground; Height (m) = tree height; Live Crown Ratio (0-1) = (total height – height to base of tree crown)/total height ; Phloem thickness (mm) = average phloem thickness at site of resin flow collection.
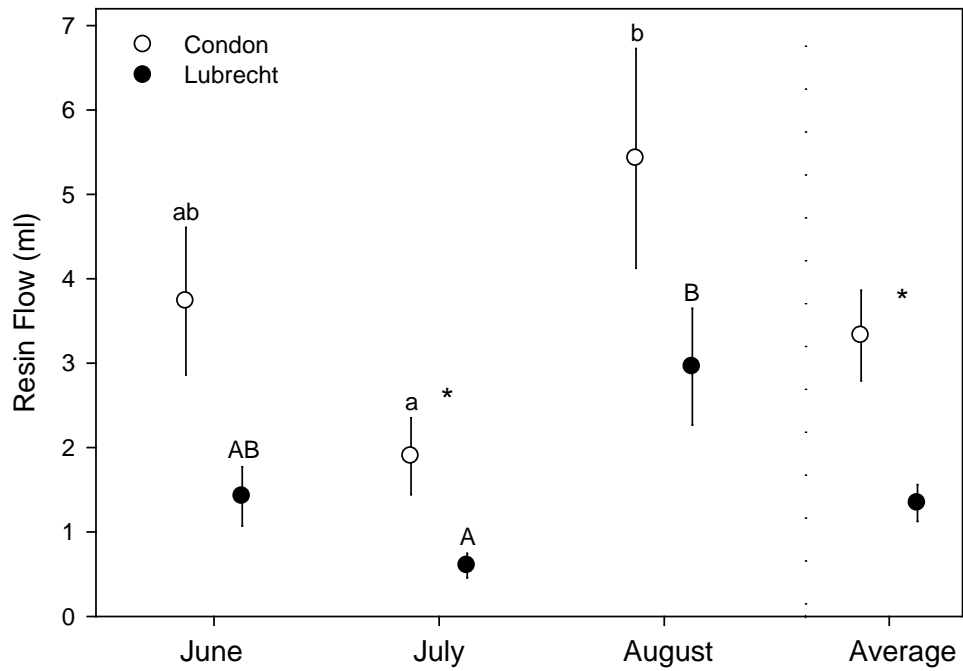
Figure 1. Monthly and seasonal 2011 average resin flow (± std. err.) for Condon and Lubrecht Inland Empire Tree Improvement Cooperative Sites. Only trees sampled one time included in analysis (n = 103). Asterisks denote significant differences (α=0.05) between sites within a given month and for season average. Different letters denote significant differences between months within a site, lowercase = Condon and uppercase = Lubrecht.
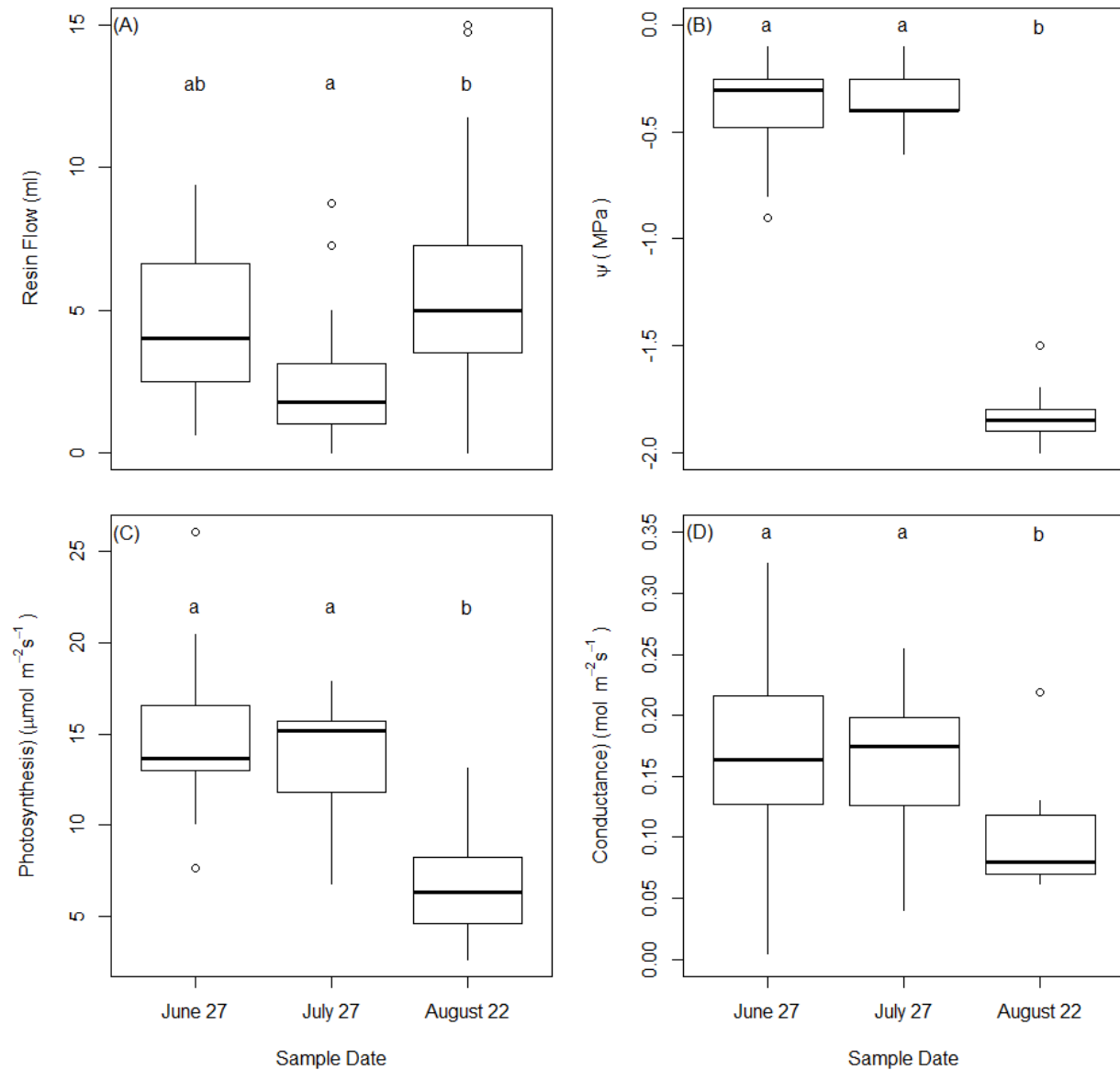
Figure 2. (A) Average monthly 24 – hour resin flow sampled approximately 1.37 m above ground ($F_{2,41}$ = 5.69, P = 0.0066). (B) Average monthly xylem water potential (between dawn and 0700 h) ($F_{2,37}$ = 270.68, $P < 0.0001$), (C) net photosynthetic rate ($F_{2,41}$ = 23.12, $P < 0.0001$), and (D) stomatal conductance ($F_{2,41}$ = 9.05, P = 0.0006) of 1-year old ponderosa pine needles at Condon site. N = 15 (June, July) and N = 14 (August). Different letters within plots indicate month are significantly different (α = 0.05). Boxes denote first and third quartiles, lines the median, and whiskers the 1.5 inter-quartile range (IQR). Circles indicate outliers that > 1.5 X IQR.

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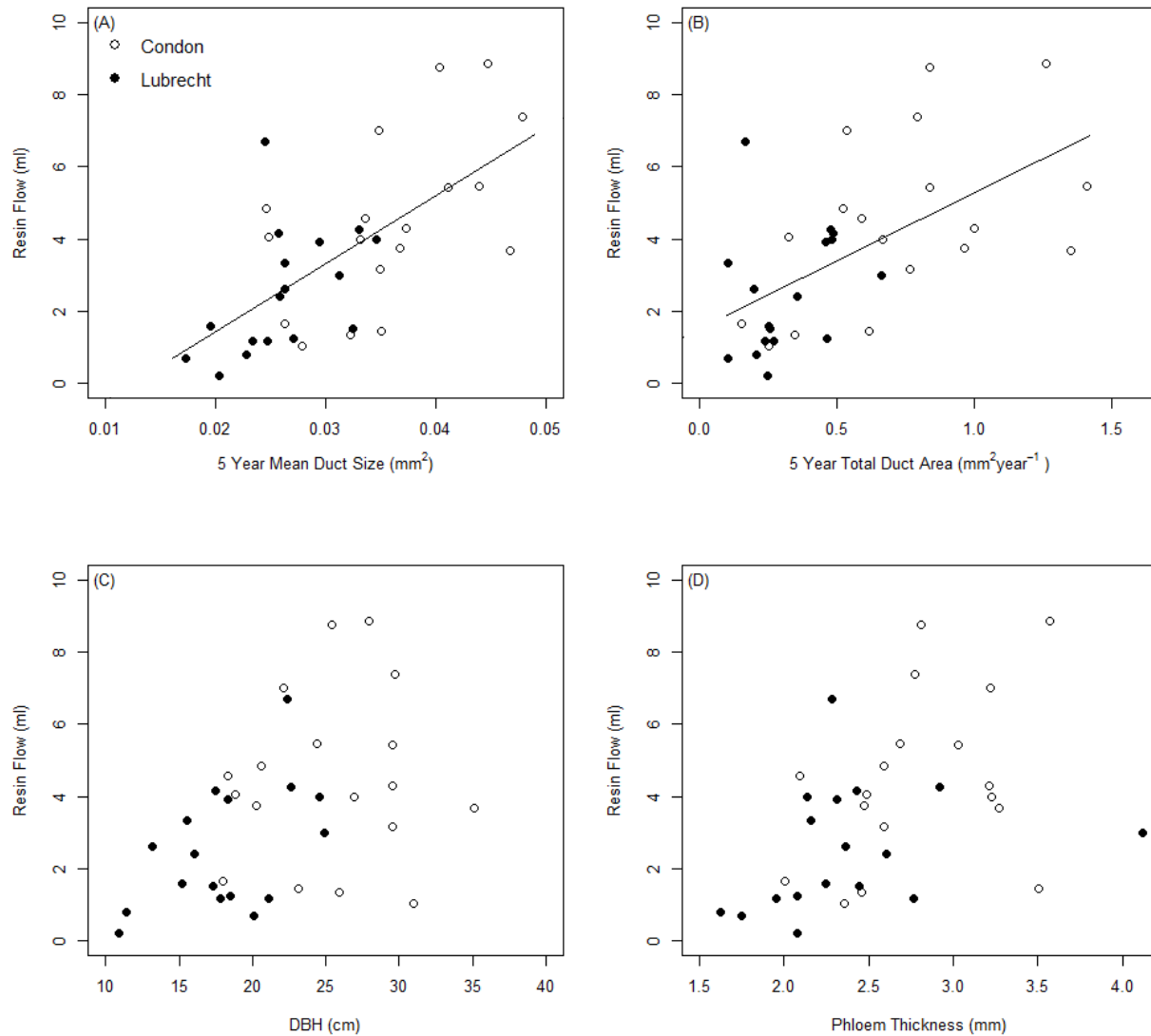
Figure 3. Average monthly resin flow (ml) as a function of (A) 5-year mean duct size ($F_{1,10} = 12.47$, $P = 0.0054$; site: $F_{1,10} = 0.04$, $P = 0.8388$), (B) 5-year total duct area ($F_{1,10} = 7.38$, $P = 0.0217$; site: $F_{1,10} = 0.49$, $P = 0.5002$), (C) DBH ($F_{1,10} = 3.31$, $P = 0.0988$; site: $F_{1,10} = 0.87$, $P = 0.3734$), and (D) phloem thickness ($F_{1,10} = 4.75$, $P = 0.0543$; site: $F_{1,10} = 2.44$, $P = 0.1496$) by site for trees sampled three times total in 2011 (n=35).

36
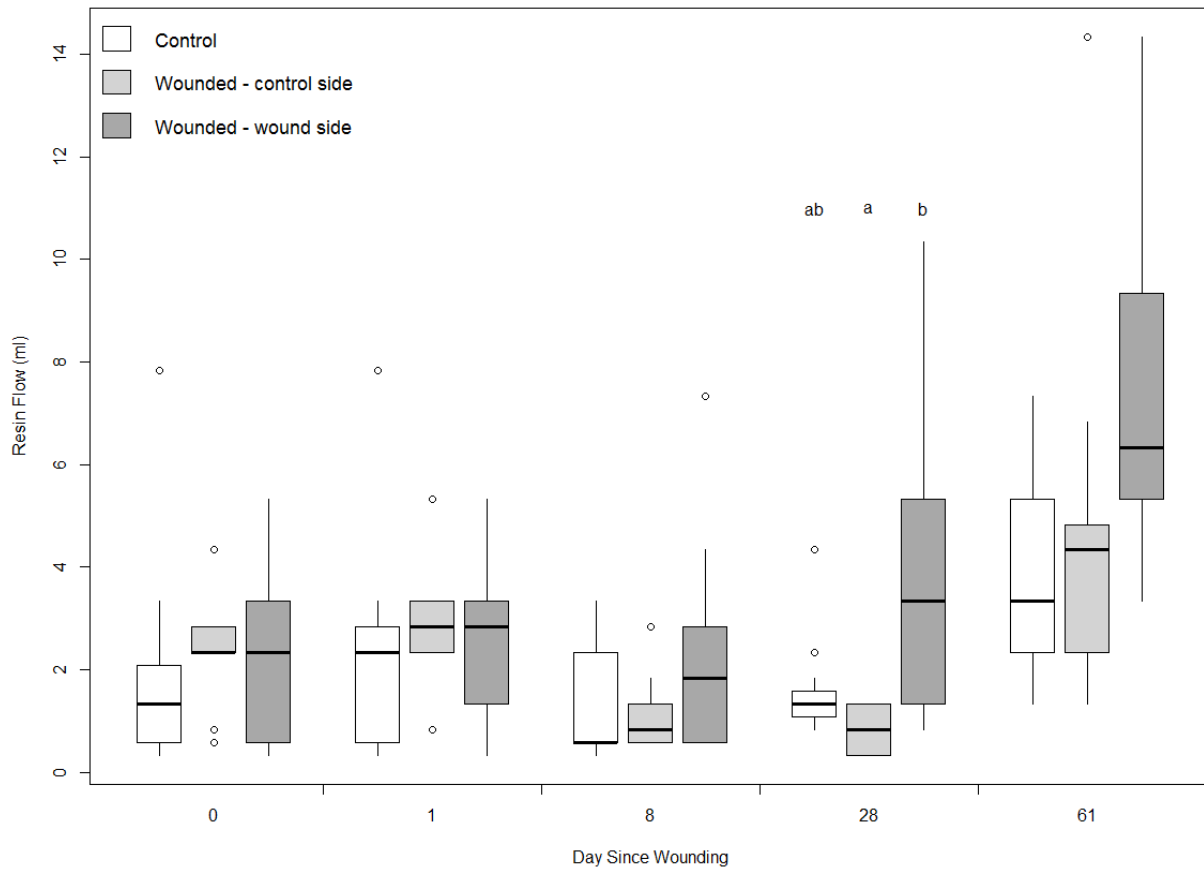
Figure 4. Ponderosa pine 24-hours resin flow by wounding treatment at Lubrecht Experimental Forest, MT. Trees were wounded on Day 0 after measuring constitutive resin flow. Control = tree with no mechanical wounding; Wounded-control side = opposite side of tree with mechanical wounding; Wounded-wound side = side of tree with no mechanical wounding (see *Treatment Description* for additional details). Different letters indicate resin flow is significantly different between treatments within a time step ($\alpha = 0.05$). Boxes denote first and third quartiles, lines the median, and whiskers the 1.5 inter-quartile range (IQR). Circles indicate outliers that > 1.5 X IQR.
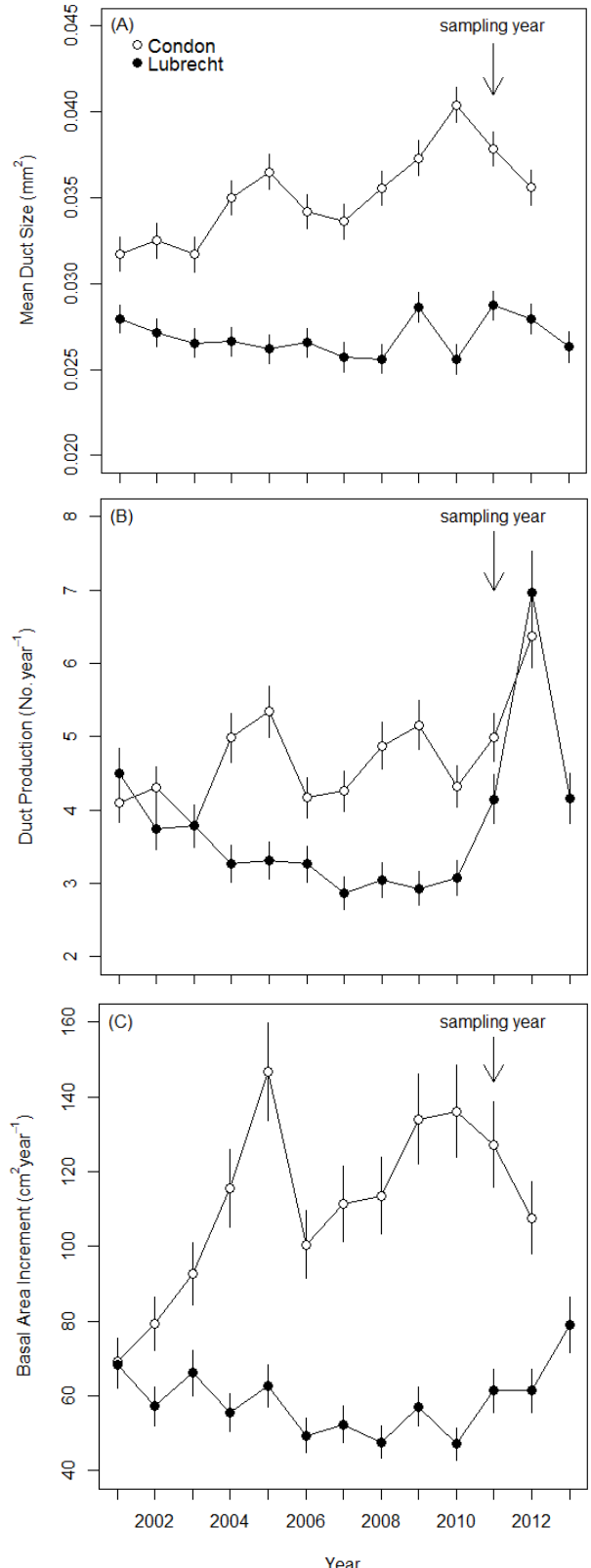
Figure 5. Annual (A) mean duct size, (B) number of ducts, and (c) basal area increment for trees at Condon and Lubrecht. Arrow indicates year of resin flow sampling (2011).

