

CHARACTERISTICS OF WHITEBARK PINE (*PINUS ALBICAULIS*) GROWTH & DEFENSE IN
DISTURBANCE-PRONE, HIGH-ELEVATION, MONTANE ECOSYSTEMS OF THE NORTHERN
ROCKY MOUNTAINS

by

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DEDICATION

I dedicate this work to my incredible wife Katherine and beautiful daughter Mary Olive. Kat, you have diligently stood by me through nearly a decade of academia – through the many moves, the many highs and lows, and the constant uncertainty of what comes next – all while being a continuous source of love and inspiration. I cannot thank you enough, my darling. Thank you for your love and for taking care of me. You are my soulmate, and I could never have done this without you. You make me a better person and I cannot imagine a life without your beautiful smile greeting me at the start and end of each day. Our little Mary Olive surprised us with her arrival right in the middle of this dissertation program and I could not have asked for a better gift. Mary Olive, my little angel, thank you for teaching me to see the world in a beautiful new way and to truly appreciate the little moments. I love you with all my heart. I believe in you and know that you can accomplish all of your dreams. You are so special and have so much to offer the world. Please never underestimate yourself – realize your value and know that your Daddy loves you and supports you fully, wherever life may take you. Strive to make the world a better place and bring kindness, love, and happiness wherever you go.

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ABSTRACT

Whitebark pine (*Pinus albicaulis*) is a high-elevation conifer, recognized as a foundation species due to the numerous ecological benefits it provides in subalpine environments. In whitebark pine and other conifers, resin-based defenses have long been recognized as the primary mechanism by which trees respond to bark beetle attacks and several studies have linked resin duct properties to survivorship during periods of increased beetle activity. Utilizing a unique dataset of whitebark pine collected on the Flathead Indian Reservation in northwestern Montana, we set out to investigate the following research questions: (1) Are there differences in physiology (tree growth and resin duct anatomy) between trees that persisted through recent mountain pine beetle outbreaks and trees that died? (2) Does constitutive resin chemistry differ between whitebark and co-occurring lodgepole pine and are there relationships between tree growth, resin duct anatomy and resin chemistry? (3) Does competition influence constitutive resin chemistry in either whitebark or lodgepole pine? and (4) Is whitebark pine growth and/or resin duct anatomy constrained by warmer and/or regionally drier conditions? We found that whitebark pine trees that have persisted through recent stand-level disturbance produced fewer but larger resin duct structures with greater duct area compared to trees that died. We also detected important differences in the chemical composition of resin between whitebark and lodgepole pine that generally support field observations, whereby under endemic scenarios mountain pine beetle preferentially select lodgepole pine, while under outbreak scenarios, beetles successfully colonize whitebark pine trees. We found complex relationships between tree growth, resin duct anatomy and constitutive resin chemistry that present beetles with many permutations of resin-based defenses, while competition, particularly with Engelmann spruce (*Picea engelmannii*) can further influence constitutive resin chemistry. Lastly, we found that whitebark pine across our study sites are experiencing increased growth and defense under warmer and regionally drier conditions. Whitebark pine at our study sites exhibit differing strategies in the allocation of resources toward growth and defense. Our results support the idea that maintaining genetic variability promotes diverse response strategies to a complex array of biophysical stressors that might leave a species vulnerable to extinction across its range.

INTRODUCTION

An increase in the incidence of large and damaging fires in the western U.S. highlight the need to revise forest management policies and implement adaptive strategies to support resilient forest ecosystems (Franklin and Agee 2003, Stephens and Ruth 2005, Westerling et al. 2006, Littell et al. 2009, van Mantgem et al. 2009, McWethy et al. 2019). Insect and disease outbreaks and extremes between wet and dry years are occurring in concert with changing fire activity, leading to disturbance synergies with profound ecological and social consequences (Loehman et al. 2017). Developing management strategies to mitigate the impacts of these disturbance synergies requires an understanding of complex interactions between disturbances and forest ecosystem dynamics in the past and present (Kane et al. 2017). While many researchers have documented changes in disturbance regimes over the past century, this information is often limited both spatially and temporally (Conedera et al. 2009, Driscoll et al. 2010) and may not fully account for subtle but important biophysical and climatic variability across geographic gradients. Currently, results from many regional studies have been applied broadly, leading to management prescriptions that are ill-matched for the ecosystems in which they are adopted (Dawson et al. 2011, Halofsky et al. 2011). This is often the case for high-elevation mixed-conifer forest stands of the northern Rocky Mountains that experience a range of disturbance conditions (in both frequency and intensity) that are highly variable across time and space (Veblen et al. 1994, Baker 2009). Management in these diverse areas requires a multidisciplinary framework that considers numerous ecological responses to past and present perturbations within a heterogeneous matrix of vegetation and topography.

Whitebark pine (*Pinus albicaulis*) is a long-lived, high-elevation conifer that occupies subalpine ecosystems (Arno and Hoff 1989, Keane 2000, Tomback et al. 2001). Often dominating treeline, whitebark pine is considered a foundation species, with high cultural and ecological significance (Cole 1990, Mills and Doak 1993, Tomback et al. 2001). Whitebark pine is one of the few species that thrives at high altitudes (> 2,000 m a.s.l.) where they stabilize erosive slopes; promote snow accumulation; increase snowpack retention and effectively modulate moisture loss in subalpine regions, helping provide a consistent supply of water to lower elevations throughout summer months (Arno and Hoff 1989, Tomback and Kendall 2001, Bockino and Tinker 2012). Whitebark pine seeds are highly nutritious and an important food source for several species of wildlife, including grizzly bears (*Ursus arctos horribilis*), black bears (*U. americanus*), and numerous small mammals and birds. Seeds are predominately dispersed by the Clark's nutcracker (*Nucifraga Columbiana*), a bird which caches seeds broadly across alpine landscapes, including disturbed areas. In these environments, forgotten seeds often germinate, forming whitebark pine forests of tomorrow. Whitebark pine is an important pioneer species, as the hearty seedlings colonize areas and ameliorate site conditions, promoting additional vegetative growth through tree island initiation (Tomback et al. 1993, Tomback et al. 2016). However, extensive whitebark pine mortality due to the introduction of a highly aggressive exotic pathogen white pine blister rust (*Cronartium ribicola*) and extensive mountain pine beetle (*Dendroctonus ponderosae*) outbreaks caused by shifting climate, along with future projections of continued population declines due to warming, led the U.S. Fish & Wildlife Service to list whitebark pine as "threatened" under the Endangered Species Act in December 2020. Managers tasked with supporting and maintaining the viability of whitebark pine into the

future need information on how whitebark forests respond to climate and disturbance synergies across biophysical gradients throughout its range.

While insects, fire, disease, and secondary effects of drought (including increased tree stress and predisposition to other disturbances) have contributed to recent mortality of whitebark pine, these processes also play an important role in the long-term establishment and persistence of northern Rocky Mountain whitebark pine forests. However, historical records detailing the relationship between disturbance and whitebark pine ecosystem dynamics are particularly lacking. For example, a metanalysis of paleoecological data suggests that the abundance of *Pinus* subgenus *strobus*, as inferred from pollen percentages, was greater (particularly at lower elevations) during the Holocene summer insolation maximum (~9 kyrs BP) when summer temperatures were warmer, bark beetle activity was prevalent, and fire activity was high in the western U.S. (Iglesias et al. 2015). This research suggests possible complex interactions between temperature, fire, and insect dynamics that have developed over thousands of years, highlighting a need to better understand how five needle pines (*P. strobus* subgenus) have persisted in the past and will respond to multiple disturbances in the future. Research investigating the physiological response of whitebark pine to fire frequency, increased competition, and timing / severity of beetle outbreaks across a range of diverse environmental gradients is essential for developing effective management targets. This is especially true given that current species risk assessments for whitebark pine rely on bioclimatic models that include little information on potential physiological adaptability of the species to interactions and feedbacks between changing climatic and disturbance regimes. In addition, previous research evaluating natural physiological defenses in whitebark to insect attacks has traditionally been conducted in either

“pure” high-elevation stands (particularly around the Greater Yellowstone Ecosystem) or in common-garden experiments (Raffa et al. 2013, Raffa et al. 2017, Warwell and Shaw 2017, Bullington et al. 2018, Six et al. 2018, Mason et al. 2019), which often fail to capture the complex suite of interacting factors that influence mixed-conifer stands where whitebark pine co-occurs. Our study sites, located within the Flathead Indian Reservation in northwestern Montana, are characterized by mixed stands of whitebark pine, lodgepole pine (*P. contorta* var *latifolia*), subalpine fir (*Abies lasiocarpa*), and Engelmann spruce (*Picea engelmannii*) with differing legacies of fire, insects, and pathogens. While these types of ecosystems are common within the northern Rocky Mountains, little research has explored disturbance interactions within these mixed whitebark stands (Kichas et al. 2020, Kichas et al. 2021).

Better understanding how physiological characteristics of whitebark pine (specifically the allocation of resources to growth and defense) influence survival following the synergistic effects of multiple disturbances is critical for resource managers tasked with managing and supporting the long-term viability of this foundation species. Across many species of North America pines, research shows that during beetle attacks, even at peak epidemic levels, there are mature individuals that remain uncolonized by beetles, and that these resistant individuals may have different defense traits (Strom et al. 2002, Hood et al. 2016). For instance, Strom et al. (2002) found oleoresin flow from progeny of mature loblolly pines (*P. taeda*) that "escaped" mortality during an outbreak of southern pine beetle (*Dendroctonus frontalis*) was higher compared to the general population. Other studies have demonstrated the importance of resin ducts as defense traits in ponderosa pine (*P. ponderosa*), piñon pine (*P. edulis*), limber pine (*P. flexilis*), lodgepole pine, and whitebark pine (Kane and Kolb 2010, Gaylord et al. 2013,

Ferrenberg et al. 2014, Hood et al. 2016, Kichas et al. 2020). Survivorship of individuals within such diverse populations suggests an intrinsic level of resistance that can be genetically passed on to the survivors' progeny (de la Mata et al. 2017, Six et al. 2018). This is supported by research which suggests that the capacity to produce defensive features (such as resin ducts) and the composition of chemical defenses (e.g. chemotypes) are under strong genetic control, resulting from site-specific selective pressures that moderate growth and defense relationships over time (Hannrup et al. 2004, Rosner and Hannrup 2004, Moreira et al. 2014, Moreira et al. 2015, Westbrook et al. 2015, Moreira et al. 2016).

Evaluating relationships between tree growth, resin duct development and oleoresin chemistry can provide valuable insight into overall capacity for these trees to resist and defend against stressors that are projected to increasingly impact this important species. Utilizing a unique dataset of whitebark pine collected on the Flathead Indian Reservation in northwestern Montana, we set out to investigate the following research questions:

- 1) Are there differences in physiology (tree growth and resin duct anatomy) between trees that have persisted through recent mountain pine beetle outbreaks and trees that have died?
- 2) Does constitutive resin chemistry differ between whitebark and co-occurring lodgepole pine and are there relationships between tree growth, resin duct anatomy and resin chemistry for either species?
- 3) Does competition influence constitutive resin chemistry in either whitebark or lodgepole pine?

- 4) Is whitebark pine growth and/or resin duct anatomy constrained by warmer and/or regionally drier conditions?

Previous whitebark pine research has primarily focused on the relationship of mortality to white pine blister rust and mountain pine beetle (Logan et al. 2010, Bockino and Tinker 2012, Millar et al. 2012, Retzlaff et al. 2016, Tomback et al. 2016). However, little is known about how physiological characteristics of whitebark pine resource allocation to growth and defense influence survivorship to the interacting effects of climate and compounding disturbances (Millar and Delany 2019). In addition, strategies for maintaining whitebark pine stands rely heavily on bioclimatic models that project substantial losses in the distribution of whitebark pine stands throughout the northern Rocky Mountains. These models, however, fail to capture long-term physiological adaptability of this unique species, and as such, are potentially misleading. Our results address these knowledge gaps by identifying physiological mechanisms by which whitebark pine respond to complex disturbance interactions within these unique montane environments.

CHAPTER TWO

CONSIDERING CONSTITUTIVE AND INDUCED DEFENSES, CHEMICAL
COMPOSITION, AND MORPHOLOGICAL RESIN DUCT DEFENSES IN RELATION TO
WHITEBARK PINE (*PINUS ALBICAULIS*)

Mounting evidence suggests recent increases in tree mortality resulting from fire exclusion, changes in climate, pathogen and insect activity, and complex interactions among these disturbance events (Mattson and Haack 1987, Westerling et al. 2006, van Mantgem and Stephenson 2007, van Mantgem et al. 2009, Allen et al. 2010, Schwandt et al. 2010, Tomback and Achuff 2010, Roy et al. 2014). As such, there is a growing interest to better understand the role of tree defense and physiological responses of trees as they relate to mortality.

However, tree physiology and disturbance regimes vary substantially across both time and space, which complicates our ability to make generalized assumptions for inter- and intra-specific species response to biotic and abiotic processes. This is made more difficult by the substantial complexity of defense anatomy and diversity of chemical compounds that are produced by plants, which vary across populations, individuals, and plant organs (Trowbridge 2014, Jamieson et al. 2017). In addition, defensive structures and chemical compounds are influenced by a host of biotic and abiotic factors that collectively inflate variability and confound the evolutionary origin and development of secondary metabolites. Coevolution of chemical defenses in response to herbivores and pathogens over millions of years has resulted in a myriad of complex relationships whereby insect and pathogen activity is often intimately linked to the presence, diversity, and concentration of specific chemical compounds in host plants (Smith 2000, Six 2012, Jamieson et al. 2017). However, the production of physical defense structures

and chemical compounds is energetically expensive, which necessitates important trade-offs between metabolic differentiation to growth versus resource allocation to defensive features (Stamp 2003, Trowbridge 2014).

While there have been several studies that have looked to quantitatively assess growth and defense characteristics for a range of species, general inconsistencies in methodologies and research findings both within and across species reflect a need for standardization of metrics and ongoing refinement of analytical techniques. This is particularly true for long-lived organisms, such as conifers that have variable, genetically-mediated metabolic processes and context-specific responses to their local environment that may select for unique combinations of defensive traits over time (Cobb et al. 1994, Mitton et al. 1998, Rosner and Hannrup 2004, Moreira et al. 2016).

Resource Allocation to Growth and Defense

Physical and chemical defenses are critical for mediating plant-herbivore-pathogen interactions; however, the production and proliferation of defensive structures and chemical compounds is energetically expensive with high demand for relatively limited carbon resources (Lewinsohn et al. 1991, Stamp 2003, Moreira et al. 2014, Trowbridge 2014). Given adequate resource availability, plants will preferentially allocate resources toward primary production, including diameter and height growth as well as development of leaves, cones, and coarse- and fine-root networks (Trowbridge 2014, Jamieson et al. 2017). However, plants simultaneously utilize carbon reserves in the production of secondary metabolites, such as anatomical features and chemical compounds (e.g., terpenoids, alkaloids, and phenols) to protect crucial tissues from damage by herbivory and pathogens. As such, there are important trade-offs regarding resource

allocation between growth and defense, which remains to be fully elucidated given the complex biosynthetic pathways involved in cell differentiation and chemical synthesis (Trowbridge 2014). Many hypotheses have been proposed to characterize observed biophysical processes relating to these trade-offs, including the resource allocation hypothesis (RAH), as well as the growth-differentiation balance hypothesis (GDBH). The resource allocation hypothesis argues that plants in stressful, resource-poor environments will exhibit naturally slow growth rates and will invest comparatively more carbon into the development of defensive features and chemical compounds to limit the potential loss of expensive tissues, which would be difficult to replace under such circumstances (Coley et al. 1985, Stamp 2003, Endara and Coley 2011, Moreira et al. 2014, Moreira et al. 2015, Moreira et al. 2016). This hypothesis has been supported by several studies across a range of deciduous trees and shrubs, which tend to have lower growth rates and higher levels of constitutive defenses in resource poor environments (Endara and Coley 2011). For instance, research by Moreira et al. (2015) provides support for this hypothesis as the authors found increased resin duct density and increased relative area (a metric that combines duct density and duct size) as well as decreased growth rates in juvenile maritime pine (*Pinus pinaster*) under a phosphorous limited setting. Additional work by Moreira et al. (2014) looked at defense characteristics in a common greenhouse experiment for 18 Pinaceae species whose native habitats occupy broad latitudinal (31°) and elevational (2300 m) gradients. The researchers found increased constitutive defenses (stem resin and needle phenolics) and decreased inducible defenses for species that naturally occur at higher latitudes and elevations, where environmental conditions correspond with slower growth rates, suggesting a heritable pattern of increased primary defenses in more resource-limited environments.

Alternatively, the GDBH suggests that investment in defensive features may occur with relatively minimal trade-offs to growth under resource-limited conditions (such as reduced moisture or nutrient availability) whereby growth is constrained by resource limitations, but photosynthesis continues to produce carbon compounds and this ‘carbon surplus’ may then be allocated toward physical and chemical defenses. This hypothesis has some observational support, as resin flow has been observed to increase in some species under moderate water stress, where growth would be more limited, but photosynthesis would continue to produce carbon resources, while decreasing under severe moisture stress, where assimilation would be insufficient in meeting basic metabolic demands for primary production (Loomis 1932, Herms and Mattson 1992, Stamp 2003, Gaylord et al. 2007, Gaylord et al. 2013, Hood and Sala 2015, Moreira et al. 2015). Anatomical resin ducts also tend to form in the latewood when water availability is generally reduced, which would follow this logic (Rigling et al. 2003). The GDBH has also been supported in various studies, although research is largely context dependent, given wide variability in methodologies and protocols (Ferrenberg et al. 2015). For instance, Gaylord et al. (2013) found that resin flow in twigs of piñon pine decreased under extreme (3 year) drought stress, as trees were unable to assimilate carbon for resin defenses, as well as under water-additive treatments, as the well-hydrated trees preferentially allocated resources to growth over defense, whereas resin production increased under moderate water stress, corresponding with intermediate growth. Similarly, the work by Moreira et al. (2015) also lends support for the GDBH as the maritime pines responded to phosphorous limitation through decreased growth with a corresponding increase in defensive features. Research by Hood and Sala (2015) also broadly support this hypothesis as the researchers found increased resin flow in ponderosa pine

trees during periods of seasonal moisture stress, where oleoresin production was greater in August (less moisture availability) than in July.

Central to each hypothesis is the larger idea that resource allocation towards defense enhances plant fitness and that inter- and intra-specific variability can be explained by trade-offs between growth and defense, whereby external factors including resource availability, abiotic stress, and herbivore pressures all contribute to investment in defensive systems (Moreira et al. 2014, Trowbridge 2014, Ferrenberg et al. 2015, Jamieson et al. 2017). However, results of many studies looking to establish evidence for either hypothesis are often contradictory, as the nature of biophysical mechanisms involved in resource allocation to growth and defense are highly complex, as well as difficult to test in both field / greenhouse studies and lab experiments (Trowbridge 2014, Jamieson et al. 2017). In addition, the hypotheses themselves are not mutually exclusive and it is likely that aspects of both are correct depending on the plant species considered as well as environmental conditions influencing resource allocation, such as climate, topography, intra- and inter-specific competition, as well as a host of other biotic and abiotic pressures (Stamp 2003, Trowbridge 2014). Research addressing either hypothesis tends to be limited to small number of species in early developmental phases (e.g., seedlings) with minimal data for intermediate and mature ages classes (Ferrenberg et al. 2015).

Constitutive and Induced Defense

The production of resin ducts and storage of oleoresin compounds has been researched for a variety of species and has generally been distilled into two broad categories: constitutive (primary) defensive features, which are always present and provide a relatively constant barrier to attack by herbivores and pathogens, and induced (secondary) defenses, which are formed

locally in response to biotic disturbance (Lewinsohn et al. 1991, Moreira et al. 2014, Trowbridge 2014). The degree to which certain plants will exhibit constitutive versus induced defenses is largely controlled by evolutionary history, with pines (*Pinus* spp.) having characteristically greater constitutive defenses while true firs (*Abies* spp.) tend to exhibit greater inducibility (Lewinsohn et al. 1991, Phillips and Croteau 1999). However, the degree to which species reflect constitute versus induced defenses is strongly moderated by environmental conditions and biotic pressures and can therefor vary substantially within and among populations (Ferrenberg et al. 2017). In addition, the plethora of external factors acting on individuals and populations will collectively select for defensive phenotypes over time and may lead to incongruencies regarding what would otherwise be expected based purely on molecular phylogeny (Moreira et al. 2015). While pines and true firs broadly represent two extremes of defense phenotypes many taxa exist along a gradient of constitutive and inducible defenses and the two are not mutually exclusive (Phillips and Croteau 1999). For instance, many species of pine will biosynthesize additional defensive features, such as traumatic resin ducts and phenolic compounds, in response to insect attack or fungal inoculation (Klepzig et al. 1995, Paine et al. 1997, Phillips and Croteau 1999). These induced responses are regulated by hormones of jasmonic and salicyclic acid, which collectively signal plant defense systems. This process may take days to weeks and is inherently complicated due to antagonistic crosstalk between signaling pathways that can vary substantially within and across species (Siemens et al. 2009, Moreira et al. 2014, Liu et al. 2017b). Synthetic compounds of jasmonic and salicyclic acid have been successfully used in greenhouse experiments to stimulate induced defense and have provided an effective tool for assessing the

development of inducible defenses, which are characteristically difficult to assess in natural settings (Moreira et al. 2014, Moreira et al. 2015, Moreira et al. 2016, Liu et al. 2017b).

Constitutive and induced defenses are the primary barrier to beetle-fungal complexes and consist of intricate anatomical resin duct structures located throughout cortex, xylem and secondary phloem tissues, with the relative frequency and distribution of these structures varying largely across taxa (Wu and Hu 1997, Warren et al. 1999). These systems contain axial and radial tube-like resin ducts, surrounded by secretory parenchyma cells that produce viscous, free-flowing liquid compounds which are quickly mobilized to isolated areas in response to damage from mechanical injury, abiotic stress, or biological agents (Raffa and Berryman 1983, Paine et al. 1997, Nagy et al. 2000, Franceschi et al. 2005, Rodríguez-García et al. 2014). Preformed resin is synthesized and stored within the lumen of resin ducts, which are lined by thin-walled secretory epithelial cells (Phillips and Croteau 1999, Franceschi et al. 2000, Nagy et al. 2000, Baier et al. 2002). Parenchyma cells, containing toxic polyphenolic compounds, are located throughout the secondary phloem and play an important role in mitigating insects and fungal activity due to their ability to immobilize enzymes produced by microorganisms (Bell 1981, Paine et al. 1997, Franceschi et al. 1998, Warren et al. 1999).

There are many types of resin duct structures, including cortical resin ducts, resin ducts in the secondary xylem and phloem, as well as traumatic resin ducts and resin blisters (multicellular sac-like structures), which are produced in response to isolated injury (Bannan 1936, Lewinsohn et al. 1991, Wu and Hu 1997, Nagy et al. 2000, Baier et al. 2002). However, the nature of these structures varies widely among and within species as does the relative rate of resin synthesis and storage methods for oleoresin compounds (Bannan 1936, Phillips and Croteau 1999, Nagy et al.

2000). Pines for instance exhibit an elaborate system of constitutive resin structures which extend in the vertical, radial, and transverse directions throughout the xylem and phloem, often intersecting to form complex networks capable of quickly transporting large volumes of preformed and synthesized resin to various areas of the tree in response to physiological demands (Lewinsohn et al. 1991, Phillips and Croteau 1999, Perrakis and Agee 2006, Wainhouse et al. 2009). On average, species of pine also produce a much larger quantity of resin than other species such as true firs and many genera in the Cupressaceae family, although some species of pine (e.g., ponderosa) are more prolific resin producers than others (e.g., Great Basin bristlecone pine) (Lewinsohn et al. 1991, Wu and Hu 1997, Phillips and Croteau 1999, Smith 2000, Wainhouse et al. 2009, Bentz et al. 2017).

In addition to intra- and inter-species variation there is supportive evidence that developmental stage (ontology) and phenology also have a significant influence on the relative proportion of carbon allocated to resin-based defenses (DeAngelis et al. 1986, Imaji and Seiwa 2010). For instance, Wainhouse et al. (2009) found that Corsican pine (*P. nigra* spp. *laricio*) produced more resin duct structures as the trees aged, compared to Sitka spruce (*Picea sitchensis*), which did not exhibit any notable differentiation in resin duct production across an age gradient. Lodgepole pine (*P. contorta*) has also been found to have fewer chemical defenses in older stem tissues, whereas chemical defenses increase in older white spruce (*P. glauca*) foliage (Quiring 1992, Goodsman et al. 2013). Moreira et al. (2014) found that constitutive resin defenses in the stems and needles of 18 species in Pinaceae decreased for taxa that characteristically occupy lower latitudes and elevations that correspond with higher growth-rates, while inducible defenses increased. In general, slow growth rates associated with reduced vigor

tend to correlate with reduced resin-based defenses, which can increase susceptibility to mortality from insects and pathogens (Kane and Kolb 2010, Perrakis et al. 2011, Ferrenberg et al. 2014, Hood and Sala 2015, Hood et al. 2015, Hood et al. 2016).