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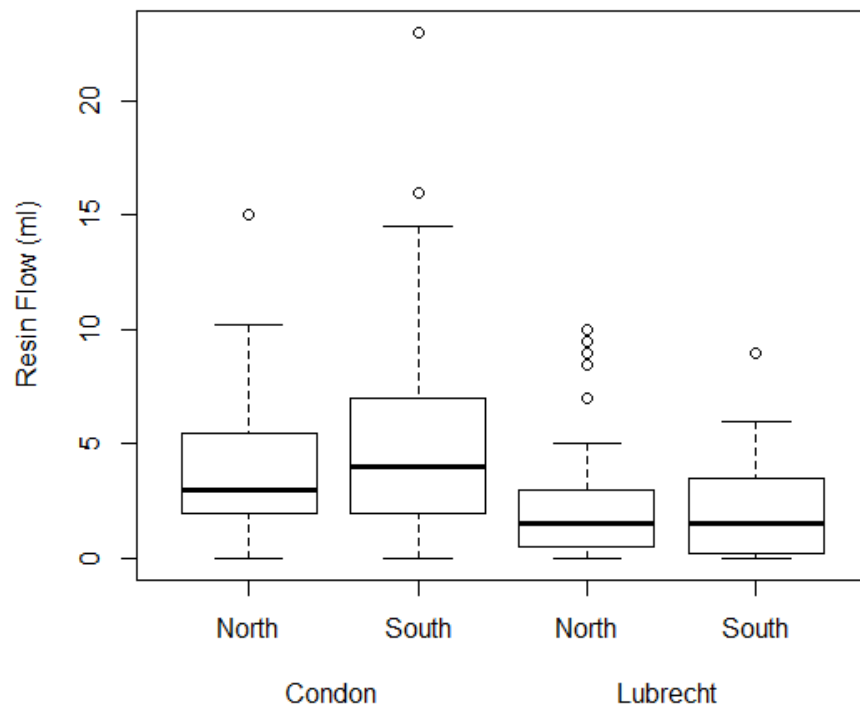
Supplemental Figure 1. Resin flow by site and aspect. Resin flow was different between ($F_{1,193} = 18.24$; P<0.0001) but not by aspect with site ($F_{1,193} = 1.18$; P=0.2793).

Supplemental Figure 2. Tree with mechanical wounding treatment.
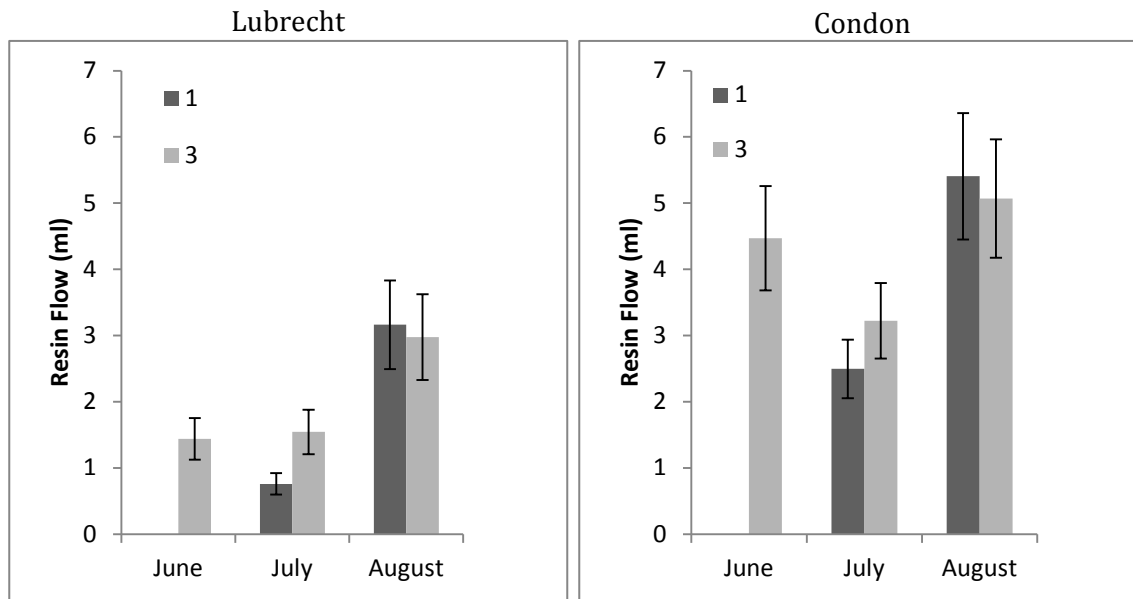
Supplemental Figure 3. Mean resin flow by sampling period and site for trees sampled two and three times compared to trees only sampled once. Resin flow between trees sampled once versus multiple times did not differ at either site (Sample time: Lubrecht $F_{1,47.69} = 1.78$, $P = 0.1887$; Condon $F_{1,50.02} = 0.25$, $P = 0.6221$). Bars are standard errors.

Supplemental Table 1. F-statistics for sampling time (June, July, August) as a function of measured resin duct variables. Sampling time did not affect any measured resin duct variable or growth.

Variable	Lubrecht			Condon		
	DF	F	P	DF	F	P
Duct Production	52	0.79	0.3782	52	2.44	0.1247
Total Duct Area	52	1.01	0.3195	52	1.24	0.2716
Duct Density	52	0.39	0.5341	52	0.63	0.4323
Relative Duct Area	52	1.17	0.2838	52	0.92	0.3428
Duct Size	52	0.66	0.4214	52	0	0.9875
Basal Area Increment	52	1.04	0.3119	52	0.54	0.4638

Supplemental Table 2. P-values and degrees of freedom of treatment and sampling date comparisons of resin flow wounding study. Comparisons in bold are statistically different (α = 0.05).

Treatment	Day since wounding	Treatment	Day since wounding	DF	Adj P
Treatment Comparisons by Sampling Date					
Control	0	Wounded	0	73.67	0.9978
Control	0	Wounded control	0	73.67	0.9619
Wounded	0	Wounded control	0	122	1
Control	1	Wounded	1	73.67	0.9992
Control	1	Wounded control	1	73.67	0.9279
Wounded	1	Wounded control	1	122	1
Control	8	Wounded	8	73.67	0.9949
Control	8	Wounded control	8	73.67	1
Wounded	8	Wounded control	8	122	0.9984
Control	28	Wounded	28	73.67	0.7789
Control	28	Wounded control	28	73.67	0.9109
Wounded	**28**	**Wounded control**	**28**	**122**	**0.0064**
Control	61	Wounded	61	73.67	0.6768
Control	61	Wounded control	61	73.67	1
Wounded	61	Wounded control	61	122	0.9235
Sampling Date Comparisons by Treatment					
Control	0	Control	1	122	1
Control	0	Control	8	122	1
Control	0	Control	28	122	1
Control	**0**	**Control**	**61**	**122**	**0.0305**
Control	1	Control	8	122	0.9789
Control	1	Control	28	122	1
Control	1	Control	61	122	0.2536
Control	8	Control	28	122	0.988
Control	**8**	**Control**	**61**	**122**	**0.0033**
Control	28	Control	61	122	0.2103
Wounded	0	Wounded	1	122	1
Wounded	0	Wounded	8	122	1
Wounded	0	Wounded	28	122	0.9607
Wounded	**0**	**Wounded**	**61**	**122**	**0.0035**
Wounded	1	Wounded	8	122	0.9993
Wounded	1	Wounded	28	122	0.9996
Wounded	**1**	**Wounded**	**61**	**122**	**0.0264**
Wounded	8	Wounded	28	122	0.774
Wounded	**8**	**Wounded**	**61**	**122**	**0.0006**
Wounded	28	Wounded	61	122	0.3258
Wounded control	0	Wounded control	1	122	0.9999
Wounded control	0	Wounded control	8	122	0.7754
Wounded control	0	Wounded control	28	122	0.182
Wounded control	0	Wounded control	61	122	0.7334
Wounded control	1	Wounded control	8	122	0.2197
Wounded control	**1**	**Wounded control**	**28**	**122**	**0.0166**
Wounded control	1	Wounded control	61	122	0.9953
Wounded control	8	Wounded control	28	122	0.9998
Wounded control	**8**	**Wounded control**	**61**	**122**	**0.0057**
Wounded control	**28**	**Wounded control**	**61**	**122**	**0.0001**

CHAPTER 2: LOW-SEVERITY FIRE INCREASES TREE DEFENSE AGAINST BARK BEETLE ATTACKS

ABSTRACT

Induced defense is a common plant strategy in response to herbivory. Although abiotic damage such as physical wounding, pruning, and heating can induce plant defense, the effect of such damage by large-scale abiotic disturbances on induced defenses has not been explored, and could have important consequences for plant survival facing future biotic disturbances. Historically, low-severity wildfire was a widespread, frequent abiotic disturbance in many temperate coniferous forests. Native *Dendroctonus* and *Ips* bark beetles are also a common biotic disturbance agent in these forest types and can influence tree mortality patterns after wildfire. Therefore, species living in these disturbance-prone environments with strategies to survive both frequent fire and bark beetle attack should be favored. One such example is *Pinus ponderosa* forests of western North America. These forests are susceptible to bark beetle attack and frequent, low-severity fire was common prior to European settlement. However, since the late 1800s frequent, low-severity fires have greatly decreased in these forests. We hypothesized that non-lethal, low-severity wildfire induces defenses in *P. ponderosa* to better resist bark beetle attack, and that lack of low-severity fire relaxes tree defense in forests dependent on frequent, low-severity fire. We first compared axial resin duct traits between trees that either survived or died from bark beetle attacks. Next, we studied axial ducts using tree cores with crossdated chronologies in several natural *P. ponderosa* stands before and after an individual wildfire and also before and after an abrupt change in fire frequency in the 20th century. We show that trees killed by bark beetles invested less in resin ducts relative to trees that survived attack, suggesting that resin duct-related traits provide resistance against bark beetles. We then show low-severity fire induces resin duct production, and finally, that resin duct production declines when fire is excluded. Our results demonstrate that low-severity fire can trigger a long-term induced resistance that may increase tree survival from subsequent herbivory.

INTRODUCTION

Plant survival depends on the ability to accurately sense and respond to environmental cues. In addition to daily and seasonal stress caused by temperature, drought, and nutrient limitation, plants must also cope with periodic disturbance events and attacks by pathogens and herbivores. To survive these biotic and abiotic challenges plants often use a dynamic array of induced chemical, physical, and mechanical defenses that can also affect subsequent herbivory (Karban and Baldwin 2007). Because disturbance is common in many ecosystems around the world (Pickett and White 1985), abiotic disturbance-mediated induced defense has the potential to greatly impact subsequent herbivory patterns over large landscape scales. For example, hurricanes increased *Conocarpus erectus* susceptibility to herbivory (Spiller and Agrawal 2003), and wildfire altered foraging pressure from snowshoe hares and pine squirrels, thus indirectly driving regional-scale variation in tree defense in members of the Pinaceae and *Betula* species (Smith 1970, Bryant et al. 2009). Additionally, disturbance removal decreased allocation to *Acacia* defense (Young and Okello 1998). These studies suggest that the frequency and severity of disturbance via physical wounding can affect plant defense and influence response to subsequent herbivory.

Here, we focus on the effects of low-severity wildfire on tree defense against attack by bark beetles. We use the term low-severity wildfire to describe fires in which the majority of mature trees survive, but trees have varying levels of injury as a result of burning. Wildfire and native bark beetles are dominant disturbance agents in conifer forests of North America, and have interacted for millennia to drive forest composition and structure (McCullough et al. 1998, Parker et al. 2006). However, domestic livestock grazing, road building, cessation of Native American burning, and organized fire suppression since the late 1800s have greatly reduced low-severity fires in many western North American forests (Pyne 1982). This makes coniferous forests an ideal system to study how changes in a large-scale, abiotic disturbance may affect plant resistance to herbivory. Historically, bark beetle and fire interactions have been largely studied from the perspective of disturbance-driven changes in stand structure. This body of research is often contradictory and important questions remain unanswered (Jenkins et al. 2014). For instance,

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frequent, low-severity fire maintains low density stands where surviving trees have more resources available for growth and defense compared to dense stands (Schoennagel et al. 2004), thus potentially increasing resistance to bark beetle attacks (Fettig et al. 2007). However, low- and mixed-severity fires are often followed by pulses in bark beetle attacks that result in additional tree mortality (Hood and Bentz 2007, Davis et al. 2012). Importantly, this pulse is short-lived and little evidence exists that fires lead to bark beetle outbreaks (Lombardero and Ayres 2011, Powell et al. 2012). Non-lethal fire injuries could provide a predictable cue to mount an induced defense to better survive attacks following fire, which may explain these fluctuating patterns of post-fire bark beetle attack success. The ability to sense reliable cues of increased herbivory risk and induce defenses may confer a selective advantage over plants that do not respond effectively to these cues (Karban and Baldwin 2007).

Many phytophagous bark beetles (Coleoptera: Curculionidae, Scolytinae) in the genus *Dendroctonus* attack and kill healthy host trees in order to reproduce (Raffa and Berryman 1983, Paine et al. 1997). This strategy creates a complex relationship between insect and host tree. Bark beetles attack conifers and bore through the bark into the phloem, where they mate, deposit eggs and inoculate the tree with fungal symbionts. Larvae feed on phloem as they develop, eventually pupating and becoming adults. This feeding destroys tree transport tissues and kills the host. Trees respond to attacks with constitutive and induced toxic allelochemicals contained in resin that can kill small numbers of attacking beetles. Bark beetles, however, can use tree allelochemicals to produce aggregation pheromones that attract conspecifics, resulting in a coordinated mass attack capable of overwhelming tree defenses. Because of this required cooperative beetle behavior, tree susceptibility is highly related to bark beetle population dynamics. At endemic levels, only physiologically stressed or weakened trees are available hosts. Periodically, populations erupt to epidemic levels, providing sufficient numbers of beetles to overwhelm the defenses of healthy trees (Raffa et al. 2008, Boone et al. 2011).

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Conifers, particularly species in the Pinaceae, have evolved high levels of constitutive and induced defenses to survive challenges from bark beetles (Franceschi et al. 2005). Resin stored in constitutive ducts in the secondary xylem is an important component of the defense system, acting first as a physical deterrent against attacking beetles. An interconnected network of axial and radial resin ducts enables resin to flow to an attack or wound site (Lewinsohn et al. 1991). Upon attack, many conifers rapidly alter the chemical composition of resin to become more toxic to bark beetles and to compartmentalize symbiotic fungi introduced during attack (Paine et al. 1997, Six and Wingfield 2011), increase *de novo* resin production, and form axial traumatic resin ducts in secondary xylem to increase connectivity within the resin duct system (Lewinsohn et al. 1991, Hudgins and Franceschi 2004). In pines, where induced ducts have full resin biosynthesis capability (Lewinsohn et al. 1991), induction of resin ducts after wounding would increase resin storage and production capacity available for subsequent attacks. Therefore, resin ducts are likely an important component of pine defense from bark beetles because they are sites of resin storage, synthesis, and delivery. Indeed, resin ducts are positively correlated with resistance to bark beetles in several pine species (Kane and Kolb 2010, Ferrenberg et al. 2014) and to resin flow (Blanche et al. 1992, Rodríguez-García et al. 2014).