The high degree of interspecific variability in natural settings is attributable to several factors, including age, genetics, and growing conditions. Conifers are locally adapted to climate and edaphic controls, which may lead to considerable variation in defensive properties across taxa even when among-population gene flow is present (Cobb et al. 1994, Mitton et al. 1998, Neale and Savolainen 2004, Ferrenberg et al. 2015, Moreira et al. 2015). For instance, tree vigor (relative growth rate) has been shown to influence defensive structures and chemical compounds. Hood and Sala (2015) found that faster growing ponderosa pine (*P. ponderosae*) invested relatively less carbon into resin ducts (lower production per year of growth) but had larger overall resin ducts and produced volumetrically greater amounts of resin compared to slower-growing individuals. Similar results were presented by Hood et al. (2015) and Hood et al. (2016), whereby ponderosa pine that survived bark beetle attacks had faster growth rates (30% wider ring widths in the 30 year period prior to attack) and increased resin duct size (20% larger ducts in surviving trees), and ponderosa pines in areas of reduced stand densities (through experimental reduction treatments involving thinning and prescribed fire) exhibited increased growth rates (2.4 x greater BAI in most recent five years of growth) and greater proportional resin defenses (33% larger ducts in most recent five years and a near doubling in resin duct production). Ferrenberg et al. (2014) also found an increase in resin duct size that corresponded with increased growth rates for both limber pine (*P. flexilis*) and lodgepole pine trees in Colorado. In another study in Crater Lake, Oregon, Perrakis et al. (2011) found that old

ponderosa pine (> 200 years) that were assigned greater crown class vigor ratings exhibited greater ring widths and higher overall resin flow four years after prescribed fire treatments. However, not all studies support a correlation between increased growth rates and increased resin-based defenses against bark beetle induced mortality. For instance, Kane and Kolb (2010) found that while resin duct density and size were greater in ponderosa pine that survived beetle attacks, growth rates between live and dead trees did not differ and growth metrics were not a significant factor in mortality models, whereas resin duct density and size were the best predictors for ponderosa pine mortality. Ferrenberg et al. (2014) found mixed results for lodgepole pine and limber pine, with decreased growth rates associated with less robust resin duct production for lodgepole pines that were killed by bark beetles (over a five-year period prior to tree death), while limber pines killed by bark beetles exhibited greater growth rates and had fewer resin duct structures (over the past 15-20 years prior to tree death), suggesting a trade-off between resource allocation to growth and defense. Although it should be noted that studies that assess growth rates utilize varying combinations of growth metrics, including ring widths, radial growth rates, as well as basal area increments (BAI), over varying time periods (e.g., growth over five, ten, and 20 years) which can complicate interpretation and cross-study comparisons and highlights a need for standardization of methodologies relating to growth and defense research.

While increased resin production may enhance a tree's ability to survive and overcome environmental stressors, the many complexities and high degree of variability in the production of resin ducts and synthesis of resinous compounds complicate our ability to accurately quantify these relationships, particularly in older trees (Baier et al. 2002, Rosner and Hannrup 2004,

Gaylord et al. 2007, Kolb et al. 2007). The notable variability in carbon trade-offs between growth and defense hinders our ability to make general assumptions and further highlights the need for additional research across a range of species and geographic areas (Warren et al. 1999, Baier et al. 2002, Gaylord et al. 2007, Wainhouse et al. 2009).

Chemical Composition and Variability of Oleoresin

Oleoresin acts as a mechanical defense by repelling or entrapping herbivores and as a chemical defense, as oleoresin compounds contain diverse chemical assemblages, including mixtures of terpenoids, which are collectively biosynthesized from five-carbon isoprene compounds (Trapp and Croteau 2001, Mumm and Hilker 2006, Trowbridge 2014, Ferrenberg et al. 2015). Key among these are diterpenes and diterpene acids, which influence viscosity and adhesiveness, as well as volatile terpenes, such as monoterpenes and sesquiterpenes, which act as solvents for transport of molecularly heavier non-volatile diterpenes (Phillips and Croteau 1999, Franceschi et al. 2005, Schaller 2008). When exposed to the atmosphere, volatile terpenes evaporate, resulting in an accumulation of semi-crystalline resin acids, which polymerize (through oxidation) to form a physically hard barrier to insects and pathogens (Phillips and Croteau 1999).

In general, production of defense structures, including preformed and wound-induced resin ducts and oleoresin compounds, is slow (occurring over weeks to months to years) and metabolically demanding, due to biological constraints of cell division and cell differentiation (Moreira et al. 2014, Hood and Sala 2015). Such processes are also resource intensive and the allocation of carbon to development of such features represents a net loss, as resin cannot be reassimilated or further metabolized after formation (Franceschi et al. 2005, Moreira et al. 2014).

However, other chemical compounds produced in various plant organs, such as water-soluble phenolic compounds in xylem and cortical tissues, may be biosynthesized *de novo* through rapid ‘plastic’ change in the metabolism of secretory cells and reassimilated following damage (Lewinsohn et al. 1991, Bonello et al. 2006, Salminen and Karonen 2011, Moreira et al. 2014). These various compounds have interacting, complimenting properties against certain aspects of the beetle-pathogen complex with terpenes and phenolics constituting the primary chemical defense in temperate conifers (Smith 2000, Raffa et al. 2013). However, the diversity of specific chemical compounds within each of these broad groupings is remarkable, with over 22,000 compounds having been described while many have yet to be recognized (Smith 2000, Trowbridge 2014).

The specific role these secondary metabolites play in defense to herbivory and pathogens is poorly understood for a wide variety of species and represents an area of active research (Trowbridge 2014, Jamieson et al. 2017). For instance, terpene compounds have been shown to disrupt nervous-systems and basic metabolic processes in many types of insects (Smith 2000, Seybold et al. 2006, Trowbridge 2014) and some compounds, such as phytoecdysones (a type of triterpene) are thought to mimic insect-molting hormones, which may disrupt the development cycle of larva (Trowbridge 2014). Diterpenes have been found to inhibit fungal activity while certain phenolics (e.g., stilbenes and phenylpropanoid) impede the synthesis of beetle aggregation pheromones and tend to have strong anti-fungal properties, particularly for fungi associated with bark beetles (Hayes and Strom 1994, Hammerbacher et al. 2013, Raffa 2014, Raffa et al. 2017).

Within the broader groupings of defensive chemical compounds monoterpenes are common and abundant in conifers, particularly pines, and have been recognized as a key defense to pathogens and insect activity due to their strong insecticidal properties (Smith 2000, Raffa et al. 2017). Monoterpenes are primarily controlled by genes in the xylem tissue and include olefins (alkene) compounds of heptane, undecane, α -pinene, β -pinene, δ -3-carene, sabinene, myrcene, limonene, β -phellandrene, with various stereoisomers, such as (+)- α -pinene and (-)- α -pinene (Lewinsohn et al. 1991, Smith 2000). Monoterpene synthase (cyclase) varies across species but is generally greater in species with more organized resin-containing structures, such as pines, and lower in species with less organized structures, including true firs and species of Cupressaceae, such as western red cedar (*Thuja plicata*) and redwood (*Sequoia sempervirens*) (Lewinsohn et al. 1991). High concentrations of monoterpenes have been associated with increased tree survival during beetle attacks, although monoterpene concentrations and diversity are widely variable within and across species and may have little to no overlap for taxa within the same genera (Zhao et al. 2010, Boone et al. 2011, Ferrenberg et al. 2017). For example, Ferrenberg et al. (2017) compared monoterpenes across an elevational gradient of lodgepole pine, ponderosa pine, and limber pine in the Colorado Rocky Mountains and found that overall concentrations were greater in lodgepole pine followed by ponderosa pine with the lowest concentrations occurring in limber pine (*P. flexilis*). In contrast, monoterpene diversity showed an opposite trend, with the greatest diversity occurring in limber pine, followed by ponderosa pine and lodgepole pine respectively. Within the sampled populations, inter-specific monoterpene composition and abundance were greatest for lodgepole pine, followed by limber pine and then ponderosa pine (Ferrenberg et al. 2017). The researchers also considered tree size, age, and growth rates and

found mixed results, with monoterpene concentrations decreasing with increased size in lodgepole pine but increasing with increased age and higher growth rates. No significant patterns were found for tree properties and monoterpene concentration and diversity in ponderosa pine and limber pine, however, all three pines exhibited reduced monoterpene concentrations at higher elevations (Ferrenberg et al. 2017). Such incongruent results suggest that monoterpene synthesis is controlled by multiple mechanisms that differ across species and varies in both time and space. In addition, while certain properties of some chemical compounds are reasonably well understood resin chemistry has also been found to change over short temporal windows (e.g., growing season) as well as over longer timescales in response to disturbance, edaphic changes, or competitive interactions following successional trajectories (Campbell and Taylor 2007, Trowbridge 2014, Ferrenberg et al. 2017). Monoterpene concentrations can also change as trees age. For instance, the monoterpene myrcene and limonene have been shown to increase with increasing age, while camphene, α -phellandrene, and gammaterpinene tend to be absent in young trees but increase as the tree ages (Smith 2000). The complex interplay of these different factors limits our ability to generalize chemical defenses and warrants species- and site-specific examinations.

Insect-Pathogen-Climate Influences and Interactions

Physical and chemical defenses are heritable but are also shaped by complex biotic and abiotic factors that can have significant impacts on host-pest relationships and on larger ecosystem function across time and space (Trowbridge 2014). For instance, bark beetles (Curculionidae, Scolytinae) have coevolved with host species and have developed complex behaviors, whereby the insects will actively exploit certain monoterpenes as synergists or

precursors in the synthesis of communication and location (e.g., aggregation and host-identification) pheromones (Seybold et al. 2006). For example, mountain pine beetle (*Dendroctonus ponderosae*) has been found to utilize the monoterpene (–)- α -pinene as a precursor to the synthesis of their aggregation pheromone (–)-trans-verbenol, and the monoterpene myrcene as a chemical catalyst in this process (Seybold et al. 2006, Raffa 2014, Hood et al. 2016, Keeling 2016). β -myrcene and δ -3-carene have also been found to synergize pheromone synthesis as well as increase host-attraction by increasing the flight response of mountain pine beetle (Seybold et al. 2006, Borden et al. 2008). Similarly, adult mountain pine beetles will exploit β -phellandrene to enhance host-recognition (Huber et al. 2000, Miller and Borden 2000). However, certain monoterpenes may also act as attractants for bark beetle predators or enhance predator response to bark beetle pheromones (Seybold et al. 2006). For instance, Mizell et al. (1984) reported that increases in the monoterpenes α - and β -pinene increased the flight response of the predaceous checkered beetle (*Thanasimus dubius*) to aggregation pheromones produced by the southern pine beetle (*D. frontalis*) in *P. taeda* trees of the southeastern U.S. Similarly, Erbilgin and Raffa (2001) found that (–)- α -pinene, (+)- α -pinene, and δ -3-carene increased the flight response of *T. dubius* in the Great Lakes Region of the U.S. However, these relationships are highly complex, as Erbilgin and Raffa (2001) also found that increased concentrations of the monoterpene β -myrcene interrupted the response of *T. dubius* to bark beetle pheromones, which highlights the delicate balance of monoterpene diversity, insect ecology, and ecosystem function.

Bark beetles have a known symbiotic relationship with a variety of Ascomycota fungi, including species in the genus *Ophiostoma* and *Grosmannia* that are vectored into trees during

attack through highly specialized exoskeletal structures (mycangia) in the beetles (Six 2003, Harrington 2005). These fungi play a complex role in catalyzing tree mortality by inhibiting transport of water and nutrients through xylem and phloem tissues (Harrington 2005, Keefover-Ring et al. 2016). The fungi further promote beetle establishment by breaking down cellulose and lignin and creating extensive networks of mycelium, which the beetles actively utilize as a food source to acquire various amino acids, vitamins, and nutrients (Beaver 1989, Six 2003, 2012). The beetle-pathogen complex has necessitated development of defensive compounds that can collectively act against each of these agents separately and further increases the complexity of resin chemistry. For instance, Keefover-Ring et al. (2016) found that ponderosa pine that were inoculated with *G. clavigera* responded through a relatively rapid (within 17 days) increase in concentrations of monoterpenes (22 x increase), sesquiterpenes (57 x increase), and diterpenes (35 x increase), and that these responses were far less pronounced in trees that were mechanically wounded without inoculation. Given these multifaceted, interacting relationships determining the concentrations and diversity of monoterpenes for variety of native and naïve hosts is important in understanding insect ecology, yet this information is critically lacking for a wide variety of species (Raffa et al. 2017).

In addition to the chemical composition of oleoresin, physical resin duct properties (such as resin duct density and duct size) have been shown to have a significant influence on bark beetle related tree mortality. For instance, in a study in northern Arizona, Kane and Kolb (2010) found that ponderosa pine that survived drought and bark beetle attacks (primarily by *Ips* spp.) produced more resin ducts (29-54% increase) with a greater overall duct density (30% increase), duct width (13% increase) and relative duct area (51-66% increase) compared to trees that were

killed. Similarly, Hood et al. (2015) showed that ponderosa pine that survived bark beetle attack (primarily by *Dendroctonus* and *Ips* spp.) across Montana, Utah, Idaho, and Oregon produced larger resin ducts (20% increase), had greater resin duct area per ring (24% increase) and allocated more area to ducts (15% increase) compared to trees that were killed. Ferrenberg et al. (2014) found more nuanced results among lodgepole and limber pines, whereby lodgepole pines and limber pines had more resin ducts (21% and 18% respectively) over the most recent ten years of growth compared to trees that died during bark beetle attack. In addition, lodgepole pines had greater duct density per annual ring (8%) although this result was not statistically significant, while surviving limber pines had greater duct density (37%) over the most recent ten-year growth period. Interestingly, the researchers did not find any difference in resin duct size between lodgepole pines that survived compared to those that died, while surviving limber pines were found to have smaller resin ducts than trees that died. These results are somewhat surprising as resin duct size has been shown to be an important factor in other studies, although research has largely focused on lower elevation species, such as ponderosa pine, which highlights the need to consider these variables across a range of different species and environments.

Climate has also been shown to have significant influence on the development of defensive structures and chemical compounds (Gaylord et al. 2013). Under drought scenarios, increased stomatal closure can reduce carbon assimilation, which can reduce resource availability for production of resin ducts and oleoresin compounds (Sala et al. 2010, Gaylord et al. 2013). Several studies have analyzed resin flow as a surrogate for defense and observations generally support reduced resin flow under drought conditions (Gaylord et al. 2007, Jactel et al.

2012, Gaylord et al. 2013). However, resin flow is considerably variable within and across species as viscosity is highly dependent upon air temperature, with increased viscosity occurring under higher temperatures, and can thus exhibit substantial daily-to-seasonal variability (Gaylord et al. 2007, Gaylord et al. 2013, Rodríguez-García et al. 2014). This has led many researchers to consider vertical resin ducts in the xylem as a proxy for defense, given their persistence and broad correlation with resin production and storage of oleoresin compounds (Kane and Kolb 2010, Gaylord et al. 2013, Ferrenberg et al. 2014, Gaylord et al. 2015, Hood et al. 2015, Hood et al. 2016). For instance, Gaylord et al. (2013) found that experimental 3-year drought reduced both the size and density of resin ducts in piñon pine (which corresponded with increased mortality), however, no treatment effect was detected on resin flow, which led the authors to suggest that resin flow is highly compartmentalized within pinon pines and drawing inference regarding tree-based defenses from isolated samples of resin flow should be avoided.

Drought has also been correlated with increased bark beetle activity that may amplify tree mortality, although the magnitude of bark beetle activity can vary widely depending on intensity and duration of the drought event, further highlighting the complex biotic and abiotic interactions that can cumulatively influence defense properties over time and space (Mattson and Haack 1987, Jactel et al. 2012, Gaylord et al. 2013). In addition, the timing and variability of seasonal changes in precipitation and temperature have been shown to influence resin duct development (Wimmer and Grabner 1997, Vázquez-González et al. 2021). For instance, in a study of climate, tree growth, and resin duct production in Norway spruce (*P. abies*), Wimmer and Grabner (1997) found that resin duct density increased during periods of above-average temperatures, particularly from June to August, while production decreased during periods of above-average

precipitation from May to July. This suggests a nuanced and complex relationship between resource acquisition and allocation of carbon to resin-based defenses.

Abiotic disturbances are also an integral component in the development of defensive features. For example, fire has been shown to have a measurable impact on the formation of oleoresin and resin ducts and may potentially influence resin chemistry by altering nutrient availability (Cannac et al. 2009, Perrakis et al. 2011, Hood et al. 2015, Hood et al. 2016). For instance, Hood et al. (2015) found that total resin duct area increased by 80% one-year post-fire for a site in Montana and by 87% two years after fire for a site in Utah, although the timing of the response varied due to interannual variability in precipitation and temperature. This research also showed that resin duct area decreased by 15% following fire cessation (due to fire suppression) at sites in both Idaho and Oregon, suggesting that the absence of fire in fire-adapted ecosystems may diminish resin defenses over time. In another study, Perrakis et al. (2011) investigated resin flow four years following prescribed fire treatments of varying intensities (in spring and fall burn operations) for old-growth ponderosa pine and found that resin flow increased across burned trees, with a peak in resin flow two years after the prescribed fires. A previous study in Cascade ponderosa pine (Perrakis 2008) compared resin flow across various fire treatments (prescribed fire and bole charring) and mechanical fire-surrogate treatments (crown pruning and root trenching) and found that resin flow only increased in the fire treatments, suggesting that induced resin response may only be stimulated through fire-related injuries or through cambial heating.

To contrast, in a series of experimental treatments using various combinations of thinning and prescribed fire in ponderosa pine dominated forests of the northern Rocky Mountains, Hood

et al. (2016) did not find any increase in resin duct properties (production, size, and area) or in long-term (12 year) resin flow in a ‘prescribed fire only’ treatment. In addition, the researchers observed decreased concentrations of monoterpenes (myrcene, $(-)\text{-}\alpha\text{-pinene}$, 3-carene, and terpinolene) compared to replicants of thinning and combined ‘thinning-and-burning’ treatments (Hood et al. 2016). However, the authors note that fire season and the type of fire (e.g., low-intensity prescribed fires) may have contributed to a lack of an induced resin response, as the prescribed fire treatments were implemented in the late spring, whereas naturally occurring wildfires in the northern Rockies tend to ignite under drier conditions in the late summer and early fall that may increase intensity and stimulate production of resin defenses (Hood et al. 2016). In addition, resin chemistry has also been found to change with time since fire, which could explain some of these differences (Campbell and Taylor 2007). In another study, Powell and Raffa (2011) found that fire-induced injuries had a measurable impact on monoterpene concentrations in both constitutive and induced phloem tissues. Regardless of the severity of injury (low, moderate, or high) monoterpene concentrations throughout induced tissues were reduced (by as much as half) while concentrations in constitutive tissues remained relatively stable. The authors found a reduction in 4-allylanisole, which has been associated with inhibition of bark beetle aggregation pheromones, in both constitutive and induced tissues, and a simultaneous increase in $(-)\text{-}\alpha\text{-pinene}$, which is a precursor for pheromone synthesis. This suggests that fire-injured trees may be less chemically defended against subsequent bark beetle colonization and that fire injuries may stimulate a chemically more favorable environment for bark beetle success. However, reductions in the monoterpene $\beta\text{-myrcene}$ could confound this effect, as $\beta\text{-myrcene}$ has been shown to synergize pheromone biosynthesis, which suggests that

more research is needed to better understand these intricate processes. These seemingly nuanced and contradictory results highlight the complexity of interacting physiological response to disturbance interactions and environmental variability and illustrate a need for additional analyses that consider such interactions across a range of species and environments.

Quantification and Analysis

Resin duct structures and oleoresin flow represent effective metrics for quantifying tree defense and overall resilience to pathogens and insects (Stamp 2003, Gaylord et al. 2007, Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015, Hood et al. 2016). Research suggests that carbon allocation to resin duct production may be better measures of vigor than carbon allocation to radial growth, which can have significant impacts on tree survival during disturbance events (Gaylord et al. 2007, Wainhouse et al. 2009, Kane and Kolb 2010, Ferrenberg et al. 2014). However, methods for analyzing resin ducts and oleoresin production vary substantially and there is a general lack of uniformity in researching this topic (Nagy et al. 2000, Baier et al. 2002, Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015). For instance, some researchers have focused on vertical resin ducts in the xylem (Nagy et al. 2000, Kane and Kolb 2010, Ferrenberg et al. 2014, Hood and Sala 2015, Hood et al. 2015, Hood et al. 2016) and radial resin ducts (Rodríguez-García et al. 2014) while others have used resin ducts in cortical tissues (Moreira et al. 2015) or in needles (Moreira et al. 2014, Moreira et al. 2016, Liu et al. 2017b), with considerable variation in what properties are measured and analyzed (e.g., duct production, density, size, and area). Such inconsistencies in resin duct methodology complicates efforts to draw conclusions across research. For example, work by Kane and Kolb (2010) and Ferrenberg et al. (2014) compared resin duct properties and growth metrics between

trees that survived and those that died following bark beetle attacks, however, their resin duct metrics did not specifically account for ring area, which is a point argued by Hood and Sala (2015) to develop a standard method for quantification of resin duct measures.

Analysis of resin duct structures and oleoresin flow can ultimately lead to a better understanding of tree physiology and their numerous coping strategies in responding to disturbance agents. However, relatively little research has been conducted given the numerous types of resin ducts and the various physical and chemical properties associated with these defense features, particularly as they relate to growth and defense characteristics (Warren et al. 1999, Wainhouse et al. 2009, Kane and Kolb 2010). Many researchers for instance have sought to analyze vertical and radial resin ducts in the secondary xylem (Blanche et al. 1992, Wimmer and Grabner 1997, Lombardero et al. 2000, Smith 2000, Baier et al. 2002, Rosner and Hannrup 2004) yet relatively little investigation has been conducted as to the nature of resin ducts originating in secondary phloem tissue (Warren et al. 1999, Franceschi et al. 2000). Resin systems within the phloem are fundamental in the initial defense of trees to insects and pathogen attack as they contain many important phenolic compounds, which help mitigate spread of these organisms (Klepzig et al. 1995, Franceschi et al. 2000, Baier et al. 2002). Similarly, only two studies have looked to relate the chemical composition (e.g., monoterpene concentrations and diversity) of oleoresin compounds with morphological characteristics of resin duct structures (Hood et al. 2016, Mason et al. 2019). When considering the broad spectrum of species that exhibit different strategies and priorities in the development of these types of primary defense systems there is a great deal of opportunity to analyze these features to better understand their overall function and variability among and within genera. In general, changes in the physical and

chemical properties of resin ducts and oleoresin compounds relating to ontogeny and phenology is poorly understood for a wide range of species and will require more nuanced research across a broader range of habitats (Boege and Marquis 2005, Barton and Koricheva 2010, Muola et al. 2010, Ferrenberg et al. 2015).

Hood and Sala (2015) established an effective framework for the quantification of key resin duct metrics in a study analyzing constitutive and induced resin response to simulated disturbance. Specifically, the authors categorized vertical resin ducts in secondary xylem tissues into standardized measures, where resin duct properties are related to growth rate and non-standardized measures, where resin duct properties are measured independent of growth rate (Table 1) (Hood and Sala 2015). Such a classification of key metrics for assessing resin-based defenses can help unify methodologies relating to resin duct analysis and future research should adopt this strategy to develop a more holistic view of the morphological characteristics of resin duct defenses. In addition, various physical and environmental conditions have been shown to influence resin duct formation and resin duct properties, including genetics, nutrient availability, drought and seasonal changes in precipitation and temperature, disturbance, as well as tree growth rates and herbivore pressures (Figure 1). Given the high degree of variability in analyzing resin-based defenses future research should consider this suite of biotic and abiotic factors that can influence resin-based defenses.

Table 1. Metrics for quantifying vertical resin duct properties in secondary xylem tissues of conifers. From Hood and Sala (2015).

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 ²
Relative Duct Area (% annual ring)	Standardized	Total duct area divided by ring area ² x 100

¹Type: Unstandardized – adjusted for growth rate; Standardized – unadjusted for growth rate.

²Ring Area: (ring width x core diameter).

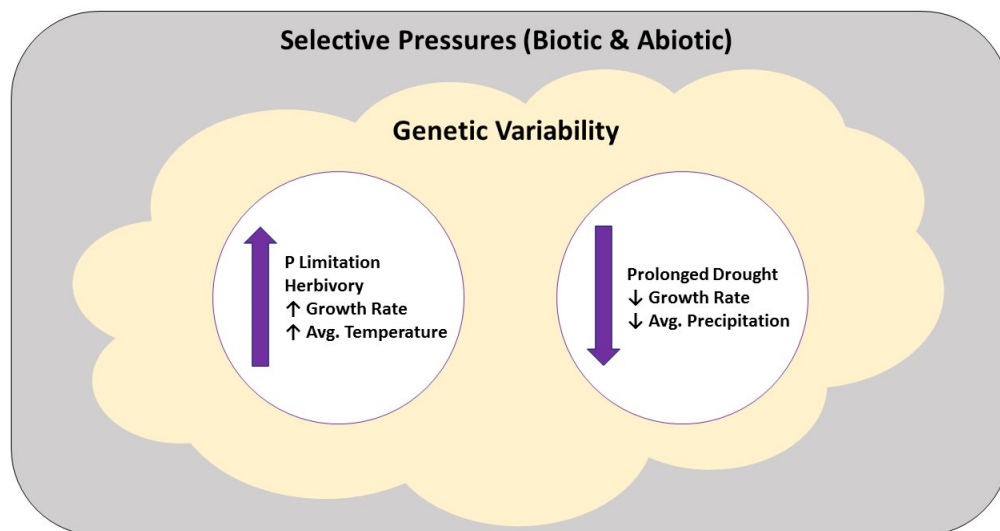


Figure 1. Conceptual diagram reflecting factors that can shape resin duct properties (broadly categorized as duct size, area, production, and density). *Note:* Amorphous lines for Genetic Variability are meant to highlight the considerable differences within and among species that can shape heritable resin duct properties. Adopted from Ferrenberg et al. (2014).