Research to date on fire-induced defense in conifers has largely focused on resin flow as a defense trait. Fire increases resin flow in *Pinus* spp. (Santoro et al. 2001, Wallin et al. 2004, Lombardero et al. 2006, Knebel and Wentworth 2007, Cannac et al. 2009), and this response may last up to 4 years (Perrakis *et al.* 2011). The underlying mechanism for this long-term response is unknown, as are the consequences of increased resin flow on post-fire bark beetle attack success. We speculated that frequent, low-severity fire induces defense by increasing axial resin duct production. As opposed to increases in *de novo* resin flow from pre-existing resin ducts, an increase in resin ducts has long-term consequences for defense because they remain functional for years after forming (Lewinsohn *et al.* 1991). Therefore, trees with more resin ducts should be able to respond to attacks with more resin, due to a larger resin reservoir, and for longer, due to larger biosynthetic capability, compared to trees with fewer ducts. Because trees

retain resin ducts in secondary xylem for centuries and proxies of fire occurrence in the form of fire scars allow dating with annual accuracy, the effect of disturbance on tree defense can be reconstructed to compare resin duct characteristics in historical versus contemporary forests where the frequency of low-severity fires has greatly decreased. We show that trees that survived mountain pine beetle (*Dendroctonus ponderosae* Hopkins) attack produced larger axial ducts and invested more in ducts than trees that died from beetle attack. We also show for the first time that low-severity fire increases ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) defense and that defense is relaxed in the absence of fire. This suggests that frequent, low-severity fire would induce pine defense to allow a heightened defense response against subsequent bark beetle attacks.

METHODS

STUDY SITES

We used five sites to address the following questions (see Appendix A for more detailed site descriptions): 1) Are resin ducts a biologically meaningful trait related to tree resistance against bark beetle attack?; 2) does a single, low-severity fire increase ponderosa pine resin ducts?; 3) does fire exclusion relax resin duct defense? To address these questions, we used increment cores collected either for this study (Lubrecht and Montana sites) or available from previous fire history studies (Idaho, Oregon, and Utah sites) (Keeling et al. 2006, Heyerdahl et al. 2008, Heyerdahl et al. 2011, Heyerdahl et al. 2014) to measure axial resin ducts. Increment cores, cylinders of wood 4 to 5 mm in diameter, spanning from the bark to roughly the pith, were extracted manually with an increment borer to yield a wood cross section containing annual tree rings. We used these cores to quantify annual tree growth and resin duct production. Cores were collected at approximately 1.37 m above the ground at the Lubrecht and Montana sites, at 15 cm from the Utah and Oregon sites, and at 50 cm from the Idaho site. Reconstructing historical fire regimes from tree rings is extremely time intensive. Therefore, using these samples allowed us to choose sites with a range of known fire histories and changes in fire frequency from which crossdated

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cores were already available. General details on fire history reconstruction can be found in Falk et al. (2011).

DATA COLLECTION

<u>1) Are resin ducts related to tree resistance against bark beetles?</u> To test whether resin ducts are related to tree resistance, we sampled ponderosa pine in the University of Montana's Lubrecht Experimental Forest in 2013 in an area with recent mountain pine beetle activity (Appendix A). Beetle activity in the area was high, with between 40 to 72% of ponderosa pine in nearby long-term tree monitoring plots mass attacked and killed between 2005 and 2012. Average tree diameter of mass attacked trees was 29.5 cm (S. Hood, unpublished data). We collected two, 4.5 mm increment cores from each of 15 randomly located pairs of beetle-attacked trees (one that survived and one that died) in unburned stands with no recent history of harvesting. Paired trees were within 10 m and similar in diameter (within $\pm$ 2.5 cm, measured at 1.4 m height). Cored trees had evidence of beetle attack (i.e., pitch tubes), but tree attack density at the time of sampling varied. All dead trees were mass attacked, with copious amounts of pitch tubes and red or fading needles. Unsuccessfully attacked trees had between 5 to 50+ pitch tubes, but had healthy phloem and needles, indicating beetles had attempted to attack the tree, but were not successful. Subsequent crossdating showed all successful attacks occurred between 2008 and 2011.

<u>2) Do resin ducts increase after one fire?</u> We used the Montana and Utah sites to determine if resin ducts increase after fire (Appendix A). The Montana site included an area that burned in a 2003 wildfire. There is no record of prior wildfire at this site for at least 100 years (Gibson and Morgan 2009). In fall 2011, eight full growing seasons after wildfire, we collected 5 mm wide cores from 19 trees within the burn perimeter and also from eight unburned trees growing less than 100 m outside of the burned area. The trees inside the burned area were part of a previous study (Davis et al. 2012), and were known to have experienced, but survived the fire. At the Utah site the majority of trees established between 1850 and 1860 after an 1841 wildfire (WUN plot 15C; Heyerdahl et al. 2011). The next and last wildfire occurred

in 1899. We selected one, 4 mm wide core each from seven trees that had formed a fire scar during the 1899 fire and seven trees that had no scar (i.e., no direct fire wounding).

3) Does fire exclusion relax resin duct defense? We used the Idaho and Oregon sites to determine if resin duct production declined after fires were locally excluded, which we identified as the date of the last recorded fire at each site (Appendix A). Although we lack information on the precise local causes of these abrupt cessations in fire occurrence, they are clearly evident in the fire-scar record (Heyerdahl et al. 2008, Heyerdahl et al. 2014). The Idaho site (called site MB in Keeling et al. 2006; but COV in Heyerdahl et al. 2008) historically sustained frequent, low-severity fires that occurred every 18 years on average (range 3 to 46 years) over a 12 ha area from 1663 to 1919. At this site, we had 5 mm wide cores available from two stands (8 cores per stand) with similar physiography and fire history until a fire in 1919. After this time, there were no fires in one stand, but the other stand burned in 1960 and 1987. This allowed us to compare resin ducts between stands with and without fire since 1919. The Oregon site (LYT plot 18C; Heyerdahl et al. 2014) historically sustained frequent, low-severity fires that occurred every 12 years on average (range 6 to 18 years) over a 0.15 ha area from 1650 to 1864. The last fire occurred in 1864. We selected all 4 mm wide cores collected from trees in the plot with at least 75 years of growth before fire exclusion began (n=7).

Core Preparation and Measurement: We prepared cores collected from the Lubrecht and Montana sites using standard techniques (mounted and sanded until cellular structure was visible through a binocular microscope) and assigned the correct calendar year to each tree ring (i.e., crossdated) (Grissino-Mayer 2001). The cores we obtained from existing studies were prepared similarly (Keeling et al. 2006, Heyerdahl et al. 2008, Heyerdahl et al. 2011, Heyerdahl et al. 2014). We measured ring widths of all cores to the nearest 0.001 mm using an Acu-Gage micrometer and Measure J2X software (version 3.1, Voortech Consulting, Holderness, NH). We scanned all cores using an Epson platform scanner at 1200 dpi for processing in ImageJ (version 1.46r, US National Institutes of Health). We measured axial resin

duct size to the nearest 1 x 10^{-7} mm^2 using the ellipse tool and noted the calendar year in which each duct formed.

We calculated five resin duct metrics for each core. Three of these metrics capture annual absolute investment in resin ducts, unadjusted for ring area: (1) mean duct size, calculated as the mean size of all ducts per annual ring (mm^2); (2) duct production, calculated as the total number of ducts per annual ring (no. year^{-1}); and (3) total duct area calculated as the sum of duct area per annual ring (mm^2 year^{-1}). The other two metrics capture the annual investment in resin ducts relative to ring area: (4) duct density, calculated by dividing duct production by ring area (ring width x core diameter; no. mm^{-2} year^{-1}), and (5) relative duct area calculated by dividing total duct area by ring area and multiplying by 100 (% annual ring) (see Appendix A for years measured by site).

DATA ANALYSIS

We compared resin duct metrics and ring width between paired susceptible and resistant trees (n=15 pairs) at the Lubrecht site based on measurements from the most recent 30 years available (1981-2010). All susceptible trees died between 2009 and 2012, with 9 of 15 trees dying in 2011. Attacks occurred one year prior to death. The last common year of growth for most trees was 2010; therefore, we excluded rings produced after 2010 from the analysis. Resin duct area and production values from the same tree were summed by year to reduce incidence of zeros in the dataset, while ring width values were averaged.

Resin duct production is variable and positively correlated with summer temperature and precipitation (Rigling et al. 2003). Therefore, we examined resin duct variation over time and response time post-fire by calculating departures from average relative resin duct area eight years before and after the single fires at the Montana and Utah sites. For each tree, we calculated the annual departure of total relative resin duct area, where positive departures indicate an increase in duct area for that year compared

to the 16-year core average. Based on our departure analysis, we then compared total resin duct area before and after the single fires.

To test changes in resin duct production due to fire exclusion, we measured resin ducts each year for the entire available time span (1663 – 2006) from 16 trees at the Idaho site and compared periods before and after the last common fire in 1919 (eight trees from each stand). At the Oregon site, we measured resin ducts each year from seven trees 75 years before and after the last recorded fire in 1864 (time span 1795 – 1944). At both sites we included the five years after the last recorded fire in the before exclusion period to account for any fire effect on resin ducts.

We used general linear mixed models for all analyses (SAS Institute v. 9.3, Cary, NC, USA) and applied a zero-adjustment for rings without ducts using a log-link function after Stahel (2002). We standardized all sites to 5 mm wide core diameters for resin duct area and duct production to aid in comparisons between sites. Ring area was used as a covariate in models comparing duct size, total duct area, and duct production to account for correlations between ducts and growth, but not in models of resin duct density and relative duct area, as these variables were already standardized to ring area. Individual trees were the experimental unit. We used a lognormal distribution to stabilize residuals for all variables except duct size, which was normally distributed. Log-normal model estimates were back-transformed for the purpose of reporting mean and standard error. Pairwise differences in categorical variables were tested using Tukey's post hoc test (significance level $\alpha < 0.05$, 1-tailed test).

Total duct area and ring width were strongly and positively correlated at each site, suggesting a common influence of climate on both variables (Fritts 1976, Rigling et al. 2003). Therefore, we investigated the role of climate in resin duct production apart from any effect of fire. For this analysis we used the most recent 30 rings (after 1980, the period of instrumental data) on cores from Idaho, Montana, and Oregon, plus seven cores from younger trees at the Oregon site. Utah data was excluded from analysis, as we did not have resin duct data for this time period. We used PRISM climate data (PRISM

Climate Group, Oregon State University, http://prismmap.nacse.org/nn/, accessed 10/24/13) to relate climate to total resin duct area. Annual monthly total precipitation (PPT, mm), maximum temperature (T_{max}, °C) and 1981-2010 30-year normal PPT and T_{max} were used in the analysis. We used July climate data, as that was the month showing most significant correlations between total resin duct area, PPT, and T_{max} (data not shown) and also because mid-summer is typically when the transition to latewood begins as trees become water stressed. Resin ducts are typically found in the earlywood-latewood transition and latewood (Rigling *et al.* 2003); therefore, conditions in July will likely affect resin duct production. We calculated departure from 30-year normal July PPT and T_{max} by subtracting the 30-year normal values from yearly data. We used mixed model analysis to examine total resin duct area as a function of departure from 30-year normal July PPT and T_{max}.

RESULTS

1) Are resin ducts related to tree resistance against bark beetles? Tree ring area was positively related to duct size, total duct area, and duct production, and was a significant covariate in the models for each of these variables ($P < 0.0001$; Appendix B). After accounting for ring area, trees that survived bark beetle attack had produced approximately 20% larger ducts ($F_{1,14} = 18.25$, $P = 0.0004$) during the previous 30 years than trees that died from attack (Fig. 1A). In addition, resistant trees had approximately 24% more resin duct area per ring and had allocated 15% more area to ducts than susceptible trees (Total Duct Area (mm^2 year^{-1}): $F_{(1,14)} = 9.83$, $P = 0.0037$; Relative Duct Area (%): $F_{(1,14)} = 4.01$, $P = 0.0325$; Fig. 1C-D). Trees did not differ in duct production ($F_{(1,14)} = 1.75$, $P = 0.1038$; Fig. 1B) after accounting for ring area or in duct density ($F_{(1,14)} = 0.03$, $P = 0.4274$; Fig. 1E). In the 30 years before attack, surviving trees tended to grow faster than trees killed by beetles, with approximately 20% wider ring widths than susceptible trees ($F_{1,14} = 2.71$, $P = 0.061$; Fig. 1F).

2) Do resin ducts increase after one fire? Based on our departure analysis, we compared total resin duct area one year before and after the fire for the Montana site and two years before and after the fire for the Utah site (Fig. 2). Total resin duct area was 80% greater after fire at the Montana site (fire x time: $F_{1,25} =$

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7.45, $P = 0.0115$) and 87% greater at the Utah site (fire: $F_{1,12} = 3.6$, $P = 0.0071$), after accounting for ring area (Fig. 2 insets; Appendix C). Patterns of increase following fire were similar at both sites, but timing of response differed slightly (Fig. 2). There was considerable interannual variability at both sites, but the departure direction for the burned and unburned trees was generally in agreement for all years, except after fire. At the Montana site, burned trees had the greatest duct area one year after fire and returned to unburned tree levels the next year, whereas the unburned, control trees showed no corresponding increase. Burned trees at the Utah site showed a two year increase in total resin duct area before returning to unburned tree levels. However, resin duct area the first year post-fire did not differ from unburned tree levels.

Total duct area was positively related to precipitation ($F_{1,65} = 15.68$, $P = 0.0002$) and maximum temperature ($F_{1,65} = 12.79$, $P = 0.0007$) departure from 1981-2010 30-year July normal values (Appendix C), with total duct area per ring increasing with warmer and wetter conditions.

3) Does fire exclusion relax resin duct defense? Duct area decreased by approximately 15% in the period after fire exclusion at both the Idaho (period: $F_{1,14} = 10.6$, $P = 0.0057$) and Oregon (period: $F_{1,4} = 10.07$, $P = 0.0338$) sites (Appendix B). At the Idaho site with no fire since 1919, duct area declined after fire ceased (Fig. 3; $P = 0.0027$), whereas at the site that continued to burn throughout the 20th century there was no change in duct area between the two periods (Fig. 3; $P = 0.9854$). Duct area also declined in the years following fire exclusion at the Oregon site ($P = 0.0338$). Ring area was a significant covariate in explaining duct area at both sites (Idaho Site: $F_{1,4795} = 1177.47$, $P < 0.0001$; Oregon Site: $F_{1,871} = 68.79$, $P < 0.0001$; Appendix C).

DISCUSSION

Our results indicate that resin duct traits are related to tree resistance from beetle attack. Trees that died from beetle attack had smaller axial resin ducts that occupied a lower percentage of ring area relative to trees that survived the attack. This difference was consistent for 30 years prior to attack.

Because ducts remain functional for years and synthesize, store, and conduct resin (Lewinsohn et al. 1991), increases in allocation to ducts results in a long-term increase in a tree's defense capacity against bark beetle attack. Resin ducts are under strong genetic control, but are also a plastic trait influenced by climate and wounding, as shown here and by others (Rigling et al. 2003, Rosner and Hannrup 2004, Rodríguez-García et al. 2014). However, we note that the relationship between resin duct anatomy and resin flow is not fully understood, and flow is likely also influenced by additional factors such as radial resin ducts (Rodríguez-García et al. 2014), not measured in this study.

Our results generally support those of Kane and Kolb (2010) and Ferrenberg et al. (2014), who found a positive association between resin ducts and tree survival in ponderosa pine, limber pine (*Pinus flexilis* James) and lodgepole pine (*P. contorta* Douglas ex Louden). Though these studies reported positive correlations between growth and resin duct production and area, they did not account for ring area when reporting resin duct differences between susceptible and resistant trees. This makes direct comparisons with our study difficult and highlights the need to develop standardized methodology and reporting of resin duct characteristics. Our results go a step further than Kane and Kolb (2010) by pairing attacked resistant and susceptible trees of close proximity and similar size. This reduced potential confounding factors of tree location and size that can influence beetle host selection and behavior (Boone et al. 2011) and ensured that survival was not due to lower likelihood of attack.

Resin duct production was positively related to tree growth for all our sites, suggesting that faster growing trees are more resistant to bark beetles (Appendix B-C). Resistant trees also tended to have faster growth than susceptible trees (Figure 1). However, we also found resistant trees invested more per unit area of xylem in resin ducts compared to susceptible trees (Figure 1D). Kane and Kolb (2010) also found resin duct properties were more important than tree growth for predicting resistance to bark beetles. Several studies have reported positive correlations between resin ducts and tree growth (Rigling et al. 2003, Rosner and Hannrup 2004, Kane and Kolb 2010, Ferrenberg et al. 2014, Rodríguez-García et al.

2014) and between resin yield and growth (Roberds et al. 2003, McDowell et al. 2007). These results suggest that faster growing trees may be more resistant to bark beetles, but as we show, variation in resin duct properties also greatly influences tree resistance.