Research Significance for Whitebark Pine

Whitebark pine (*P. albicaulis*) is a foundation species in the northern Rocky Mountains that has been shown to influence soil hydrology, increase snowpack retention, and promote vegetative establishment in subalpine settings (Keane and Arno 1993, Campbell and Antos 2000,

Kendall and Keane 2000, Tomback and Kendall 2001, Kim et al. 2003, Bockino and Tinker 2012). Whitebark pine also serves as an important food source for grizzly bears and a host of other wildlife (Arno and Hoff 1989, Tomback and Kendall 2001). However, there is growing concern that whitebark pine may be largely extirpated from its current habitat over the next century due to cumulative impacts of bark beetles, fire suppression, increased competition, and the invasive pathogen white pine blister rust (*Cronartium ribicola*), which has already decimated populations (by as much as 80%) in some areas (Keane and Arno 1993, Kendall and Keane 2000, Macfarlane et al. 2013). In addition, modeling efforts show that the species may be significantly reduced in the Greater Yellowstone Ecosystem in the next century based on changing habitat suitability (Chang et al. 2014). Given these collective biotic and abiotic pressures there is a critical need to develop a more comprehensive assessment of growth and defense characteristics relating to this important species.

In this context, Raffa et al. (2013) and Raffa et al. (2017) looked at the chemical composition of oleoresin in both lodgepole pine (the native host for mountain pine beetle), as well as whitebark pine (a naïve host) to determine how bark beetles may interact with these populations. The research found that whitebark pine is a chemically more suitable host than lodgepole pine, which may exacerbate mortality under projected epidemic scenarios. For instance, the researchers found that whitebark pine has thicker phloem on average compared to lodgepole pine, which would make it more habitable for overwintering beetle populations. Whitebark pine was found to have greater concentrations of the monoterpene (–)- α -pinene, a precursor for pheromone biosynthesis (2.4 x increase), as well as myrcene and δ -3-carene, which catalyze pheromone production (1.6 x and 2.1 x increase, respectively), and lower concentrations

of the phenylpropanoid 4-allylanisole, which inhibits pheromone production (2.3 x decrease) compared to lodgepole pine (Raffa et al. 2017). To contrast, lodgepole pines had greater overall concentrations of monoterpenes in both constitutive and induced tissues and responded to simulated attack by producing significantly more 4-allylanisole (3.8 x increase) to inhibit bark beetle aggregation and less myrcene (0.56 x decrease), which catalyzes pheromone synthesis (Hayes and Strom 1994, Raffa et al. 2017). Lodgepole pines also had greater concentrations of nitrogen and calcium, which have been shown to be important minerals in signaling induced defenses (Rantty et al. 2016, Raffa et al. 2017). Collectively, these findings suggest that lodgepole pines have more thorough chemical defenses against mountain pine beetle while whitebark pine are chemically more susceptible to successful bark beetle colonization, likely resulting from a less continuous and more fragmented historic exposure of the naïve host to this disturbance (Raffa et al. 2013, Raffa et al. 2017). However, despite the chemical suitability of whitebark pines for colonization, the researchers found that under endemic scenarios mountain pine beetles will preferentially attack lodgepole pines, although this preference decreased with increasing proportions of whitebark pine at the stand level (Raffa et al. 2013, Raffa et al. 2017). This is likely attributable to the greater concentrations of the monoterpene β -phellandrene in lodgepole pines (2.6 x increase), which adult pine beetles exploit in host-recognition (Miller and Borden 2000, Raffa et al. 2017).

The implications of increased susceptibility of whitebark pine to bark beetles is concerning, given projections for enhanced bark beetle activity over the next several decades. Temperature has been shown to exert a strong control on the regulation of life-cycle development in mountain pine beetle populations. For instance, prolonged periods of

temperatures less than -40°C will kill nearly all (~99-100 %) of beetle populations, regardless of developmental stage, whereas changes in climate that result in increased summer seasons and decreased winter periods have well researched correlations with increased bark beetle activity, as many species of these insects are able to reproduce for longer time periods under these warmer conditions (Buotte et al. 2016, 2017). A key area of concern is an observed trend in increased winter minimum temperatures, which is particularly pronounced in the northern Rocky Mountains (Pederson et al. 2009, Pederson et al. 2011, Pederson et al. 2013) and which has been correlated with recent, epidemic outbreaks (Williams and Liebhold 2002, Bentz et al. 2010). Continued increases in winter temperatures has the potential to increase the climate suitability for increased brood production, which could exacerbate tree mortality throughout the broader range of whitebark pine over the next century (Chang et al. 2014, Buotte et al. 2016, 2017).

Increased temperatures in the winter combined with an earlier onset of spring have led to decreased winter snowpack in the northern Rocky Mountains and a shift to longer and drier summer seasons, which can amplify stress in trees (Westerling et al. 2006, Pederson et al. 2009, Pederson et al. 2011, Pederson et al. 2013). This drought-induced stress can reduce tree responsiveness and increase susceptibility to fire, insects and pathogens (Rigling et al. 2003, Smith et al. 2005, Fettig et al. 2007, Maloney et al. 2008, Kane and Kolb 2010). Environmental stressors in general reduce vigor, inhibit growth, and lead to premature mortality within older age classes (Perrakis and Agee 2006). Mortality in these older cohorts of whitebark pine is particularly concerning due to well-documented rates of high mortality in younger age classes as a result of the rampant spread of white pine blister rust, which preferentially targets younger trees and cone-bearing branches (Kim et al. 2003, Tomback and Achuff 2010).

The overall pressures influencing whitebark pine mortality warrant special consideration for this important species, yet relatively little research exists relating growth and defense characteristics across a range of habitats. Understanding resin defense systems is of particular importance in this regard as these advanced biochemical structures represent the primary defense mechanism of whitebark pine to biotic disturbance. Knowledge of resin duct structures and oleoresin production can provide valuable insight into overall defensibility of these trees to damaging agents, which is especially important given the sensitivity of whitebark pine to unforeseeable changes in disturbance regimes. In addition, linkage of morphological characteristics of resin ducts with resin chemistry (e.g., monoterpene concentrations and diversity) is an area of limited research and one that could benefit from future studies.

CHAPTER THREE

WHITEBARK PINE (*PINUS ALBICAULIS*) GROWTH AND DEFENSE IN RESPONSE TO MOUNTAIN PINE BEETLE OUTBREAKS

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

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Whitebark pine (*Pinus albicaulis*) growth and defense in response to mountain pine beetle outbreaks

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ABSTRACT

Whitebark pine (*Pinus albicaulis*) is a critical forest species of Northern Rocky Mountain upper subalpine ecosystems, yet little is known about the physiological response of whitebark pine to disturbance (e.g. fire, bark beetles, and pathogens) across a range of diverse environmental gradients. Resin-based defenses have long been recognized as the primary mechanism by which conifers respond to attack by bark beetles and pathogens and several studies have linked resin duct properties to survivorship during periods of increased beetle activity. However, to our knowledge, no studies have compared axial resin ducts in the secondary xylem of whitebark pine across pairs of living and dead whitebark pine trees to better understand survivorship following multiple disturbances including mountain pine beetle and white pine blister rust. We found a clear distinction in growth and defense characteristics between live and dead whitebark pine. Across our study sites on the Flathead Indian Reservation in northwestern Montana, live whitebark pine produced larger resin ducts with a greater annual investment in resin-based defenses than whitebark pine that died. Resin duct size, duct area, and relative duct area were all greater in live whitebark pine (by 56%, 48%, and 57%, respectively) and these were the most important variables influencing whitebark pine survivorship. In contrast, whitebark pine that had died grew faster over time (22% larger ring widths) than their live counterparts and also produced more resin duct structures (20% more ducts on average). Whitebark pine at our study sites exhibit differing strategies in the allocation of resources toward growth and defense, with the majority of survivors of recent disturbance investing more in defensive structures than growth. Our results support the idea that maintaining genetic variability and the associated suite of differing physiological traits promotes diverse response strategies to a complex array of biophysical and biological stressors that might leave a species vulnerable to extinction across its range.

1. Introduction

Whitebark pine (*Pinus albicaulis*) is a critical forest species of U.S. Northern Rocky Mountain subalpine ecosystems (Arno and Hoff, 1989, Keane, 2000, Tomback et al., 2001). Often dominating treeline, whitebark pine is considered a keystone species, with high cultural and ecological significance (Cole, 1990, Mills and Doak, 1993, Tomback et al., 2001). Whitebark pine is one of the few species that thrives at high altitudes (> 2000 masl) where they stabilize erosive slopes; promote snow accumulation; increase snowpack retention and effectively modulate moisture loss in subalpine regions, influencing water availability at lower elevations (Arno and Hoff, 1989, Tomback and Kendall, 2001, Bockino and Tinker, 2012). Whitebark pine seeds are highly nutritious and an important food source for several species of wildlife, including grizzly bears (*Ursus arctos horribilis*), black bears (*U. americanus*), and a number of small mammals and birds. Seeds are predominately dispersed by the Clark's nutcracker (*Nucifraga columbiana*), often cached in disturbed areas, such as recently burned landscapes, where, if forgotten, the cached seeds germinate to become the whitebark pine forests of tomorrow. Whitebark pine is an important pioneer species, as the hearty seedlings colonize areas and ameliorate site

conditions, promoting additional vegetative growth through tree island initiation (Tomback et al., 1993, Tomback et al., 2016). However, there is growing concern that whitebark pine may be largely extirpated from its current habitat over the next century due to cumulative impacts of climate change, insect-related mortality, changing fire regimes, increased competition from shade-tolerant species, and the invasive exotic pathogen white pine blister rust (*Cronartium ribicola*). These stressors, primarily blister rust, have already decimated populations (by as much as 90%) in some areas of the Northern Rocky Mountains (Keane and Arno, 1993, Kendall and Keane, 2000, Macfarlane et al., 2013, Shanahan et al., 2016, Amberson et al., 2018).

While insects, fire, disease, and secondary effects of drought (including increased tree stress and predisposition to these disturbances) have contributed to recent mortality of whitebark pine, these processes also play an important role in the long-term establishment and persistence of Northern Rocky Mountain whitebark pine forests. However, historical records detailing the relationship between disturbance and whitebark pine ecosystem dynamics are particularly lacking. For example, a metaanalysis of paleoecological data suggest that the abundance of *Pinus* subgenus *strobus*, as inferred from pollen percentages, was greater (particularly at lower elevations) during the Holocene

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summer insolation maximum (~9 k.y.a.) when summer temperatures were warmer, bark beetle activity was prevalent, and fire activity was high in the Western U.S. (Iglesias et al., 2015). This research suggests a possible complex interaction between temperature, fire, and the insect dynamics that has developed over thousands of years and the need to better understand how five needle pines (*P. strobus* subgenus) have persisted in the past and will respond to multiple disturbances in the future. Research is needed to better understand the physiological response of whitebark pine to fire frequency and severity and beetle outbreaks across a range of diverse environmental gradients. This is especially true given that current species risk assessments for whitebark pine rely on bioclimatic models that include little information on disturbance processes and physiological adaptability of whitebark pine to interactions and feedbacks between changing climatic and disturbance regimes.