We show for the first time that low-severity fire increased resin duct defenses in ponderosa pine and that these defenses declined when fires ceased. We suggest this fire-caused stimulation of resin ducts is similar to systemic induced resistance (SIR) (Bonello et al. 2006, Eyles et al. 2010) and results in increased tree survival following bark beetle attack by increasing the potential for resin synthesis and storage in resin ducts (Fig. 4). SIR is a well-defined plant response involving specific defense signaling responses, in which a stimulus amplifies dormant signaling proteins and leads to priming and a more rapid defense response to subsequent attack (Koornneef and Pieterse 2008, Conrath 2011). However, it is not known if fire-induced resistance involves the same signaling mechanisms as SIR and to what degree the response is truly systemic, as opposed to localized to the tree bole. Our results suggest that in forested ecosystems with frequent, low-severity fire regimes, fire-induced resistance may serve to increase defense against bark beetle attack in the long-term. In this way, trees exposed to fire are better able to survive bark beetle attacks if injuries levels are low (Lombardero et al. 2006, Kane and Kolb 2010).

In trees with moderate levels of fire-caused injuries, fire-induced susceptibility may increase mortality from bark beetles (Fig 4). Indeed, immediately following fire, bark beetle-caused tree mortality can be substantial, up to 25% tree mortality beyond the direct effects of fire (Hood and Bentz 2007), and trees with moderate injury levels were more likely to be mass-attacked and killed by beetles compared to trees with low and high injury levels (Davis et al. 2012). Trees with low injuries can quickly mount a defense response. In contrast, high levels of crown scorch and basal cambium kill resulting from mixed- and high-severity fires can limit phloem transport, photosynthetic potential, and reduce resources necessary for compartmentalizing basal wounds and mounting an energetically demanding defense response. These trees with high injury are also poor hosts for bark beetle development due to low

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substrate quality (Powell et al. 2012). The pulse of bark beetle mortality after fire is typically short-lived, persisting only one or two years (Lombardero and Ayres 2011, Davis et al. 2012, Powell et al. 2012). We know of no study showing a long-term bark beetle population increase attributable to fire. This is likely due to a combination of a depletion of susceptible host trees within one to two years after fire and a decline in beetle attack success in trees with fire-induced resistance.

Studies of post-fire tree mortality and bark beetles interactions have also found similar patterns of fire-induced susceptibly followed by fire-induced resistance (Lombardero et al. 2006, Knebel et al. 2008, Lombardero and Ayres 2011). For instance, fire can alter induced resin composition (Powell and Raffa 2011) and temporarily decrease resin flow prior to subsequent increases above pre-fire levels (Lombardero et al. 2006, Lombardero and Ayres 2011). In trees with low levels of fire-caused injury, increases in resin ducts and resin flow, combined with declines in attack success, also suggest a switch from increased susceptibility to increased resistance. Indeed, there is widespread evidence of increased long-term resin flow after fire in several pine species (Santoro et al. 2001, Wallin et al. 2004, Lombardero et al. 2006, Knebel and Wentworth 2007, Cannac et al. 2009, Perrakis et al. 2011). The exact timing of this switch depends on the specific bark beetle and tree species involved, growing season length, and post-fire climate. Initial increases in resin flow could occur by *de novo* resin synthesis in existing ducts and then continue to increase if fire increases resin ducts, as axial resin duct density is positively related to resin flow (Blanche et al. 1992). Therefore, because resin ducts remain functional for years and provide more sites for resin production and storage, the one- or two-year increase in resin ducts after fire that we report is consistent with the long-term increase in resin flow found in previous studies. Rodríguez-García et al. (2014) reported an increase in axial resin ducts after tapping for resin collection in maritime pine (*Pinus pinaster* Aiton) and suggested that this response in pines is a form of SIR.

There was a difference in timing of resin duct increase after fire between the Montana and Utah sites. These site differences may be due to a number of factors, including differences in fire-caused tree

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injury and differences in post-fire climate. We had detailed records of fire occurrence immediately after the fire at the Montana site, but not at the Utah site. The Utah site burned in 1899, making it impossible to know exactly which trees burned within the fire perimeter and the extent of tree injury. While the unburned trees had no fire scars, fire may have burned around some of the trees and stimulated resin duct production without killing cambium, thus causing the increase in ducts in unburned trees one year post fire. Potentially higher wounding levels at the Utah site could have limited wood growth and resin duct formation immediately after a fire, thus delaying response time. Post-fire climate may also have influenced tree response time. At both sites the induction response occurred the year when precipitation, which has been shown to enhance resin duct production (Rigling et al. 2003), was near normal. Annual precipitation at the Montana site was near average one and two years post-fire. At the Utah site, one year post-fire was the eighth driest year on record (between 1895-2012; PRISM Data Explorer, http://prismmap.nacse.org/nn/), while the second year post-fire had near normal precipitation.

Increased resin duct production after fire is likely a direct response to wounding by fire. However, changes in microclimate due to frequent, low-severity fire may also enhance resin duct production via changes in stand structure. Low-severity fire reduces tree density, which reduces water stress in surviving trees, and increases temperatures due to increased solar radiation (Sala et al. 2005). Consistent with the results of Rigling *et al.* (2003), we found that resin duct area was positively correlated with both warmer and wetter conditions and with ring width. Therefore, increases in tree radial growth in response to higher water availability (Fritts 1976), combined with warmer temperatures may also further stimulate resin duct production beyond the effects of fire alone. While favorable environmental conditions may increase duct production, they do not explain the sharp spike in resin ducts observed immediately after fire in our study. Changes in stand structure after fire persist for years. Therefore, if these changes were driving the observed increase in resin ducts we would predict a persistent increase in resin ducts over time rather than a spike after fire we observed. Additional research is needed on both pre-fire and post-fire climate and fire severity feedbacks on resin duct production. Fire severity is increasing in some regions due to climate

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change (van Mantgem et al. 2013). Severe water stress before or after fire may impair tree growth and wound repair, leading to higher tree mortality either due to direct effects of the fire or subsequent bark beetle attacks, which are also impacted by changes in climate (Bentz et al. 2010).

Knowledge of the effects of low-severity fire on tree defense and ensuing stand susceptibility to bark beetle attack is important not only to increase our understanding of the ecological role of frequent fire, but may also have management implications. Uncharacteristically high stand densities are now common in many fire-dependent forests, such as low elevation ponderosa pine forests, as a result of decades of fire exclusion (Keeling et al. 2006, Ryan et al. 2013). Recent increases in fire severity and bark beetle outbreaks have been attributed to these uncharacteristic conditions (Raffa et al. 2008, Stephens et al. 2009). The realization of fire's important role in many forest types has increased efforts to reduce stand densities and the probability of high-severity wildfire in forests that historically sustained low-severity fire regimes, primarily through mechanical thinning, sometimes followed by prescribed burning. Our results demonstrate that low-severity fire can trigger a long-term induced resistance that may increase tree survival against bark beetle attacks. These findings suggest that excluding fire from forests with frequent, low-severity fire regimes might lead to increased bark beetle attack success due to a relaxation of tree defenses. Therefore, the long-term outcomes of burning and thinning may differ as the benefits of thinning alone are limited to those mediated via changes in stand structure and tree physiology, but exclude potential direct effects of fire on tree defense. While our study only examines the effect of fire on ponderosa pine, our findings likely extend to other conifer ecosystems where disturbance is dominated by frequent, low-severity fire and native bark beetles. Our results highlight the need to understand how and by which mechanisms specific disturbances influence long-term tree defense and resistance to bark beetles.

Acknowledgements

Figure 1. Axial resin duct properties (A-E) and ring width (F) (mean ± SE) of resistant versus susceptible ponderosa pine based on the most recent, common 30 calendar years of tree growth (1981-2010). Susceptible trees were successfully attacked by mountain pine beetle between 2008 and 2011 and killed, while resistant trees survived attack. One-tailed significance values indicate susceptible trees have lower duct properties compared to resistant trees. A, B, and C are adjusted means after accounting for ring area as a covariate based on a 5 mm core diameter. D and E are standardized to ring area; therefore, ring area was not a covariate in the model. ns (non-significant) $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
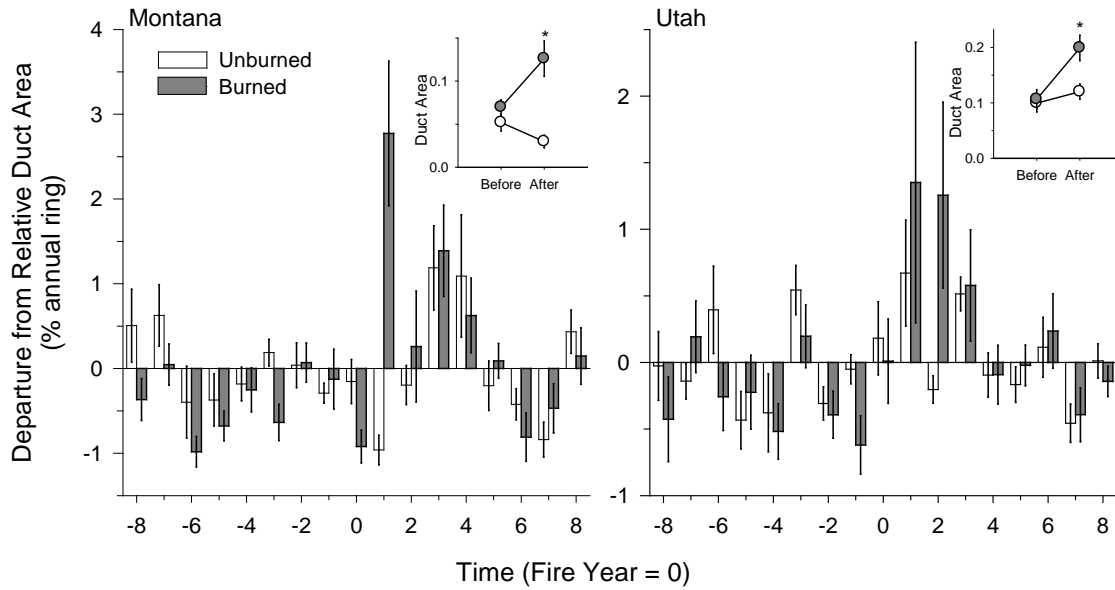
Figure 2. Departure (± SE) from average total axial resin duct area by year for burned and unburned ponderosa pine trees at Montana and Utah sites. Fire occurrence is denoted as time = 0, negative values are years before fire and positive values are years after fire. Insets show total resin duct area (mm^2 $year^{-1}$; adjusted mean ± SE) after accounting for ring area based on 5 mm wide samples. Inset for the Montana site total duct area is 1-year before and after fire, and inset for the Utah site total duct area is the 2nd year before and after fire. One-tailed significance values indicate duct area increased after fire on burned trees * $P \leq 0.05$.
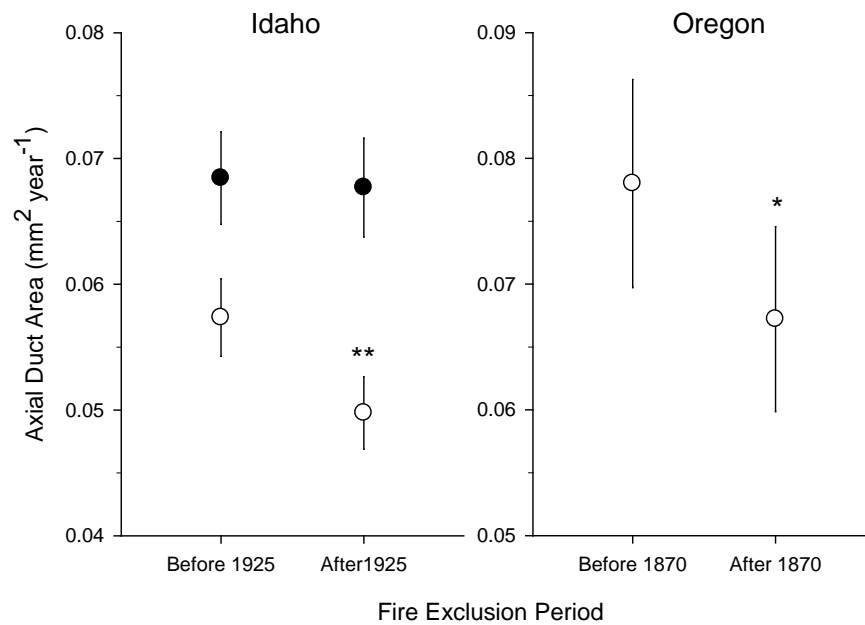
Figure 3. Ponderosa pine axial resin duct area (mm^2 year^{-1}; adjusted mean ± SE) before and after fire exclusion in Idaho and Oregon after accounting for ring area based on a 5 mm core diameter. We defined fire exclusion as the period following the last recorded fire at a site, determined from tree-ring reconstructions. The Idaho site was divided into two areas, o = fires excluded; ● = fires not excluded. One-tailed significance values indicate duct area decreased after fire exclusion * $P \leq 0.05$; ** $P \leq 0.01$.

(A) Low tree injury

Resistance

Fire

Constitutive resistance

Fire

FIRE-INDUCED RESISTANCE
↓ Attack success due to:
↑   Resin duct production
↑   Resin flow
?   Induced resin chemistry

FIRE-INDUCED SUSCEPTIBILITY
↑ Attack success due to:
→   Resin duct production
↓/→ Resin flow
↓   Induced resin chemistry

Tree Death

(B) Moderate – high tree injury

Time
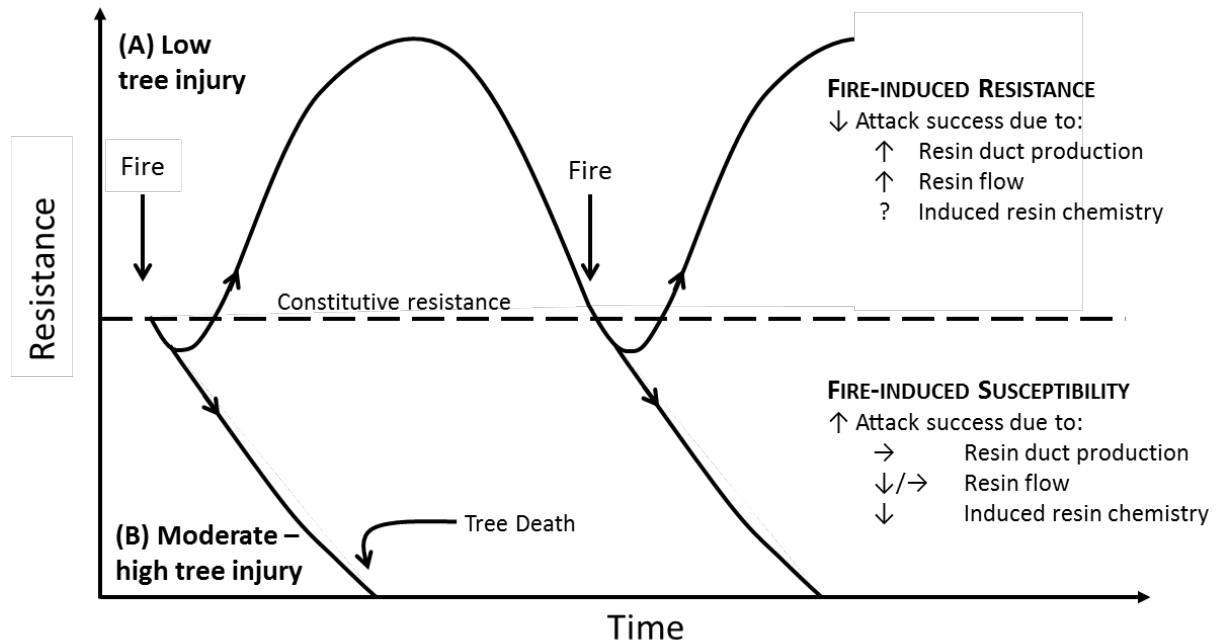
Figure 4. Conceptual model of fire-induced resistance in conifers. (A) For trees with low levels of injury fire causes a brief (days – 1 growing season) reduction in resistance to bark beetle attacks (i.e., Fire-induced Susceptibility) followed by a period of increased resistance that lasts for several years (i.e., Fire-induced Resistance) before returning to constitutive levels as induced resin ducts lose connectively due to annual tree growth. The exact timing and magnitude of this switch is dependent on the specific bark beetle and tree species involved, growing season length, and post-fire climate. (B) Fire increases susceptibility to attack for trees with moderate to high levels of injury and the probability of tree death. Symbols denote the following responses: → no change; ↑ increase; ↓ decrease; ? unknown.

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Appendix A. Location and description of the sites used in each analysis. Cores used in analyses from

Idaho, Oregon, and Utah sites had previously been collected for studies of fire history (Heyerdahl et al.

2008; Heyerdahl et al. 2011, Heyerdahl et al. 2014).

Site Name	Location	Geographic Coordinates	Number of trees	Last Recorded Fire	Years Included in Analysis
Test 1: Resin ducts and tree resistance					
Lubrecht	Lubrecht Experimental Forest, MT	46.8914 -113.434197	30	Not Applicable	1981-2011
Test 2: Duct response to one fire					
Montana	Lolo National Forest, MT	46.8263 -114.1503	27	2003	1995-2011
Utah	Ashley National Forest, UT	40.5419 -110.6390	14	1899	1891-1907
Test 3: Duct response to frequent fire					
Idaho	Frank Church – River of No Return Wilderness Area, ID	45.4155 -115.5185	8 8	1919 1987	1663-2006 1680-2006
Oregon	Ochoco National Forest, OR	44.2810 -120.2992	7	1964	1795-1944
Test 4: Duct response to climate					
Montana	Same as above	Same as above	27	Same as above	1981-2010
Idaho	Same as above	Same as above	16	Same as above	1981-2006
Oregon	Same as above	Same as above	14	Same as above	1981-2008

Appendix B. F-values and significance of models comparing axial duct properties and ring width to the status of paired resistant and susceptible ponderosa pine based on the most recent, common 30 calendar years of tree growth (1981-2010). Susceptible trees were successfully attacked by mountain pine beetle between 2008 and 2011 and killed, while resistant trees survived attack. Models of duct size, duct production, and total duct area include ring area as a covariate. Relative duct area and duct density are standardized to ring area; therefore, ring area is not a covariate in the model.

Effect	Duct Size (mm^2)	Duct Production $(no.\ year^{-1})$	Total Duct Area $(mm^2\ year^{-1})$	Relative Duct Area (% annual ring)	Duct Density $(no.\ mm^{-2}\ year^{-1})$	Ring Width (mm)
Status	**18.25*****	1.75	**9.83****	**4.01***	0.03	2.71
Pair	1.67	1.23	1.62	0.74	0.93	**3.69***
Ring Area	**29.40*****	**159.29*****	**162.33*****	NA	NA	NA

F-values in bold indicate significance effects at *$P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. NA = not

applicable.

Appendix C. Model results comparing total duct area (mm^2 year^{-1}) before and after one fire, before and after frequent fire, and with climate. One fire: Montana and Utah sites, where fire = burned and unburned; time = before fire and after fire; ring area = ring width*increment core diameter. Frequent fire: Idaho and Oregon sites, where fire = fires excluded, fires not excluded; period = before and after fire exclusion; ring area = ring width * increment core diameter. PPT_depart = departure from 30-year normal July precipitation. Tmax_depart = departure from 30-yearnormal July maximum temperature. 30 –year normal = 1981 – 2010.

Effect	DF	F Value	P-value
Duct Response to One Fire			
Montana			
Time	25	0	0.9594
Fire	25	33.04	**<.0001**
Fire * Time	25	7.45	**0.0115**
Ring area	25	17.56	**0.0003**
Utah			
Time	12	10.49	0.0822
Fire	12	3.60	**0.0071**
Fire * Time	12	3.04	0.1065
Ring area	12	6.03	**0.0303**
Duct Response to Frequent, Low-severity Fire			
Idaho			
Fire	14	10.30	**0.0063**
Period	14	10.60	**0.0057**
Fire * Period	14	8.76	**0.0104**
Ring area	4795	1177.47	**<0.0001**
Oregon			
Period	4	10.07	**0.0338**
Ring area	871	68.79	**<0.0001**
Duct Response to Climate			
PPT_depart	65	15.68	**0.0002**
Tmax_depart	65	12.79	**0.0007**
PPT_depar*Tmax_depar	65	1.24	0.2692

CHAPTER 3: FORTIFYING THE FOREST: ROLES OF TREE DEFENSE, FIRE, AND STAND STRUCTURE IN RESISTANCE TO A BARK BEETLE OUTBREAK

ABSTRACT

In areas shaped by multiple natural disturbances, changes in a disturbance regime may cause shifts in species composition, structure and function, and reduce ecosystem resilience to other overlapping disturbances. For example, since the late 1800s the frequency of low-severity fire in many low-elevation coniferous forests in western North America has been greatly reduced relative to the historical fire regime, which was characterized by frequent (<35 years), low-severity fire where the majority of trees survived the fire. This has led to changes in species composition, increases in stand density, decreases of resource availability, and increased susceptibility to forest insect pests. In response, ecosystem management treatments are often implemented to mitigate these negative effects and increase forest resilience. However, whether management treatments increase resilience to disturbance can only be evaluated in the presence of actual disturbance events. We capitalized on an existing replicated study of fire and stand density treatments in a *Pinus ponderosa-Pseudotsuga menziesii* (ponderosa pine-Douglas-fir) forest in western Montana, U.S. that experienced a naturally occurring mountain pine beetle (MPB; *Dendroctonus ponderosae*) outbreak five years after treatment. Treatments included a control, prescribed burn, mechanical thin, and thin-burn. We explored whether treatment effects on tree-level defense and stand structure affected resistance to MPB. We hypothesized that untreated controls would have the least resistance, followed, in order, by the burn-only, thin-only, and thin-burn treatments. Mortality from MPB was highest in the denser control and burn-only treatments, with approximately 50% and 20%, respectively, of ponderosa pine killed during the outbreak, compared to almost no mortality in the thin-only and thin-burn treatments. Thinning treatments, with or without fire, dramatically increased growth and resin ducts (a component of tree defense) relative to control and burn-only treatments. Prescribed burning did not increase resin ducts, but burning alone caused changes in resin chemistry that may have

reduced MPB communication and lowered attack success. While ponderosa pine remained dominant in the thin and thin-burn treatments after the outbreak, the high pine mortality in the control and burn-only treatment caused a shift in species dominance to Douglas-fir. The large Douglas-fir component in both the control and burn-only due to fire exclusion, coupled with the high pine mortality from MPB has likely reduced resilience of this forest beyond the ability to return to a ponderosa pine dominated system in the absence of further fire. This suggests that excluding frequent fire from this system has greatly decreased resistance to bark beetle outbreaks, and that management in fire-dependent pine forests that reduce tree density increase resilience in the short term, while the reintroduction of fire may also be important in the long term.

INTRODUCTION

Forest land management goals often include increasing ecosystem resilience (Hobbs et al. 2014), yet actual management prescriptions rarely examine potential effects from multiple threats of resilience, such as wildfire, insects, disease, or climate change. In ecosystems shaped by frequent natural disturbance, altered disturbance regimes are likely to affect responses to other overlapping natural disturbances, potentially yielding "ecological surprises" (Paine et al. 1998) and reduced ecosystem resiliency (Folke et al. 2004). Limited resources to implement treatments heighten the need to develop treatment prescriptions in the context of historical disturbance regimes from a social-ecological resilience perspective (sensu Folke 2006), in which the focus is on the adaptive capacity of the system to absorb multiple disturbances and the understanding of system feedbacks and cross-scale interactions. Management may also affect ecosystem resistance, an attribute of ecosystem resilience and defined as the "difficulty of changing the system" (Folke et al. 2004).

Wildfire and bark beetles (Coleoptera: Curculionidae, Scolytinae) are two of the largest disturbance agents in temperate coniferous forests worldwide and have interacted for millennia to drive forest composition and structure (McCullough et al. 1998, Parker et al. 2006). As such, they provide an ideal system to study disturbance interactions and how altered regimes of one disturbance may affect resilience

to another disturbance. Anthropogenic changes to historical fire regimes have altered flammability, changing the intensity, extent, and effects of subsequent fire in regions around the world (Bond and Keeley 2005, Flannigan et al. 2009, Ryan et al. 2013). Many North American forests are dependent on fire for persistence on the landscape (Agee 1998). Factors such as domestic livestock grazing, road building, cessation of Native American burning, and organized fire suppression since the late 1800s have greatly reduced fire frequency, with the most striking impacts on ecosystems dependent on frequent, low-severity fire (Pyne 1982, Keeley et al. 2009). Widespread exclusion of wildfire in these areas with historical low and mixed-severity fire regimes poses serious concerns from both an ecological and social viewpoint. Ecologically, many of these forests now have increased fire severity when wildfire does occur due to species composition shifts to shade-tolerant species and increased density (Hanberry 2014), causing such deleterious effects as increased tree mortality and erosion rates and reductions in carbon storage and nitrogen availability (Keeley et al. 2009). Socially, increased fire intensity and severity results in enormous annual costs in fire-fighting expenditures, loss of homes, degraded municipal watersheds, and human health risks from poor air quality (Stephens and Ruth 2005, Westerling et al. 2006, Hurteau and Brooks 2011).

Native bark beetles are the largest mortality agent to North American coniferous forests (Logan et al. 2003), with the majority of tree mortality caused by only a few of the hundreds of North American bark beetle species (Bentz et al. 2010). These aggressive bark beetles typically occur at endemic population levels, causing limited tree mortality due to specialized host tree physical and chemical defenses that reduce attack success (Franceschi et al. 2005, Seybold et al. 2006). Beetles can overcome host defenses through pheromone-mediated cooperative behavior, in which beetles mass attack trees to deplete defenses in order to successfully reproduce (Berryman 1972, Six and Wingfield 2011). Periodically widespread regional climatic triggers can allow populations to irrupt to outbreak levels during which beetles kill large extents of coniferous forests (Raffa et al. 2008). Relative to previously recorded outbreaks, recent bark beetle outbreaks are more synchronous and are causing more tree

mortality over larger areas (Bentz et al. 2009). These higher severity outbreaks have been attributed to direct and indirect effects of climate change and past land management practices (Bentz et al. 2009, Bentz et al. 2010). Land management practices that reduced forest heterogeneity such as widespread harvesting in the early 1900s and fire exclusion also increase host availability and susceptibility to outbreaks (Parker et al. 2006, Bentz et al. 2009).

The US enacted several policies beginning in 2000 providing funding for treatments designed to reduce fire severity and encourage healthy forests resistant to insects and disease, and targeted land with a historic frequent, low-severity fire regime as these areas have been most effected by past wildfire exclusion (Stephens and Ruth 2005). Such treatments often include either mechanical thinning, prescribed burning, or a combination of the two and several recent reviews and meta-analyses describe these treatment alternatives and effectiveness. Modelled fire behavior simulations after treatment suggest that thinning or a combination of thinning and burning is most effective to restore low-severity fire behavior conditions in areas with prior extended periods of fire exclusion (Fulé et al. 2012). Thinning can also be an effective preventive treatment to reduce tree mortality from bark beetles (Fettig et al. 2007, Fettig et al. 2014b), although it is less clear that it can stop bark beetle outbreaks once they begin (Fettig et al. 2014a, Six et al. 2014). By necessity, most of the studies so far test treatment efficacy at increasing resiliency from fire or resistance to bark beetles through modeling exercises. However, modelling fire behavior and bark beetle outbreaks is subject to high uncertainties and limited accuracy assessments (Bentz et al. 1993, Cruz and Alexander 2010, Cruz et al. 2014). Continued improvements to hazard models requires better understanding of the underlying physiological mechanisms that affect higher-order, stand- and landscape-level processes and feedbacks, especially to extrapolate to novel climatic conditions (Heffernan et al. 2014). Yet only a few studies exist examining linkages of treatment impacts on tree physiology and resulting changes in potential susceptibility to future disturbances (Kolb et al. 1998, Wallin et al. 2003, Zausen et al. 2005).

The effects of forest management on bark beetle attack patterns have been widely studied (Fettig et al. 2007, Fettig et al. 2014b). However, most of our knowledge is based on unreplicated, case studies and virtually no studies with replicated density and fire treatments exist that were subsequently subject to an actual widespread bark beetle outbreak. This is because the availability of replicated experimental treatments from which statistical inferences can be made is minimal and also because testing treatment efficacy on minimizing fire severity and increasing tree resistance to bark beetles requires the presence of these disturbances to overlap with replicated, treated experimental areas, a rare combination. Even when a wildfire or a bark beetle outbreak begins in an experimentally replicated treatment area, these disturbances do not typically impact the entire study area due to such factors as abrupt changes in weather conditions or management responses that attempt to stop or mitigate the disturbance (i.e., fire suppression tactics, removal of beetle-attacked trees). This limits statically robust testing of treatment effectiveness to the disturbance (Fulé et al. 2012, Six et al. 2014). These limitations and uncertainties highlight the need for a better understanding of how forest management actions affect forest resilience and resistance from multiple threats (Schoennagel and Nelson 2010).

We capitalized on an existing, replicated study originally designed to increase resiliency of a ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) forest from potential wildfire that later experienced a naturally occurring mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins) outbreak. Treatments included prescribed burning, mechanically thinning, thinning + burning, and a control (no action). Historically, the area burned very frequently, but prior to treatment implementation the study site had not burned in over 100 years (Gundale et al. 2005, Grissino-Mayer et al. 2006). This offered a unique opportunity to explore the underlying tree-level physiological effects and stand-level ecological effects of treatments on resistance to bark beetle attack with far-reaching management implications for the resiliency of fire-dependent coniferous forests from multiple threats. Specifically, we hypothesized that trees in the control treatments would have the least resistance (highest mortality from MPB), followed in order by the burn-only, thin-only, and thin-burn treatments due to higher tree density. We reasoned that

the burn-only treatment would reduce tree density, but not near to historical levels due to the atypically long time since prior wildfire, but confer some increased long-term resistance to bark beetles due to fire-caused stimulation of host-tree defenses (Hood et al. In Press). The thin-only treatment would increase resistance to bark beetles by reducing tree density and the thin-burn would be most resistant due to both decreasing density and the fire-caused stimulation of tree defense.

METHODS

SITE AND TREATMENT DESCRIPTION

Our study site is located on the University of Montana's Lubrecht Experimental Forest in western Montana, US (46°53'N, 113°26'W) and is part of a national program to study the effects of silvicultural methods designed to reduce fire hazard in forests with a historical frequent, low-to-moderate severity fire regime (Stephens et al. 2009). Mean annual temperature is 7°C, ranging from a mean minimum of 0°C and maximum of 13°C. The area receives an average of 50 cm precipitation annually, about half in the form of snow (Fiedler et al. 2010). Our study site is located in a second-growth forest that established after widespread harvesting in the late 1800s to early 1900s. The forest is dominated by ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) and Douglas-fir (*Pseudotsuga menziesii* [Mirbel] Franco var. *glauca* [Beissn.] Franco), with scattered western larch (*Larix occidentalis* Nutt) and lodgepole pine (*Pinus contorta* Douglas ex Louden). Historical mean fire return interval was 7 years (range of 2 to 14 years), but fire frequency greatly declined after 1871 on the experimental forest (Grissino-Mayer et al. 2006). The study site had not burned since the late 1800s, although there was moderate grazing throughout the 1900s (Gundale et al. 2005).

Three, 36-ha blocks consisting of 4, 9-ha units each were established in 2000. Each unit within a block was assigned one of four treatments: control, burn-only, thin-only, and thin-burn (see Gundale et al. 2005, Fiedler et al. 2010 for detailed treatment prescriptions and pre-treatment conditions). In each unit, 10, 20m x 50m plots were established for sampling trees > 10 cm diameter at breast height (DBH; 1.37 m above ground). Control treatments were not manipulated. Thinning and burning treatments were designed

to restore stands towards historical conditions by reducing tree density and encourage seral, shade-intolerant species such as ponderosa pine, while also reducing hazard of stand-replacing wildfire. The goal of all treated units was to create fire-resilient forests in which 80% of basal area of overstory trees would survive a wildfire burning under 80th percentile weather conditions (Fiedler et al. 2010). In the thin and thin-burn units, a low thinning with improvement/selection cutting to favor ponderosa pine ≥ 40 cm DBH reduced average residual basal area to a target of 11 m^2 ha^{-1}. Thinning was conducted in the winter of 2001. Burn-only and thin-burn units were broadcast burned in May and June 2002. The prescribed burns were designed to reduce surface fuel loading and ladder fuels consisting of seedling and saplings.

DATA COLLECTION AND TISSUE PREPARATION
Treatment Resistance to Mountain Pine Beetle Outbreak

Preharvest measurements were completed in 2000, with annual measurements for the first four years post-treatment. These initial treatment effects through 2005 are published (Gundale et al. 2005, Six and Skov 2009, Stephens et al. 2009, Fiedler et al. 2010); hence we only describe data collected between 2005 and 2012 here. In 2005 and 2010 we measured DBH for all trees ≥ 10 cm DBH in each treatment and assessed bark beetle attack status and tree mortality. During the 2010 assessment, we observed increasing MPB activity in many units in conjunction with a large-scale regional outbreak in western Montana (Montana DNRC 2010). Bark beetle activity was low during the first several years after treatment implementation, at least through 2004 (Six and Skov 2009). We therefore sampled the site again in 2012, during which time we also collected additional data to quantify potential treatment differences in tree physiology and defense-related traits to further examine mechanisms associated with stand-level resistance to bark beetles.

We calculated several forest structural and species composition attributes using the tree data (> 10 cm DBH) from the 2005 and 2010 plot surveys, including basal area (m^2 ha^{-1}), density (trees ha^{-1}), quadratic mean diameter (QMD; cm^2) by host (ponderosa and lodgepole pine) and non-host (Douglas-fir and western larch) species for each plot for each time step. Analysis only included living trees. Basal area

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is the cross-sectional area of living trees per hectare. Density is the number of living trees per hectare. QMD is the average diameter of the mean basal area (Curtis and Marshall 2000). We also calculated the percent of host basal area and density per plot for each time step.