Better understanding how physiological characteristics of whitebark pine (specifically the allocation of resources to growth and defense) influence survival following the cumulative effects of fire, bark beetles, and white pine blister rust would provide critical information to resource managers tasked with managing this keystone species. Across all species of pine, research shows that during beetle attacks, even at peak epidemic levels, there are mature individuals that remain uncolonized by beetles, and that these resistant individuals may have different defense traits (Strom et al., 2002, Hood et al., 2016). For example, Strom et al. (2002) found oleoresin flow from progeny of mature loblolly pines (*P. taeda*) that “escaped” mortality during an outbreak of southern pine beetle (*Dendroctonus frontalis*) was higher compared to the general population. Similarly, several studies have established the importance of resin ducts as defense traits in ponderosa pine (*P. ponderosae*) and piñon pine (*P. edulis*) (Kane and Kolb, 2010, Gaylord et al., 2013, Hood et al., 2016). Survivorship of individuals within such diverse populations suggests an intrinsic level of resistance that can be genetically passed on to the survivors’ progeny (de la Mata et al., 2017, Six et al., 2018). This is supported by research that shows that the capacity to produce defensive features (such as resin ducts) and the composition of chemical defenses (e.g. chemotypes) are under strong genetic control, resulting from site-specific selective pressures that moderate growth and defense relationships over time, although there is considerable variability in this response within and across species (Hannrup et al., 2004, Rosner and Hannrup, 2004, Moreira et al., 2014, Moreira et al., 2015, Westbrook et al., 2015, Moreira et al., 2016).

Within conifers, resin-based defenses have long been recognized as the primary mechanism by which trees respond to attack by bark beetles and pathogens (Langenheim, 1994, Phillips and Croteau, 1999, Trapp and Croteau, 2001, Franceschi et al., 2005, Keeling and Bohlmann, 2006). Resin ducts are permanent anatomical features within the secondary xylem and have been shown to correspond with resin flow, such that greater total area of resin ducts facilitates increased production, storage, and mobilization of oleoresin to sites of wounding (Hood and Sala, 2015). As resin ducts are produced regularly (typically every year to every few years), they can be measured, in conjunction with tree rings, to assess resource allocation to growth and defense over time. Several researchers have linked physical properties of resin ducts to survivorship during periods of increased beetle activity (Kane and Kolb, 2010, Ferrenberg et al., 2014, Hood and Sala, 2015, Hood et al., 2015, Hood et al., 2016). For instance, in conifer forests of northern Arizona, Kane and Kolb (2010) found that ponderosa pine that persisted through drought and stand-level bark beetle activity produced more resin ducts with a greater overall duct density and relative duct area than trees that were killed. Ferrenberg et al. (2014) found that lodgepole and limber pines in Colorado both produced more resin ducts over the most recent ten years of growth compared to trees that died during a bark beetle outbreak, while Hood et al. (2015) found that ponderosa pine that survived beetle attacks in Montana produced larger resin ducts with a greater relative area.

Drought and wildfire are also thought to influence the development

of defensive traits. For example, fire has been shown to have a measurable impact on the formation of resin ducts and oleoresin and may potentially influence resin chemistry by altering nutrient availability (Cannac et al., 2009, Perrakis et al., 2011, Powell and Raffa, 2011, Hood et al., 2015, Hood et al., 2016). Further, under drought scenarios, increased stomatal closure can reduce carbon assimilation, which can reduce resource availability for the production of resin ducts and chemical compounds, which may predispose trees to insects and pathogens (Gaylord et al., 2007, Six and Adams, 2007, Sala et al., 2010, Gaylord et al., 2013). While fewer studies have investigated the impacts of drought on resin duct characteristics, Gaylord et al. (2013) found that an experimental three-year drought reduced both the size and density of resin ducts in piñon pine. Wimmer and Grabner (1997) found that Norway spruce (*Picea abies*) resin duct density increased during periods of above-average temperatures while production decreased during periods of above-average precipitation. This highlights complex relationships between physiological processes and the influence of climate that is poorly explored for many tree species.

While physical and chemical defenses are critical for mediating plant-herbivore-pathogen interactions, the production and proliferation of defensive structures and chemical compounds is energetically expensive with high demand for relatively limited carbon resources (Lewinsohn et al., 1991, Stamp, 2003, Moreira et al., 2014, Trowbridge, 2014). Given adequate resource availability, research suggests trees will preferentially allocate resources toward primary production, including diameter and height growth as well as development of leaves, cones, and roots (Trowbridge, 2014, Jamieson et al., 2017). However, in processes that are less well understood, trees simultaneously utilize carbon reserves in the production of defensive features (e.g. resin ducts) and secondary metabolites (e.g. terpenoids, alkaloids, and phenols) to protect crucial tissues from damage by herbivory and pathogens (Trowbridge, 2014, Jamieson et al., 2017). The divergent allocation of resources towards biophysical processes moderating growth and defense has long been thought to reflect an important tradeoff in plant physiology and many hypotheses have been proposed to explain different patterns observed in nature and laboratory experiments (Hermes and Mattson, 1992, Stamp, 2003, Endara and Coley, 2011, Trowbridge, 2014).

Central to these hypotheses is the broader idea that resource allocation towards defense enhances plant fitness and that inter- and intra-specific variability can be explained by tradeoffs between growth and defense, whereby external factors including resource availability, abiotic stress, and pathogen/herbivore pressures all influence investment in defensive systems (Moreira et al., 2014, Trowbridge, 2014, Ferrenberg et al., 2015, Jamieson et al., 2017). Numerous studies looking to establish evidence for specific hypotheses have found that they are not mutually exclusive and that the allocation of resources to growth and defense depends on specific species and environmental conditions (climate, topography, intra- and inter-specific competition, trait heritability, and disturbance legacies) (Stamp, 2003, Trowbridge, 2014, Mason et al., 2019).

Currently, evaluation of growth and defense tradeoffs has focused on relatively few species of limited size and age classes (Rosner and Hannrup, 2004, Ferrenberg et al., 2015). To our knowledge, only one other study (Mason et al., 2019) has considered such tradeoffs for mature whitebark pine in natural settings and no studies have compared axial resin ducts in the secondary xylem of whitebark pine across pairs of living trees and trees that were killed by varying combinations of mountain pine beetle (*D. ponderosae*) and white pine blister rust. In this study, we evaluated whether diameter growth and structural characteristics of resin ducts differed between live and dead whitebark pine trees and whether trees that lived (hereafter “live trees”) and trees that died (hereafter “dead trees”) responded similarly to climate. We hypothesized that: H1) live whitebark pines would exhibit greater and more sustained growth than dead whitebark pines during the overlap of their lifespan, and H2) currently live whitebark pines would have

increased resin duct defenses, specifically, increased resin duct size, number, and cumulative area than whitebark pines that were killed by disturbance. We anticipated that surviving individuals would have more robust constitutive defenses and that there would be a detectable difference in growth and defense anatomy compared to individuals that have died. We also investigated whether seasonal climate had any influence on whitebark pine growth and defense, as other research has shown strong influence of multiple disturbance interactions (e.g., drought and beetle attacks) acting on survivorship (Gaylord et al., 2013, Gaylord et al., 2015).

Our hypotheses are based on previous research which suggests that vigorous trees (those with increased growth and thus increased photosynthetic capacity) can fix comparatively more carbon and are thus able to allocate a greater 'surplus' of carbon annually to the development of physical and chemical defenses (Ferrenberg et al., 2014, Hood and Sala, 2015, Hood et al., 2016). However, not all research supports a correlation between greater growth rates and increased defensive capacity in trees. For example, several studies suggest an opposite strategy, whereby trees growing in resource-rich environments may invest less in constitutive defenses (such as axial resin ducts) relative to growth. It is argued that trees adopting this strategy rely more on inducible defenses, which are synthesized *de novo* in response to attack or injury and may thus allocate more annual carbon towards primary production at the expense of defense (Ferrenberg et al., 2014, Moreira et al., 2014, Moreira et al., 2015). These seemingly conflicting results suggest that growth and defense strategies employed by trees are complex and species specific, likely varying across habitat and ontogeny. Thus, more research is needed to investigate these relationships across a range of species and habitats.

The lack of empirical studies evaluating growth and defense theory for high elevation pines that experience multiple, complex disturbance stressors, highlights a need to better understand growth and defense characteristics across a range of biophysical gradients. Understanding resin defense systems is of particular importance in this regard as these advanced biochemical structures represent the primary defense mechanism of whitebark pine to biotic disturbance. Evaluating relationships between resin duct structures and oleoresin production and disturbance can provide valuable insight into overall defensibility of these trees to stressors that are projected to increasingly impact this important cultural and ecological species.

2. Methods

2.1. Site description

Data for this study were collected on two high-elevation whitebark pine sites on the Flathead Indian Reservation during the summer of 2016 as part of a larger fire history reconstruction for the Confederated Salish and Kootenai Tribes (CSKT; Fig. 1). We selected sites where whitebark pine was an important component of the canopy (> 15% canopy cover). Canopy species at both sites consist of lodgepole pine (*P. contorta* var. *latifolia*) and whitebark pine, with smaller components of subalpine fir (*Abies lasiocarpa*) and Engelmann spruce (*Picea engelmannii*). Herbaceous cover across both sites consists primarily of grouse whortleberry (*Vaccinium scoparium*), common huckleberry (*V. membranaceum*), dwarf huckleberry (*V. cespitosum*), bear grass (*Xerophyllum tenax*), and smooth woodrush (*Luzula hitchcockii*). *Menziesia* (*Menziesia ferruginea*) was also a sizable understory component at the Boulder site (BLD) but was not found at the Three Lakes Peak site (3LK). Evidence of disturbance legacies, including wildfires, insect and pathogen outbreaks were common at both sites.

The 3LK site is located in the Reservation Divide Mountains between the Flathead Indian Reservation and Missoula, Montana. Mean annual temperatures at this location are generally cool, ranging from 2° to 5° C with a 30- to 60-day frost-free period. Precipitation averages 1143 mm per year, which occurs primarily as snowfall from November through

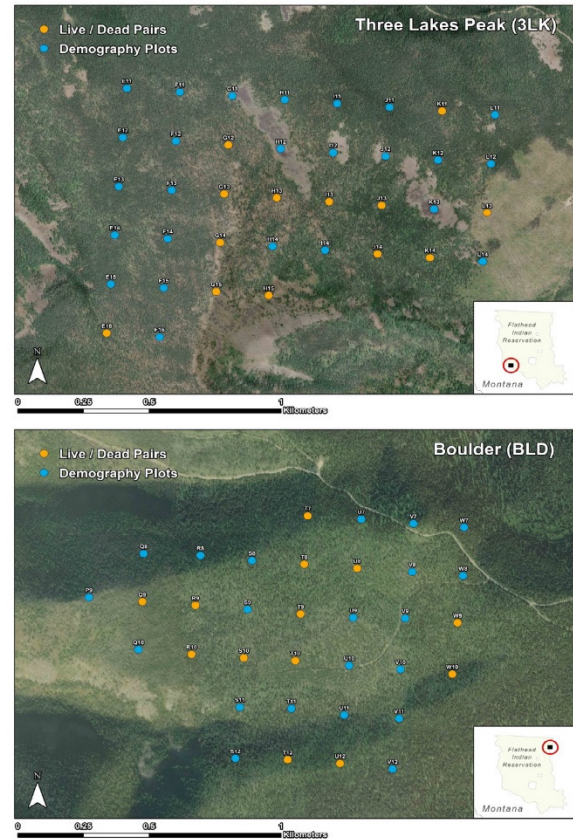


Fig. 1. Three Lakes Peak (top) and Boulder (bottom) study sites on the Flathead Indian Reservation in northwestern Montana. Permanent plots ("Demography Plots") were located on a 200 m grid as part of a fire history study for the Reservation. Plots cover approximately 100 ha at 3LK and 88 ha at BLD. Orange plots (covering approximately 24 ha at each site) had suitable pairs of live/dead whitebark pine trees (those that were closer than 20 m and less than 3 cm difference in DBH). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

March. Soils at this site are from the Phillcher series and Halloway-Waldbillig series and consist of gravelly ashy silt loams that are moderately drained. Whitebark pine is a notable tree component at 3LK, comprising 15% of all sampled individuals (225 out of 1507 trees). 3LK is characterized by complex disturbance legacies, with evidence for mixed- and low-severity fire regimes on southern and eastern aspects and lethal fire regimes on northern and western aspects. The area has also been affected by numerous large-scale bark beetle outbreaks, occurring in the 1930s, 1960s–1980s and most recently 2002–2009 (Arno and Hoff, 1989, Kipfmüller and Swetnam, 2002, Buotte et al., 2017, van de Gevel et al., 2017). The majority of whitebark pine mortality at this location was due to cumulative impacts from mountain pine beetle and white pine blister rust, which was introduced to this region of the Northern Rocky Mountains circa 1950 (Mielke, 1943, Samman et al., 2003, Geils et al., 2010). Of the 225 sampled whitebark pine trees, 73% were dead, with the majority of dead trees (88%) showing evidence of beetle activity (*J*-shaped galleries along the tree stem and/or presence of blue-stain fungi (*Grossmannia clavigera*), which is vectored by bark beetles during colonization.