Tree Physiology, Growth, and Axial Resin Ducts

We randomly selected three plots in each unit and then one ponderosa pine in each plot in 2012 with no sign of bark beetle attack (n = 3 trees/treatment). Median size of MPB host trees across all units in 2010 was 31.2 cm DBH. Therefore, we chose trees between 27-36 cm DBH to sample representative host trees in the study. Measurements took place from July 17-19, 2012, sampling one block per day. For each tree we sampled 24-hour constitutive resin flow, soil moisture, water potential, and gas exchange. We collected resin flow samples on the west and east aspects of each tree at approximately 1.37 m above ground by removing a 2.5 cm circular section of the bark and phloem using an arch punch. We then created a funnel immediately below the tapping site with silicone and attached a 50 ml vial to the tree below the funnel to collect the resin. After 24 hours we measured the volume of resin to the nearest 0.25 ml. We collected branches between 0600-0830 from the upper one-third of each tree with a 12-gauge shotgun for xylem water potential and gas exchange measurements of one-year old needles. We measured AM water potential with a Model 1000 pressure chamber (PMS Instrument Company, Albany, Oregon, USA) to quantify water stress. Photosynthesis and stomatal conductance were measured with a LiCor 6400 (LiCor, Lincoln Nebraska), at 1000 µmol m^{-2} s^{-1} light provided by an LED light source. Temperature and CO_2 concentration were maintained at 13°C and 380 µmol mol^{-1}, respectively. We were unable to measure gas exchange on four trees due to battery limitations for the LiCor. Soil moisture was measured within 2 meters of each tree base with a HydroSense Soil Water Content Measurement System (Campbell Scientific, Inc. Logan, UT, US).

To quantify annual tree growth and axial resin duct production, we extracted two, 5 mm wide increment core from each tree in October 2013 using a manual increment borer to obtain a cross section of wood containing annual rings from 2013 to the tree pith. Cores were collected at approximately 1.37 m

above the ground, below and within 3 to 6 cm the resin flow tapping site. We prepared cores using standard techniques (mounted and sanded until cellular structure was visible through a binocular microscope) and assigned the correct calendar year to each tree ring (i.e., crossdated) (Grissino-Mayer 2001). We scanned all cores using an Epson platform scanner at 2400 dpi and measured ring widths to the nearest 0.001 mm using CooRecorder v7.7 (Cybis Elekronik & Data AB, Saltsjöbaden, Sweden). We measured resin ducts in ImageJ (version 1.46r, National Institutes of Health, USA) to the nearest 1×10^{-7} mm^2 using the ellipse tool and assigned the calendar year in which each duct formed. We measured ducts formed from 1996 (5 growing seasons pre-treatment) through 2011 (10 growing seasons post-burn and year prior to resin flow sampling).

We calculated basal area increment, duct size, duct production, and total duct area, for each core based on raw ring width values and calculated the average annual value per tree from the two collected cores. We observed no age-related decline in the ring width for the section of the chronology we used for analyses (the most recent 16 years); therefore we did not detrend raw ring width values. We calculated basal area increment (BAI; cross-sectional area of secondary xylem produced per year) from ring widths and the tree diameter inside bark using dplR package v. 1.6.0 in R v. 3.0.1 (Bunn 2008). Resin ducts metrics included: (1) duct size (mean size of all ducts per annual ring (mm^2)); (2) duct production (total number of ducts per annual ring (no. $year^{-1}$)); and (3) total duct area (sum of duct size per annual ring (mm^2 $year^{-1}$)).

Constitutive Resin Flow and Monoterpene Composition

We measured resin flow on an additional 6 to 11 trees in each unit using the same methods as described above to increase sample size (n=128). To quantify constitutive monoterpene composition we collected phloem tissue from 9 to 24 trees in each unit, including all trees sampled for resin flow (n=199). We extracted 2 x 5 cm samples of phloem at approximately 1.37 m high on the east of each tree using a chisel. Tissue was placed in 15 ml plastic vials and stored on dry ice in the field and then in a -80°C freezer in the laboratory until chemical analysis. Chisels were rinsed in 70% ethanol between samples.

We extracted monoterpenes from phloem tissue and analyzed by gas chromatography (gc) using methods based on Powell and Raffa (2011). We placed finely chopped tissue into 2 ml gc vials with 1 ml hexane and agitated for 24 hours. We then filtered the hexane solution through glass wool into a second gc vial, rinsed the first vial with 0.25 ml hexane twice, and added the filtered solution into the second vial for a final volume of 1.5 ml. We added 10 µl 0.01% isobutylbenzene (IBB) to the vial as an internal standard. Phloem samples were dried for one week at 25°C and weighed.

Samples were analyzed using a Hewlett Packard 5890 Series II gas chromatograph with an Agilent Technologies Cyclodex-B column (0.25 mm diameter, 30 m) with helium as the carrier gas. Initial temperature was 60°C for 10 minutes with a 5°C rise per minute until 160°C. We ran seven gc-grade standards (Sigma-Aldrich Co., St. Louis, MO, US) of known ponderosa pine monoterpenes using the method described above to determine retention times and identify peaks of the sample chromatograms. We calculated absolute concentration of each monoterpene (µg) by integrating the area under each peak, dividing by the IBB peak area, and multiplying by the density of IBB.

DATA ANALYSIS

To compare treatment effects on physiology (water stress and gas exchange), growth, and resin duct defenses, we used general linear mixed models with a randomized block design for all analyses. Treatment units were the experimental unit (n=3) and block and block x treatment were random effects (SAS Institute v. 9.3, Cary, NC, USA). For the dependent variables of BAI and resin ducts we used the most recent five year average prior to resin flow sampling (2007-2011). We used a lognormal distribution to stabilize residuals when necessary. Log-normal model estimates were back-transformed for the purpose of reporting mean and standard error. Pairwise differences in categorical variables were tested using Tukey's post hoc test ($\alpha = 0.05$).

We tested for treatment differences in constitutive resin flow and total monoterpene composition using the same mixed model as described above, with a lognormal distribution for all variables. For

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individual compounds, we used a Kruskal-Wallis nonparametric test with Dunn's test for post hoc

multiple comparisons (Elliott and Hynan 2011) due to non-normally distributed data. We used the

average resin flow per tree for the resin flow dependent variable. For monoterpenes, we standardized

monoterpene concentrations by mass of the phloem sample (g) to calculate absolute concentration (μg g^{-1}

phloem) for each compound and summed the concentration of the seven compounds for total

concentration. We then calculated the relative concentration of each compound as percent of the total

concentration.

We then tested for treatment differences in attack success using a Kruskal-Wallis nonparametric

test with multiple comparisons (Elliott and Hynan 2011) due to non-normally distributed data. The

dependent variable was percent host killed (i.e., ponderosa and lodgepole pine), which we calculated by

dividing the number of host trees alive in 2005 by host trees killed by MPB between 2005 and 2012 for

each 0.1 ha Whittaker plot.

Lastly, we examined treatment differences in resistance to the MPB outbreak using both linear

models and ordination. We used a repeated measures general linear mixed model to test within and

among treatment differences in basal area, tree density, and QMD by MPB host species before and after

the outbreak. We specified treatment, year (2005, 2012), MPB host, and the 3-way interaction of

treatment, year, and host as fixed effects, with residuals as random effects and experimental unit as

treatment x block. We used non-metric multidimensional scaling (NMS) using the vegan library (v2.0-10)

in R v3.0.1 to both visualize how treatments had changed in structure and composition after the outbreak

and also to quantitatively test resistance to the outbreak. We used the Bray-Curtis dissimilarity index as

the multidimensional distance measure of plots from 2005 (before outbreak) to 2012 (after outbreak). We

used 2005 and 2012 plot average total basal area, density, DBH, QMD, and the percentages of host and

non-host basal area and density before and after the outbreak to determine how treatments and time

correlated with forest structure. We used stress values as a measure of the goodness-of-fit for the final

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NMS configuration. Stress values <0.05 indicate the ordination provides an excellent representation of the data with no prospect of misinterpretation, and values <0.1 indicate a good representation with little risk of false inferences (Clarke 1993). Treatment by time 95th percentile confidence interval ellipses allowed testing of statistical differences ($\alpha = 0.05$), in which no ellipse overlap indicate differences (Oksanen et al. 2013). In 2005, ponderosa pine and Douglas-fir comprised 96% of the trees sampled; therefore, for simplicity we refer to these two species only instead of host and non-host in the results and discussion.

RESULTS

TREATMENT RESISTANCE TO MOUNTAIN PINE BEETLE OUTBREAK

During the outbreak, mortality from MPB was markedly different between treatments (Figure 1; $X^2_{(3, N = 120)} = 43.33$, $P < 0.0001$). Over 44% ($\pm$ 5.8 s.e.) of the ponderosa pine > 10 DBH was killed in the control treatment and 30% ($\pm$ 5.6 s.e.) in the burn-only treatment. Mortality was much lower in the thin-burn (8% $\pm$ 3.4 s.e.) and virtually nonexistent in the thin-only (1% $\pm$ 0.6 s.e.). Median mortality in the control was 47%, compared to 18% in the burn-only and zero for the thin-only and thin-burn treatments. Mortality was variable as evidenced by the differences in mean and median mortality, but almost all plots in the control had some mortality from the beetle (Figure 1). Responses were generally consistent across blocks, indicating one block was not driving the combined study results (Supplemental Figure 1). In the control, median MPB-caused mortality exceeded 30% in all blocks. It was near 0% in all but one block of the thin-only and thin-burn treatments (the one block with higher MPB-kill in the thin-burn was still less than 10% mortality). The burn-only treatment had the largest variation among blocks, ranging from a median of 0% to almost 60% MPB kill.

The MPB outbreak had a large effect on basal area and density (Table 1; Figure 2). The high mortality from MPB in the control reduced pine basal area and density by over half, from 14.9 m^2 ha^{-1} in 2005 to 6.6 m^2 ha^{-1} in 2012 ($P = 0.0033$) and from 199 trees ha^{-1} in 2005 to 90 trees ha^{-1} in 2012 ($P = 0.0046$; Figure 2A,C). Though ponderosa pine basal area and density was also greatly reduced in the burn-only treatment, these differences were not statistically significant due to high block variability in the

burn-only (Supplemental Figure 1). Ponderosa pine in the thin-only and thin-burn treatments and Douglas-fir in all treatments showed little change before and after the outbreak (Figure 2). There were more Douglas-fir trees in the control and burn-only compared to the thin-only and thin-burn treatments (Figure 2B, D), reflecting the thinning treatment prescription of removing Douglas-fir to promote ponderosa pine. Douglas-fir tended to be larger in the thin-burn treatments compared to the other treatments, from the combination of thinning to remove Douglas-fir and the prescribed burn killing smaller, less fire-tolerant trees.

The outbreak caused a shift in species dominance in the control and burn-only treatments (Figure 3). Before the outbreak, all treatments had more ponderosa pine than Douglas-fir, though the difference was not significant in the control (basal area, $P = 0.6384$; Figure 3). After the outbreak, ponderosa pine remained the dominant species in terms of both basal area and density only in the thin-only ($P < 0.0001$; $P = 0.0195$) and thin-burn ($P < 0.0001$; $P = 0.0044$) treatments while Douglas-fir density was higher than ponderosa pine in the control ($P = 0.0241$).

The ordination analysis supported the individual tests of changes in structure before and after the outbreak (Figure 4). The final 2-D ordination configuration suggested excellent representation of the data (stress = 0.0536). Axis 1 shows a species composition gradient from ponderosa pine to Douglas-fir dominance. Axis 2 shows a tree density and size gradient from larger trees with lower density to smaller trees with higher density. Before the outbreak, the four treatments were clustered into two groups: (1) the control and burn-only and (2) the thin-only and thin-burn (Figure 4, based on overlap of solid line 95th percentile confidence ellipses). The control and burn-only were associated with more Douglas-fir, higher total tree density, and smaller trees, whereas the thin-only and thin-burn were associated with more ponderosa pine, lower total density, and larger trees. After the outbreak, the control exhibited the largest change, or least resistance, of the treatments (Figure 4). The control shifted toward Douglas-fir dominance and reduced total density. The burn-only shifted similarly to the control, but not as

dramatically. The thin-only and thin-burn treatments showed high resistance to the outbreak, with very little change in species composition occurring in either treatment.

TREE PHYSIOLOGY, GROWTH, AND AXIAL RESIN DUCTS

In 2012, at the end of the regional MPB outbreak, there were no treatment differences for any of the whole-tree physiological variables measured: water stress ($F_{3,8.14} = 2.73$; $P = 0.1123$), soil moisture ($F_{3,9.74} = 0.3$; $P = 0.8256$), photosynthetic rate ($F_{3,8.20} = 0.41$; $P = 0.7508$), and stomatal conductance ($F_{3,8.60} = 0.34$; $P = 0.8006$) (Table 2).

Treatment effects emerged for resin ducts and growth variables between the thinned and unthinned treatments (Table 2; Figure 5). Annual growth (BAI) during the most recent five years was 2.4 times higher in the thin-only and thin-burn treatments than the control and burn-only ($F_{3,32} = 18.42$; $P < 0.0001$). BAI began to increase two years after thinning and has persisted since above the control and burn-only treatments (Figure 5A). Resin duct size in the thin-only and thin-burn treatments averaged 33% larger than the control and burn-only treatments during the most recent five years ($F_{3,32.02} = 9.76$; $P = 0.0001$). In the thin-only and thin-burn treatments duct production was approximately double the production of the control and burn-only treatments ($F_{3,31.8} = 12.46$; $P < 0.0001$). Producing larger and more ducts in the thin treatments resulted in a 2.7 fold increase in total duct area per ring compared to the unthinned treatments ($F_{3,31.9} = 13.75$; $P < 0.0001$). The increase in resin duct area began immediately after the thinning treatment and has persisted for the 11 years since treatment, while duct area in the unthinned treatments has declined slightly (Figure 5B).

CONSTITUTIVE RESIN FLOW AND MONOTERPENE COMPOSITION

Resin flow varied by treatment (Figure 6; $F_{3,131.2} = 2.77$; $P = 0.0447$) and was positively related to DBH ($F_{1,122} = 7.89$; $P = 0.0058$). Ten years after the burning treatments, resin flow was higher in the control than the burn-only treatment ($P = 0.0280$). Resin flow in the thin-only and thin-burn treatments was intermediate and did not differ from either the control ($P = 0.3135$; $P = 0.3871$, respectively) or the burn-only ($P = 0.7555$; $P = 0.6678$, respectively).

Total monoterpene concentration (µg g^{-1}) differed by treatment ($F_{3,10.58} = 4.38$; $P = 0.0306$) and was lower in the burn-only compared to the thin-only and thin-burn ($P = 0.0502$, $P = 0.0688$, respectively), but not the control (Table 3). This pattern was driven by four of the seven monoterpenes tested: (-)-α-pinene, myrcene, 3-carene, and terpinoline concentrations were each lower in the burn-only compared to the thin-only and thin-burn, but not different from the control (Table 3). The exception to the pattern was limonene, which was lowest in the control compared to the thin-only and thin-burn concentrations. The concentration of (+)-α-pinene and β-pinene did not differ by treatments.

Monoterpene composition consisted of approximately 50% 3-carene for all samples. Terpinoline, limonene, (+)-α-pinene, and β-pinene each comprised about 10% of the total monoterpene content, followed by myrcene (7%) and (-)-α-pinene (3%) (Table 3). Relative concentration (%) differed by treatment only for (-)-α-pinene ($X^2_{(3, N=199)} = 8.9552$, $P < 0.0299$) (Table 3). The burn-only treatment was lower in (-)-α-pinene relative concentration compared to the other treatments, but this difference was only significant for the thin-burn.

DISCUSSION

There is widespread concern about forest resilience from forest fires, bark beetles, and climate change, but little consensus about what, if any management, is effective against these threats (Allen et al. 2010). Altered natural disturbance regimes may further decrease stability of an ecosystem and create novel conditions that complicate restoration efforts to increase resilience (Folke et al. 2004). We found that thinning treatments increased resistance to a MPB outbreak. Approximately 50% of the ponderosa pine in the control treatments was killed from MPB during the outbreak compared to 20% in the burn-only and almost no mortality in the thin-burn and thin-only treatments even though the units were surrounded by intense bark beetle pressure (Figure 1). Consistent with this and with previous studies of the positive effects of reduced density on water- and nitrogen-limited forests (Smith et al. 1997, Warren et al. 2001, Agee and Skinner 2005, Sala et al. 2005, Fettig et al. 2007), we also found a strong density effect at the individual level, where thinned treatments had increased growth (BAI) and resin ducts

compared to control and burn-only treatments (Table 2, Figure 5). This suggests that excluding frequent fire from this system has greatly decreased resistance from bark beetle outbreaks, primarily via a reduction of density (but see below), and that management treatments that reduce tree density can increase resistance to bark beetles. Bark beetle attacks are spatially dependent, with the probability of a new attack increasing around existing attacks (Raffa and Berryman 1983). Our results also suggest dense forests are more susceptible to initial attacks which can then expand. Open forests likely have fewer stressed, less defended trees and allow faster diffusion and subsequent loss of beetle-produced aggregation pheromones, thus lessening the chance of MPB patch establishment (Logan et al. 1998).

The positive effects of reduced density are also supported by the lack of physiological differences between treatments in 2012 after the outbreak killed so many trees in the control and burn-only treatments and naturally reduced ponderosa pine tree density to the mechanically thinned treatment levels. We expect growth and resin ducts will now begin to increase in the control and burn-only treatments due to thinning effects caused by mountain pine beetle, but it is still too early to determine tree growth and defense responses.

Contrary to our previous results (Hood et al. In Press) and our hypotheses, burning did not increase resin ducts or long-term resin flow. Burning has been shown to increase resin flow (Santoro et al. 2001, Wallin et al. 2004, Lombardero et al. 2006, Cannac et al. 2009, Perrakis et al. 2011), an increase that can last for up to four years (Perrakis et al. 2011). This long-term increase apparently can occur because low-severity fire induces the production of resin ducts (Hood et al. In Press), which can then contribute to resin flow as long as 10 years afterwards (Hood and Sala In Prep). At our study site, Six and Skov (2009) showed resin flow was highest in the burn-only and thin-burn treatments immediately after the prescribed fires. However, in 2012, the burn-only treatment had the lowest resin flow. The lack of long-term increase in resin flow is consistent with the lack of resin duct induction in the burning treatments. Why did the burning treatments not induce resin duct production? It is possible conditions

under which the treatments burned were too mild relative to the wildfires where we documented a fire-induced increase in resin ducts, and therefore, were insufficient to stimulate the induction. Another explanation is the difference in burning season between this study and the wildfires examined in Hood et al. (In Press). The prescribed fires in our study were set in the late Spring, yet wildfires in the Northern Rockies typically occur under drier conditions later in the growing season (Heyerdahl et al. 2008). Following logic of the growth-differentiation balance hypothesis (Lorio 1986, Herms and Mattson 1992), trees may only be able to respond to a fire stimulus and mount an increase in resin duct production later in the growing season, when defense costs are lower due to water limitations to tree growth.

Consistent with the resin duct data and changes in tree density, mortality from MPB was lowest in the thin-only and thin-burn and highest in the control and burn-only. The burn-only also had lower mortality than the control (though not statistically so) suggesting that burning alone confers some resistance to MPB. Paradoxically though, resin flow was higher in the control than the burn-only. We do not have an explanation for this result. Resin flow is highly variable (Gaylord et al. 2007, Gaylord et al. 2011) and may not be a reliable metric for defense compared to resin ducts (Hood and Sala In Prep). Another possible explanation for the reduced mortality in the burn-only treatments compared to the control is the lower levels of several specific monoterpenes in the burn-only that can benefit MPB, including (-)-α-pinene, myrcene, and 3-carene. MPB uses (-)-α-pinene and myrcene as a precursor and synergist, respectively, to the production of aggregation pheromone, and high rates of 3-carene and myrcene have been shown to increase flight response to aggregation pheromone (Seybold et al. 2006). Conflicting with our results, Powell and Raffa (2011), found burned *Pinus contorta* trees had increased proportions of (-)-α-pinene compared to unburned trees. They measured resin chemistry 1-year after burning compared to 10-years after burning in this study, so it is possible that this response changes over time. We did not measure induced resin chemistry, but it can play a large role in resistance to beetle attack (Raffa and Smalley 1995) and could also possibly explain the difference in attack rates between the control and burn-only.

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Though the thin-only and the thin-burn treatment both had the lowest mortality from MPB, there are other factors to consider when choosing whether to use prescribed burning, either alone or following thinning treatments. Density by species and the ordination results suggest the largest changes in stand trajectories occurred in the control (no overlap in pre vs. post outbreak ellipses), suggesting that burning alone increased resistance from the outbreak relative to the control. Further, we note that our analysis of the effects of burning treatments on successional dynamics is conservative because we only included trees larger than 10 cm DBH. However, burning likely killed many Douglas-fir seedlings. Therefore, there is a potentially large burning effect on recruitment that is not captured in our analyses because these seedlings have not reached census size. In addition, burning also affects nutrient cycling, spatial heterogeneity for wildlife habitat, and resilience from other disturbances which may also be important for ecosystem stability (Bond and Keeley 2005, Gundale et al. 2005, Fiedler et al. 2010, Ryan et al. 2013).

Low-elevation, seral forests such as the ponderosa pine ecosystem studied here, have been greatly impacted from altered wildfire disturbance regimes (Hood 2010). These forest types historically burned frequently and created open conditions dominated by seral, shade-intolerant species adapted to survive fire. Without frequent, low-severity fire, these forests quickly become denser and oftentimes shift in composition to shade-tolerant species (Keeling et al. 2006). Many shade-tolerant species are easily killed by fire as seedling and saplings, but become tolerant to fire as they grow larger. Therefore, if enough time lapses between fires, the shade-intolerant species will survive fire, resulting in a persistent shift in species composition even if frequent, low-severity fire returns to the system. The burn-only treatment in our study is an example of this situation: reintroducing fire after over 100 years without fire does not reduce stand density and species composition relative to controls because many of the Douglas-fir trees are now fire-tolerant (Hood and Bentz 2007, Hood 2010). This has implications for forest resilience after the beetle outbreak. The large Douglas-fir component in both the control and burn-only due to fire exclusion, coupled with the high pine mortality from MPB has shifted forest dominance to Douglas-fir – a shift that will only increase over time in the absence of further fire.

Treatments in this study were implemented about 5 years before MPB population increased, so there is a possibility of a different outcome if treatments are implemented during a MPB outbreak. There are very mixed results of the effectiveness of treatments in stopping outbreaks once they begin (Six et al. 2014), but consistent patterns of increased resistance if thinning is conducted prior to outbreaks (Fettig et al. 2007, Fettig et al. 2014a, Fettig et al. 2014b, Gillette et al. 2014). By using fully replicated forest management treatments that were subsequently challenged to a naturally occurring outbreak, our study consolidates findings from previous case studies that low density stands increase resistance to MPB attack. We further show that such effects result from the combination of increased tree defenses and forest structural changes. Additional research is needed to determine whether less than expected individual tree defense stimulation by prescribed fire is due to different seasonality and/or severity of prescribed fires relative to wildfires.

Our study highlights the importance of managing forests for multiple ecological objectives. The treatments in this study were originally designed to increase resilience from wildfire. However, fire is only one of many possible disturbances and abiotic stress, including insect pests and severe droughts. When and whether any of these disturbances will affect the treated area is virtually impossible to predict. Therefore, management decisions should be guided by broad ecological factors such as long-term ecosystem integrity and functioning in the face of climate change and multiple disturbances. Management that fosters healthy forests and mimics historical disturbance regimes will likely be the most resilient.

Table 1. Model F-statistics and significance of basal area, density, and quadratic mean diameter. Treatments included control, burn-only, thin-only, and thin-burn. Years included 2005 (before outbreak) and 2012 (after outbreak). Mountain pine beetle (MPB) host was either 1 (host) or (0) nonhost.

Effect	Num DF, Den DF	F-value	P-value
Basal Area			
Treatment	3, 8	5.42	0.0249
Year	1, 456	1.31	0.2532
MPB host	1, 456	111.64	<.0001
Treatment*year*host	10, 456	7.90	<.0001
Density			
Treatment	3, 8	19.24	0.0005
Year	1, 456	3.39	0.0660
MPB host	1, 456	37.23	<.0001
Treatment*year*host	10, 456	6.87	<.0001
Quadratic Mean Diameter			
Treatment	3, 7.929	2.48	0.1357
Year	1, 405.1	3.48	0.0627
MPB host	1, 406.6	126.69	<.0001
Treatment*year*host	10, 405.4	5.45	<.0001

Table 2. Mean (standard error) of physiological, growth, and defense variables by treatment at Lubrecht Experimental Forest, MT. Resin duct characteristics are based on a 5 mm wide sample. N = sample size per treatment.

	N	Control	Burn-only	Thin-only	Thin-Burn
Water potential$_{am}$ (mPa)	3	-1.4 (0.15)a	-1.7 (0.15)a	-1.3 (0.15)a	-1.5 (0.15)a
Soil Moisture (%)	3	7.7 (1.4)a	6.7 (1.2)a	7.5 (1.3)a	7.9 (1.4)a
Photosynthesis (μmol m^{-2} s^{-1})	3	12.9 (2.3)a	9.8(2.6)a	11.2 (2.3)a	13.3 (2.6)a
Conductance (mol m^{-2} s^{-1})	3	0.17 (0.05)a	0.13 (0.05)a	0.16 (0.05)a	0.15 (0.06)a
BAI$_5$ (cm^2)*	3	5.8 (0.7)a	7.5 (0.9)a	16.5 (2.0)b	15.1 (1.8)b
Duct Size$_5$ (mm^2)*	3	0.03 (0.002)a	0.03 (0.002)a	0.04 (0.002)b	0.04 (0.002)b
Duct Production$_5$*	3	3.2 (0.4)a	4.1 (0.5)a	7.3 (0.9)b	7.4 (0.9)b
TDA$_5$ (mm^2)*	3	0.09 (0.01)a	0.11 (0.02)a	0.27 (0.04)b	0.27 (0.04)b

* Yearly average from most recent 5 years (2007-2011) prior to resin flow sampling. BAI = basal area increment; TDA = total duct area per year; Duct production = number of ducts per year.

Table 3. Mean (std. err.) of absolute and relative concentration of individual and total constitutive ponderosa pine monoterpenes by treatment. Different letters within a column denote significant (α = 0.05) treatment differences using Dunn's test of post hoc comparisons. If no letters are present, treatment was not a significant factor. P-values are results of Kruskal-Wallis chi-square tests.