The BLD site is located approximately 74 km away in the Western

Mission Mountains on the Eastern side of Flathead Lake. Mean annual temperatures for this site are similar to 3LK, with average temperatures ranging from 1 to 6 °C with a 20- to 70-day frost-free period. This area receives slightly more precipitation than 3LK, with an average of 1397 mm annually, which occurs primarily as snowfall from November through March. Soils at this site are of the Halloway series and consist of gravelly silt loam that are also moderately drained. Whitebark pine was more prominent at this site, comprising 36% of all sampled individuals (476 out of 1320 trees). Of these, 14% were live and 86% were dead, the majority of which (71%) had evidence of bark beetle activity. This area is characterized largely by mixed- and lethal-severity fire regimes and had a similar recent history of bark beetle activity in the 1930s, 1960s–1980s and 2002–2009 (Arno and Hoff, 1989, Kipfmüller and Swetnam, 2002, Buotte et al., 2017, van de Gevel et al., 2017). White pine blister rust was also evident at this site but was not as extensive as at 3LK (unpub data).

2.2. Field sampling methods

A 200 m grid was overlain across each site using a combination of remote sensing and field surveying for optimal placement (Fig. 1). The sample grid was selected across whitebark pine stands ranging from low- to high-severity fire regimes. This gridded sampling design effectively captured much of the topographic and vegetative variability at each of the sites, providing for a more accurate assessment of disturbance legacies and microsite differences in forest structure and stand composition. Within the 200 m grid, individual plots (hereafter “macroplots”) were located 200 m apart along cardinal directions, with a total of 38 plots for the 3LK site (covering approximately 100 ha) and 34 plots for the BLD site (covering approximately 88 ha; Fig. 1).

Each macroplot was spatially referenced with Garmin Rhino 650 GPS units and plot centers were permanently marked and tagged. From the macroplot center, four 10 m wide, 100 m long belt transects were established in each cardinal direction (north, south, east, and west) (Fig. 2). The closest ten trees within each 100 m transect were sampled (to a maximum distance of 100 m) with a diameter threshold of 15 cm diameter at breast height (DBH) (Heyerdahl et al., 2014). Data collected for each tree at each macroplot included species, condition (live or dead), size (DBH), canopy base height, crown class (dominant, codominant, intermediate, or suppressed), tree height, evidence of fire. We collected increment cores from 40 sample trees per macroplot near the base of each tree (at or below 15 cm from the root collar) using Haglöl 4.3 mm diameter increment borers. A 25 m² (5.6 m diameter) was also established around plot center, where all understory vegetation was identified and estimates made for percent cover of all species (Fig. 2).

2.3. Live/dead tree pairing

To assess the influence of disturbance on whitebark pine physiology (growth and defense characteristics) live trees and corresponding dead trees (hereafter “pairs”) were identified from the macroplot data. Suitable pairs were identified based on distance (< 20 m apart) and size (< 3 cm difference DBH) to control for potential microsite differences (Kane and Kolb, 2010, Hood and Sala, 2015). As a result of these pairing criteria, only a subset of the macroplots contained suitable pairs for this analysis (Fig. 1). Overall, 30 pairs were identified at the 3LK site across 13 macroplots and 42 pairs were identified at the BLD site across 13 macroplots for a total of 144 sampled trees (72 live and 72 dead; Fig. 1). It is important to note that our live trees did not exhibit any signs of active bark beetle attack (such as pitch-tubes and canopy die-back), so our investigation of resin duct characteristics between live and dead whitebark pine trees relates to host-selection, rather than a post-selection response in live trees.

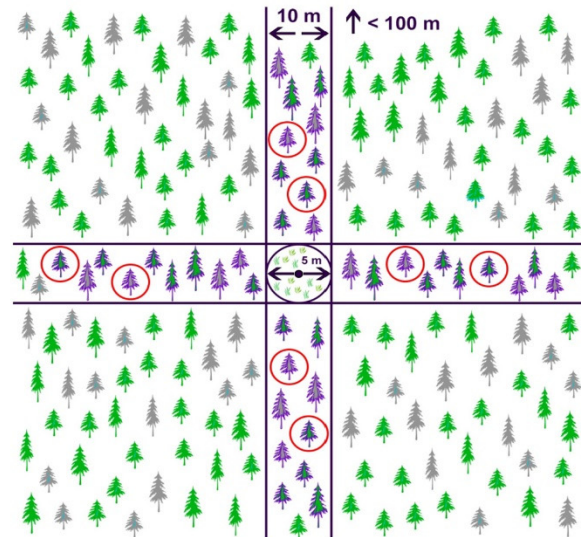


Fig. 2. Conceptual diagram of the plot sampling protocol. From plot center, four 10 m wide belt transects were extended in each cardinal direction and the closest 10 trees (> 15 cm DBH) in each transect were sampled (to a maximum distance of 100 m). A 5.6 m diameter plot was established around plot center to measure herbaceous vegetation. Coloring: Live trees (green); dead trees (grey); sampled trees for fire history study (purple highlighting); live/dead pairs of whitebark pine trees (red circles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.4. Sample preparation

Increment cores were air-dried and processed using standard dendrochronological techniques (Holmes et al., 1986, Cook and Kairiukstis, 1990, Stokes and Smiley, 1996). All ring widths were measured to the nearest 0.001 mm using a Velmex measuring stage interfaced with the recording software Measure J2X (Voortech Consulting, 2005). Tree-ring series were crossdated visually and graphically using ring-width patterns and frost rings (LaMarche and Hirschboeck, 1984). Crossdating was facilitated by developing site-specific chronologies from ring-width measurements, which were statistically checked using COFECHA (Holmes et al., 1986, Grissino-Mayer, 2001).

Individual tree cores were scanned at high resolution (1200 d.p.i.) on an Epson V550 flatbed scanner and resin duct features were measured using the program ImageJ (version 1.46r, National Institutes of Health, Bethesda, MD, USA). All resin ducts in the resulting image were measured to the nearest 1×10^{-7} mm² using the ellipse tool and the calendar year in which they formed was documented. For defense metrics, we followed the methods outlined by Hood and Sala (2015) and collected measurements across five categories, including non-relativized defense measures of resin duct size (mean size of all resin ducts per annual ring; mm²), duct production (number of ducts year⁻¹), and total duct area (sum of resin duct size: mm² year⁻¹), as well as resin duct measures that have been relativized to ring area, including duct density (number of resin ducts mm⁻² year⁻¹) and relative duct area (% annual ring) (Kane and Kolb, 2010, Hood and Sala, 2015, Hood et al., 2016).

2.5. Data analysis

To assess possible tradeoffs in the allocation of carbon towards growth and defense we evaluated relationships between resin duct metrics and radial growth for live and dead trees (Kane and Kolb, 2010,



Fig. 3. Example of increment cores from a pair of live (top) and dead (bottom) whitebark pine. Trees were paired based on distance (< 20 m) and size (< 3 cm difference in diameter at breast height). Growth and defense measures were compared across all overlapping years, as well as 20-, 10-, and 5-year periods (series of progressively shorter purple lines in the figure). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Ferrenberg et al., 2014, Hood and Sala, 2015). Following the methods of (Kane and Kolb, 2010), we calculated growth and defense metrics annually (for all overlapping years between live and dead trees), as well as in 20-, 10-, and 5-year periods corresponding to the 20-, 10-, and 5-year windows prior to mortality (Fig. 3). For growth measures, we utilized ring area (ring width * core diameter) as well as a standardized, unitless ring width index (RWI). The RWI was developed through interactive detrending in the dplR package (version 1.64) (Bunn, 2008) in R (version 3.31) (R_Development_Core_Team, 2008). For the RWI, each tree-ring series was individually fit with a negative exponential curve or a flexible spline with a frequency response of 75% the series length to remove the geometric growth trend (Fritts, 1976). Each tree-ring series was then divided by the fitted trend line, resulting in a standardized, unitless ratio (index) of actual to expected values (Cook and Peters, 1997). Conservative detrending options were selected to preserve multi-decadal variability within the ring-width series, as this was assumed to reflect possible important climate and ecological interactions - such as response to pests, pathogens, and stand dynamics that could influence growth suppressions and releases. For the majority of ring width series (87%) a negative exponential fit was selected, while a spline model was selected for 13% of the records. We chose to use RWI over basal area increment (BAI) because the assumptions of BAI are not well-suited for growth dynamics of high-elevation whitebark pine stands. For instance, some of the sampled whitebark pine were multi-stemmed trees with fused trunks (a common feature due to whitebark pine seed caching). The sampled stems had small DBH values, but ring width series from the collected increment cores reflected growth in a larger portion of the stem than we were able to measure (as we retrieved increment cores near the root collar), producing unrealistic BAI values (Maher et al., 2018).

We used Wilcoxon's paired *t*-tests to assess differences in growth and defense between pairs of live and dead whitebark pine at each site (Kane and Kolb, 2010) and applied a Bonferroni correction factor ($\alpha = 0.05/4 = 0.0125$) for all growth periods to account for multiple comparisons across similar growth periods (e.g. mean growth for total, 20-, 10- and 5-year time periods). We utilized a combination of multivariate analyses of variance, correlation analyses and regression analyses to examine relationships between annual radial growth (ring area and growth index) and defense metrics (resin duct size, production, area, density and relative area).

Next, we investigated the degree to which climate modulated growth and defense in whitebark pine and whether there were detectable differences in the response of live and dead trees to seasonal changes in climate. Estimates of local climate within the study areas were obtained from the Parameter-elevation Regressions on Independent Slopes Model (PRISM), as instrumental weather data were not available at these sites (Daly et al., 1994, Kipfmüller and Salzer, 2010, Abatzoglou et al., 2017). PRISM data were obtained at a spatial resolution of 4 km using the AN version of the dataset (Abatzoglou et al., 2017). We opted to use this version of the PRISM dataset because: 1) it is freely and publicly available, 2) it is reasonably accurate, given the sparse network of instrumental stations across our broader study region, and 3) it is a commonly utilized dataset for many dendroecological studies, particularly in areas with complex topography (Millar et al., 2007, Kipfmüller and Salzer, 2010, Guiterman et al.,

2015, Hood et al., 2015, Carnwath and Nelson, 2016, Ferrenberg et al., 2017, Slack et al., 2017, Cansler et al., 2018, Goeking and Izlar, 2018). Using the treeclim package in R (version 2.0) (Zang and Biondi, 2015) we developed 30-year moving correlation windows over two year time-step intervals to assess the influence climate on growth and defense metrics for live and dead trees. We grouped monthly climate data into seasonal windows to evaluate the more nuanced response of trees to seasonal variability. For instance, "winter" encompasses December (from the previous year) through March, while "spring" includes April and May, and "summer" is defined as June through August. Monthly climate variables obtained for this analysis included average temperature, precipitation, and the Palmer Drought Severity z-Index (z-PDSI; a measure of meteorological drought). Positive values in the z-PDSI correspond to wet (non-drought) conditions while negative values translate to drier (drought) periods. The z-PDSI metric contains no built-in autocorrelation to carry moisture from season to season. Consequently, this drought metric responds nearly instantaneously to precipitation (or lack thereof), making season-to-season moisture differences discernable, and better representing short-term and shallow soil moisture conditions (Abatzoglou et al., 2017). We opted to use z-PDSI as we were interested in how meteorological drought might influence whitebark pine growth and resin duct development. z-PDSI data were acquired using the WestWide Drought Tracker for the western Montana climate division (Abatzoglou et al., 2017).

We assessed the strength of correlations between growth and defense metrics and climate variables across the full record (1911–2004) as well as across two time intervals: early 20th century (1911–1960) and late 20th century (1961–2004). We hypothesized that increased bark beetle activity post-1960 coupled with the introduction of blister rust to the Flathead Indian Reservation around 1950 may have acted in concert with climatic variability to increase whitebark pine mortality across our sample areas in the latter 20th century. Thus, we evaluated whether correlations between climatic conditions and growth and defense metrics in the late 20th century would be different for dead whitebark pine compared to live trees. For all climate and time series analyses we established a minimum pair number (5 pairs = 10 trees) to help constrain variability at either end of the time series, where sampling depth was reduced.

Following methods of (Kane and Kolb, 2010) we developed a whitebark pine probability of mortality model using logistic regression. Live and dead whitebark pine were pooled across both sites yielding 144 samples (72 live and 72 dead). Mortality models were evaluated using an information theoretic approach, whereby a variety of candidate models were initially developed from the suite of growth and defense metrics and compared using Akaike's Information Criterion (AIC). We constructed ten models utilizing various combinations of growth and defense metrics following *a priori* assumptions (e.g. that a combination of resin duct size and duct area would collectively influence resin flow and may therefore be important variables influencing survivorship). Model parameters were limited to five variables or less and growth variables for overlapping periods were excluded within any given model to avoid issues of multicollinearity (e.g. 5-year ring area and 10-year ring area). To assess which growth and defense variables were most important in distinguishing between live and dead trees, we also developed conditional density plots using our full dataset.