| Treatment	N	(-)-α-pinene	(+)-α-pinene	Myrcene	3-carene	β-pinene	Limonene	Terpinoline	Total
				Absolute Concentration (µg g^{-1} phloem)					
Control	40	23.6 (8.8)ab	49.0 (12.5)	39.2 (13.2)ab	274.7 (73.3)ab	91.0 (59.2)	39.3(9.0)a	48.4 (9.8)ab	565.1 (159.0)ab
Burn-only	62	14.6 (3.1)a	54.1 (15.7)	22.0 (3.4)a	176.9 (29.2)a	23.9 (3.4)	43.3 (7.7)ab	44.7 (7.4)a	379.6 (64.4)a
Thin-only	30	23.5 (3.8)b	57.6 (11.5)	33.5 (3.9)b	331.0 (48.5)b	34.3 (7.4)	66.1 (11.2)ab	74.8 (10.8)b	620.8 (85.1)b
Thin-Burn	67	26.7 (3.9)b	76.3 (16.4)	34.6 (4.0)b	301.1 (41.6)b	34.9 (6.0)	77.4 (13.9)b	80.5 (14.0)b	631.6 (90.9)b
P-value		0.0018	0.0527	0.0052	0.0003	0.6411	0.0078	0.0044	0.0306
				Relative Concentration (%)					
Control		3.5 (0.4)ab	10.7 (1.6)	7.3 (0.9)	51.1 (1.8)	9.4 (1.7)	8.5 (0.9)	9.6 (0.9)	Na
Burn-only		2.7 (0.4)a	10.2 (1.1)	7.0 (0.8)	49.7 (1.6)	10.0 (1.2)	9.7 (0.7)	10.7 (0.7)	Na
Thin-only		3.4 (0.4)ab	8.1 (1.2)	7.0 (0.7)	53.7 (1.7)	6.2 (1.3)	9.9 (0.9)	11.8 (0.5)	Na
Thin-Burn		4.1 (0.3)b	10.7 (1.0)	6.4 (0.4)	49.4 (1.6)	7.1 (0.9)	10.8 (0.6)	11.4 (0.5)	Na
P-value		0.0299	0.6131	0.9713	0.1988	0.2608	0.3095	0.3512	Na
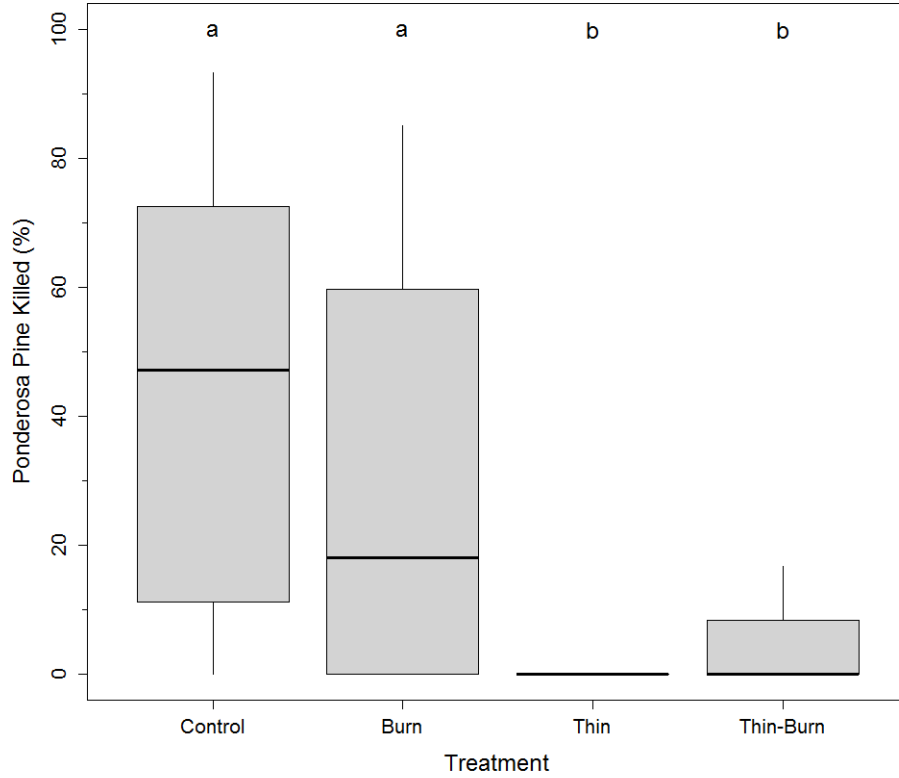
Figure 1. Percent of ponderosa pine killed by mountain pine beetle between 2005 and 2012. Different letters indicate mortality is significantly different between treatments ($\alpha = 0.05$). Boxes denote first and third quartiles, lines the median, and whiskers the 1.5 inter-quartile range (IQR).
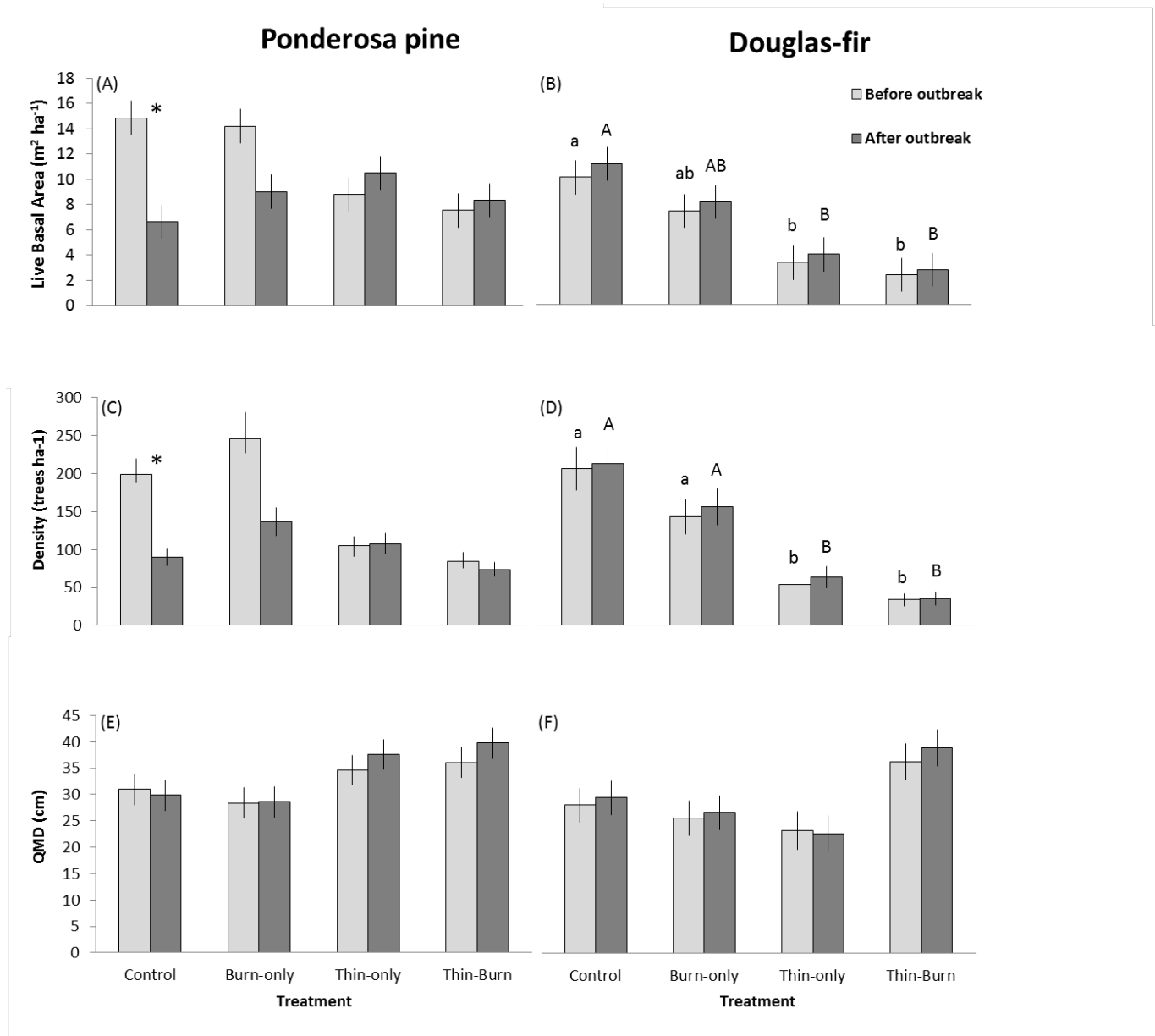
Figure 2. Mean (std. err.) of forest attributes before (2005) and after (2012) the mountain pine beetle outbreak by host (ponderosa pine) and non-host (Douglas-fir). (A) and (B) shows basal area, (C) and (D) shows density, and (E) and (F) shows quadratic mean diameter (QMD). * denotes a significant difference before and after the outbreak within a treatment. Lower case letters denote significant treatment differences before the outbreak, and uppercase letters denote significant treatment differences after the outbreak.
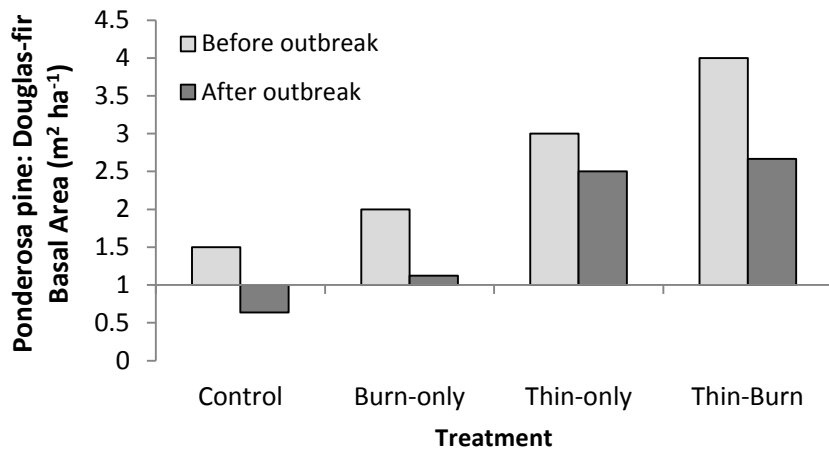
Figure 3. Ratio of host (ponderosa pine) to non-host (Douglas-fir) basal area before (2005) and after (2012) the mountain pine beetle outbreak.
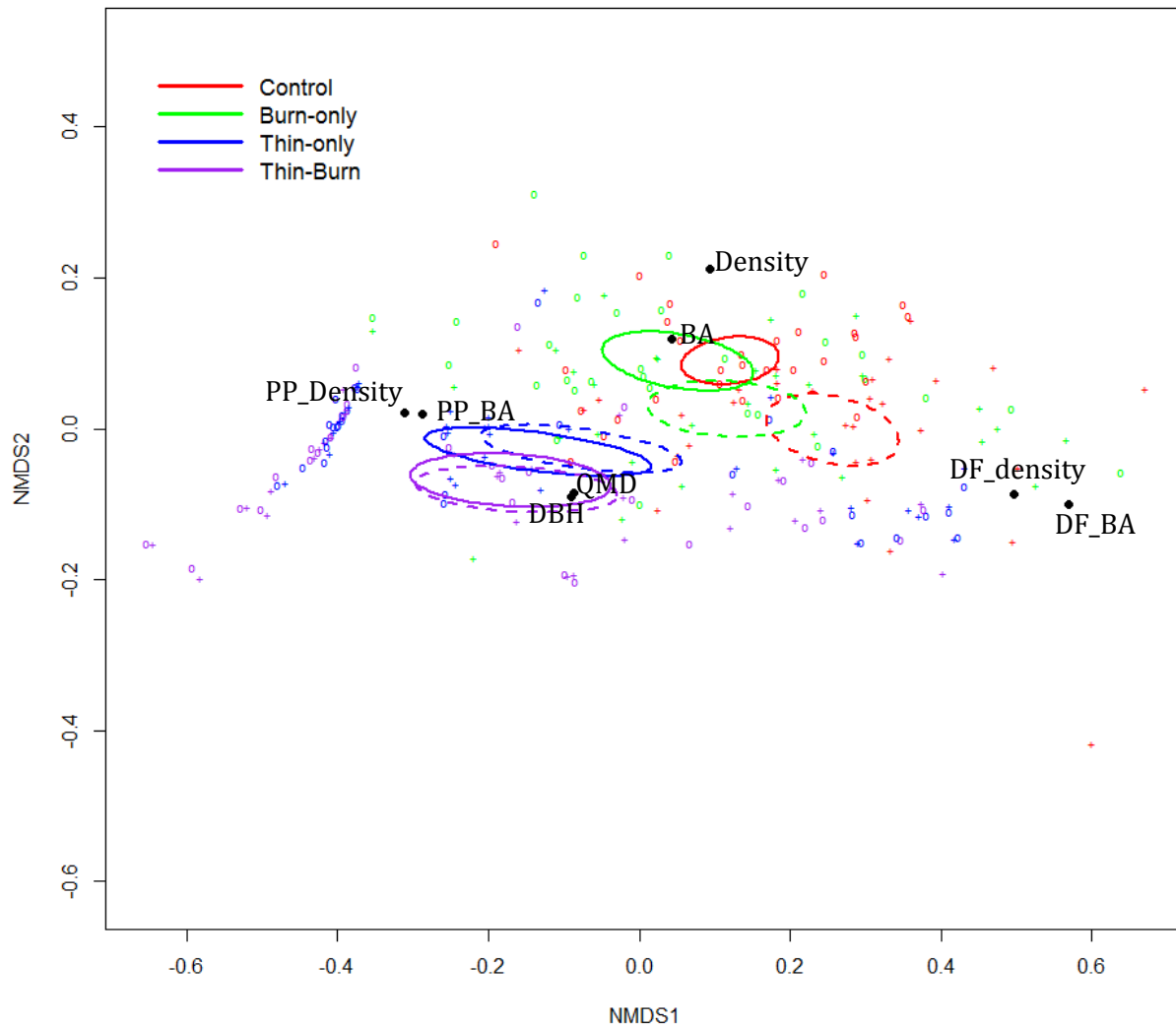
Figure 4. Non-metric multidimensional scaling (NMDS) of treatments before (solid line) and after (dashed line) the mountain pine beetle outbreak showing association with forest structural attributes. Ellipses are 95th percentile confidence intervals of each treatment and time step centroid. Individual Whittaker plots show before outbreak (o) and after (+) outbreak. Density = total trees per hectare, all species; BA = basal area (m^2 ha^{-1}), all species; DBH = diameter at breast height (cm), all species; QMD = quadratic mean diameter (cm), all species; PP_Density = percent of density composed of ponderosa pine; PP_BA = percent of BA composed of ponderosa pine; DF_Density = percent of density composed of Douglas-fir; DF_BA = percent of BA composed of Douglas-fir. Stress = 0.0536, 2 dimensions.
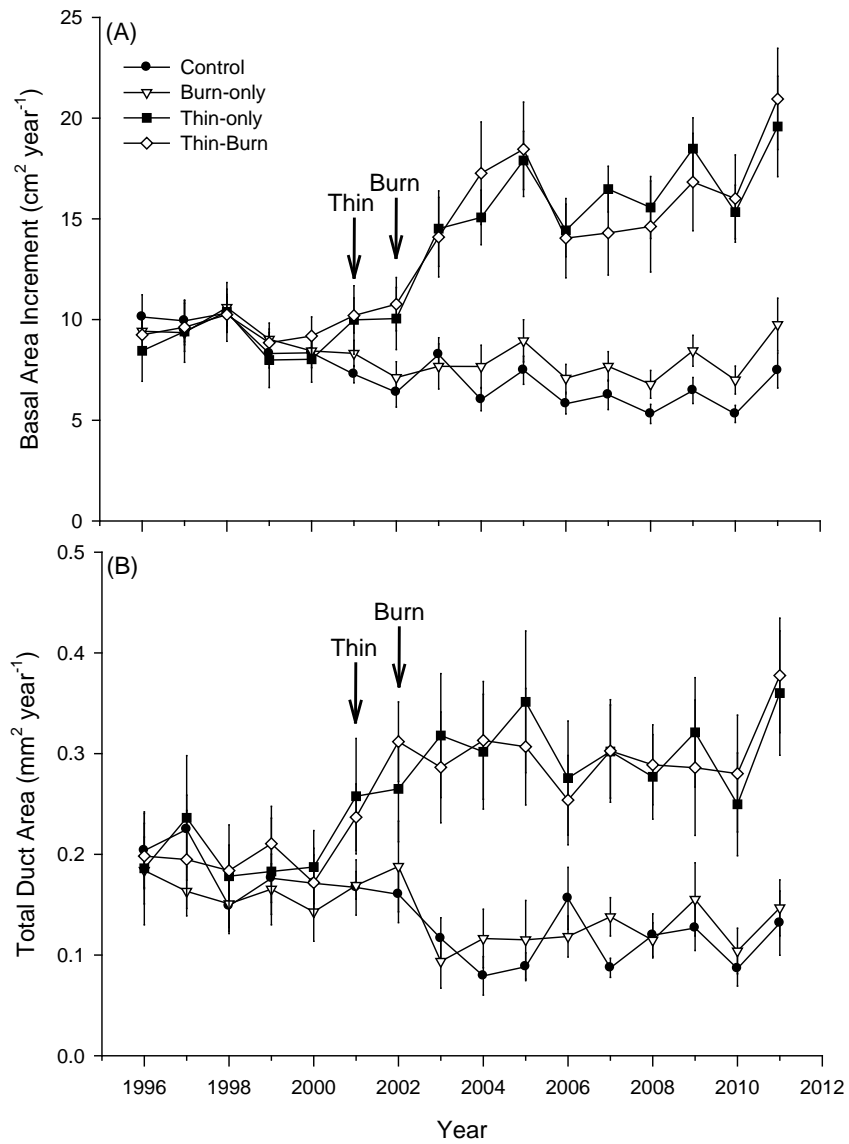
Figure 5. Yearly mean basal area increment (A) and total duct area (B) by treatment. Error bars are standard error. Arrows denote year of thinning and prescribed burn.
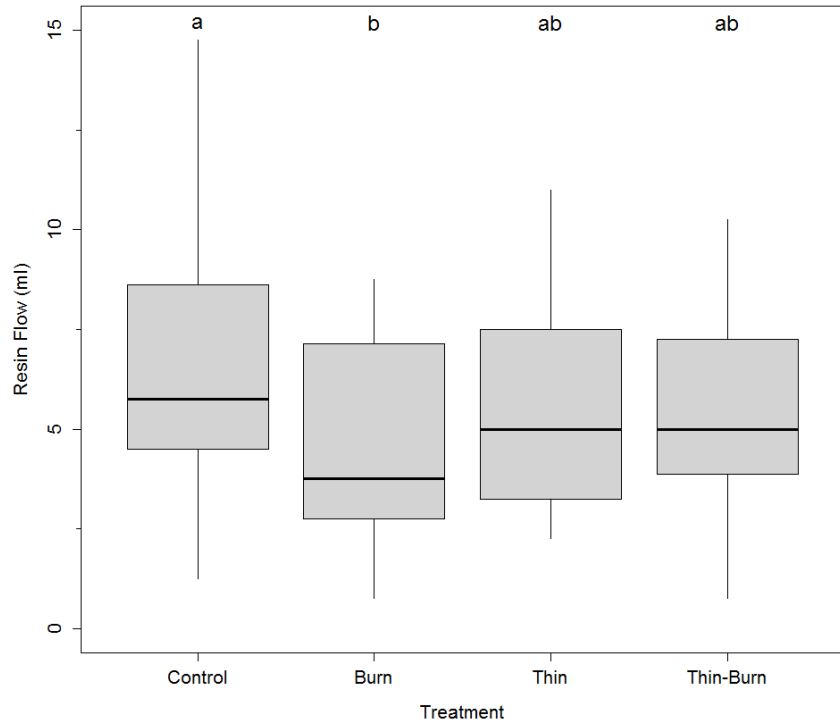
Figure 6. Average resin flow in 2012 by treatment. Different letters indicate resin flow is significantly different between treatments ($\alpha = 0.05$). Boxes denote first and third quartiles, lines the median, and whiskers the 1.5 inter-quartile range (IQR).

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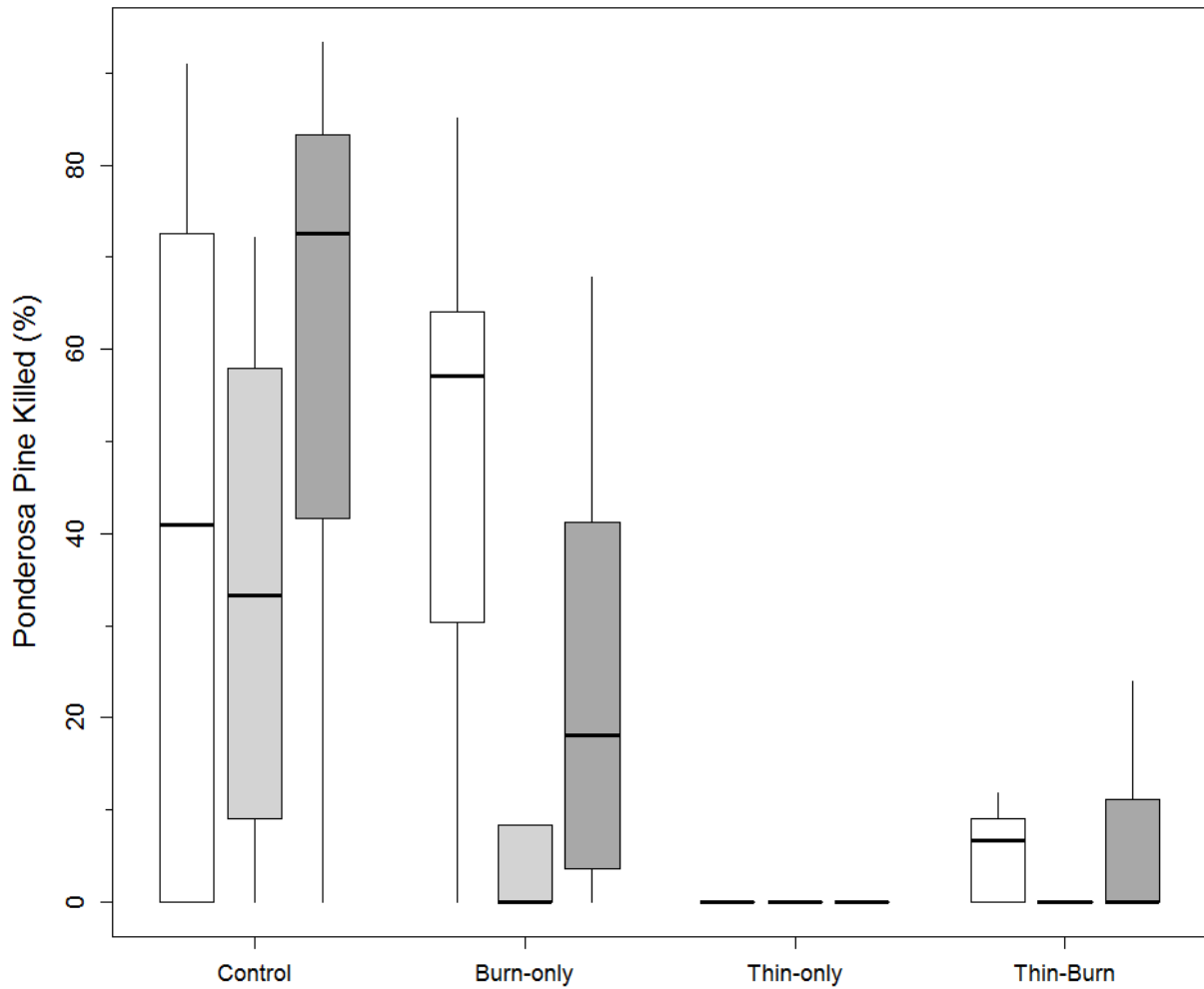
Supplemental Figure 1. Percent of ponderosa pine killed by mountain pine beetle between 2005 and 2012 by block. Block 1 = white; block 2 = light grey; block 3 = dark grey. Boxes denote first and third quartiles, lines the median, and whiskers the 1.5 inter-quartile range (IQR).