3. Results

3.1. Growth and defense between live and dead trees

Principle components analysis showed a clear distinction in past growth and defense characteristics between live and dead whitebark pine across all time periods (Figs. 4, S1). Resin duct size, duct area, duct density and relative duct area were the most important variables contributing to the first principle component (PC1), cumulatively describing 45.8% of variability within the data (Fig. 4). Growth (ring

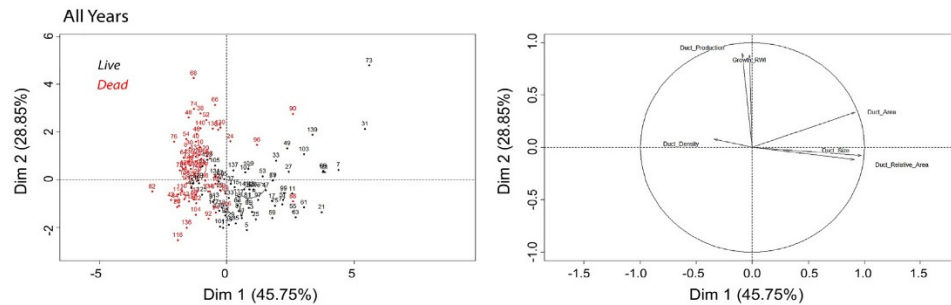


Fig. 4. Principal components analysis comparing growth and resin duct characteristics (production, size, area, density, relative area) for live and dead whitebark pine (144 samples, 72 live, 72 dead). Ellipses are 95th percentile confidence intervals for each grouping (live – black; dead – red). Variables factor map (right panel) illustrates the relative contribution of growth and defense metrics to the ordination. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Age of live and dead whitebark pine at study locations on the Flathead Indian Reservation.

	3LK		BLD	
	Live	Dead	Live	Dead
Age (Range)	72–118	41–100	94–113	44–101
Age (Average)	98 ± 1.9	75 ± 3	105 ± 0.9	77 ± 1.9

Note: Majority of trees died during late 1960s–1990s and exhibited signs of beetle activity (J-shaped galleries on tree bole and blue-staining in increment cores). Overall trends in growth and defense metrics were similar at each site, so the full suite of data was merged for analysis ($n = 144$ trees).

area) and resin duct production had the greatest contribution to PC2, cumulatively describing an additional 28.9% of variability within the data (74.7% total variability; Fig. 4) (Table 1).

Whitebark pine that had died had grown 22% faster (p -value < 0.0001) than living trees during the full record, although this pattern was driven by earlier years (1911–1975; Figs. 5a, 6a). In the 20-years preceding mortality, growth in whitebark that died declined by 26% relative to live trees, especially post-1975 (Fig. 5a). Dead whitebark pine also produced more resin ducts compared to live trees over the full record (20% greater production; p -value < 0.0001; Figs. 5b, 6b). This relationship declined (by 10%) in the 20-years preceding mortality, with the greatest difference occurring during a small interval from around 1990–2000 (Fig. 5b). However, despite producing more resin duct structures on average, the resin ducts were smaller for dead whitebark pine (56% smaller on average) compared to live trees (p -value < 0.0001; Figs. 5c, 6c). Similar to growth, duct size showed an increasing trend post-1975, where duct size in live trees continued to increase relative to dead trees (Fig. 5c).

Resin duct area was also greater in live trees across the full record (48% increase; p -value < 0.0001; Figs. 5d, 6d) and duct area showed a similar post-1975 trend, with increasing area in live whitebark pines relative to dead trees (Fig. 5d). In contrast, resin duct density was greater in dead trees (18% greater; p -value = 0.0149; Figs. 5e, 6e) and post-1975, duct density continued to increase in dead whitebark pine throughout the remainder of the record (Fig. 5e). Relative duct area (% of annual ring occupied by resin ducts) was significantly greater in live whitebark pines (57% increase; p -value < 0.0001; Figs. 5f, 6f, 7). Unlike the other growth and defense metrics, there was no clear temporal trend evident for relative duct area (Fig. 5f).

The two most significant metrics influencing tree survivorship were resin duct size (mm^2), and relative duct area (% of annual ring). Whitebark pine trees that are able to produce larger resin ducts (> 0.001 mm^2) with greater overall duct area (> 10% annual ring) had

a significantly greater chance of survival (~80%; Fig. 8).

The best mortality model included resin duct size within the 5-years prior to mortality and relative resin duct area across the full record (all overlapping years of live/dead trees; Table S1). The top five best performing mortality models all included relative duct area (for the full record), highlighting the importance of this metric in differentiating live and dead trees (Figs. 7, 8). The top performing models also included a combination of resin duct size and duct area across the 5-year and 10-year windows (Table S1). The only growth variable significant in the top five models was growth in the 5-year window (RWI; Table S1).

Resin duct production was positively correlated with radial growth for both live and dead trees, indicating that increased growth corresponds with increased resin duct production ($R^2 = 0.36$; p -value < 0.0001; Fig. S2). Duct area was also positively related to radial growth, although this relationship was stronger for dead trees (live: $R^2 = 0.16$; p -value = 0.0003/dead: $R^2 = 0.43$; p -value < 0.0001). Duct size was also positively related to growth in dead trees, though more weakly ($R^2 = 0.1$; p -value = 0.0038) with no real relationship for live trees ($R^2 = 0.02$; p -value = 0.1371; Fig. S2). For duct density, we found a negative relationship with growth for both live ($R^2 = 0.21$; p -value < 0.0001) and dead ($R^2 = 0.05$; p -value = 0.0351) trees (Fig. S2).

3.2. Influence of climate on growth and defense

We found differences in the response of live and dead whitebark pine trees to climate across our study sites, particularly in the latter 20th century (Fig. 9, Table S2). Both live and dead trees responded notably to the winter season (in particular winter precipitation and z-PDSI). For instance, growth of live trees was negatively correlated with winter precipitation (−0.41; p -value < 0.0001) and winter z-PDSI (−0.38; p -value = 0.0002), with the strength of this relationship increasing in the latter 20th century (Table S2; Figs. 9, S3). The negative correlation of growth with positive values of z-PDSI indicates reduced growth during wetter (non-drought) years. This response was much greater in live trees, driven in part by a strengthening of these relationships post-1960 for live trees and a simultaneous weakening of these relationships for dead trees during the same time period (Table S2; Figs. 9, S3). Similarly, duct production, duct size, and duct area were negatively correlated with winter precipitation and z-PDSI for live and dead trees, suggesting that during wetter winters whitebark pine trees produced fewer and smaller resin ducts, with a reduction in total resin duct area, although the overall strength of these correlations were modest (Table S2; Figs. 9, S3). These relationships were also much stronger for live trees, with a strengthening of these trends post-1960 and a simultaneous weakening of these relationships for dead trees in the latter 20th century (Table S2; Figs. 9, S3).

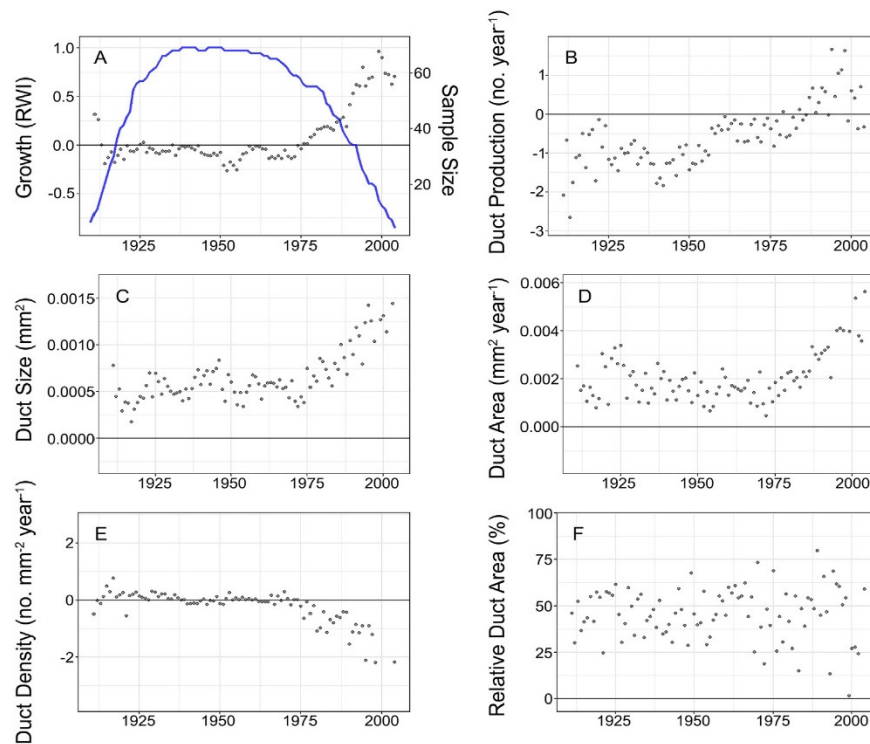


Fig. 5. Time series plots showing the difference in growth and defense metrics between live and dead whitebark pine (144 samples, 72 live, 72 dead). Each point represents the difference in growth/defense metrics for a pair of trees (1 live/1 dead). Points with positive values reflect a greater response for live trees while points with negative values reflect a greater response for trees that ultimately died (formula = [live tree metric] – [dead tree metric]). Blue line in upper-left plot illustrates sample depth across the time series. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

During the spring season, temperatures were positively correlated with growth across both live (0.36; p -value = 0.0003) and dead (0.19; p -value = 0.05) trees although this relationship was weak post-1960 for dead trees (Table S2; Figs. 9, S4). Growth was negatively correlated with spring z-PDSI for both live (-0.11 ; p -value = 0.312) and dead (-0.26 , p -value = 0.013) trees (such that wetter years were associated with reduced growth), with the strength of this relationship declining in the latter 20th century, particularly for live trees (Table S2; Figs. 9, S4). Duct production, size, and area were also negatively correlated with spring precipitation and z-PDSI for both live and dead trees, however, this shifted to positive correlations for duct size/area in live whitebark pine in the latter 20th century (Table S2, Figs. 9, S4). Duct area positively correlated with spring temperatures for live (0.24; p -value = 0.019) and dead (0.14; p -value = 0.175) trees, although this relationship faded for dead trees in the latter 20th century (Table S2; Figs. 9, S4). This indicates that whitebark pine trees produced fewer and smaller resin ducts, with a reduction in total duct area during wetter spring seasons; however, these trends have weakened substantially post-1960, possibly due to an earlier onset of spring and changing seasonality in the Northern Rocky Mountains over the past several decades (Pederson et al., 2009, Pederson et al., 2011a, Pederson et al., 2013).

We did not find any significant correlations for growth and summer precipitation or summer z-PDSI in either our live or dead trees, although there was a generally stronger relationship (increased growth with increased moisture) in the early 20th century. Resin duct production in live whitebark pine trees was negatively correlated with summer precipitation (-0.26 ; p -value = 0.012) and summer z-PDSI (-0.28 ; p -value = 0.006), such that wetter summers translated to reduced duct production. For dead trees, duct size and duct area correlated negatively with summer precipitation and z-PDSI, whereby wetter summers were associated with decreased duct size/area, although these

relationships were strongest in the early 20th century (Table S2, Fig. 9).

4. Discussion

Consistent with previous research (Kane and Kolb, 2010, Ferrenberg et al., 2014, Hood et al., 2015), we found that live and dead trees exhibited notably different growth and defense characteristics. In support of our hypothesis (H2), live whitebark pine produced larger resin ducts with a greater annual investment in resin-based defenses than the whitebark pine that died. Over the entire time series, resin duct size, duct area, and relative duct area were greater in live whitebark pine. In contrast, and counter to our hypothesis (H1), whitebark pine that died exhibited greater growth over the full time series than their live counterparts and also produced more resin duct structures on average. The increased growth was pronounced early in the record (~1919–1975) and shifted notably after 1975, whereby growth declined sharply. While those trees that died produced a greater number of resin ducts on average and consistently had a higher density of resin ducts across all time periods, the relative area of resin ducts was lower than live trees at both sites (Figs. 5–7).

Resin duct size, duct area, and relative duct area were the most important variables influencing whitebark pine survivorship (Fig. 8). Trees producing larger ducts with a greater overall percentage of annual carbon investment were far more likely to survive disturbance events at each of the study sites (Figs. 7, 8). The presence of larger resin ducts and greater overall resin duct area in live trees could be associated with an increased capacity to mobilize oleoresin in response to attack or infection and may be a factor in the ability of live trees to endure numerous disturbance events over time. Although dead trees produced more resin duct structures the ducts were smaller with less overall area, which might have been insufficient to produce, store, and mobilize adequate amounts of oleoresin in response to wounding by

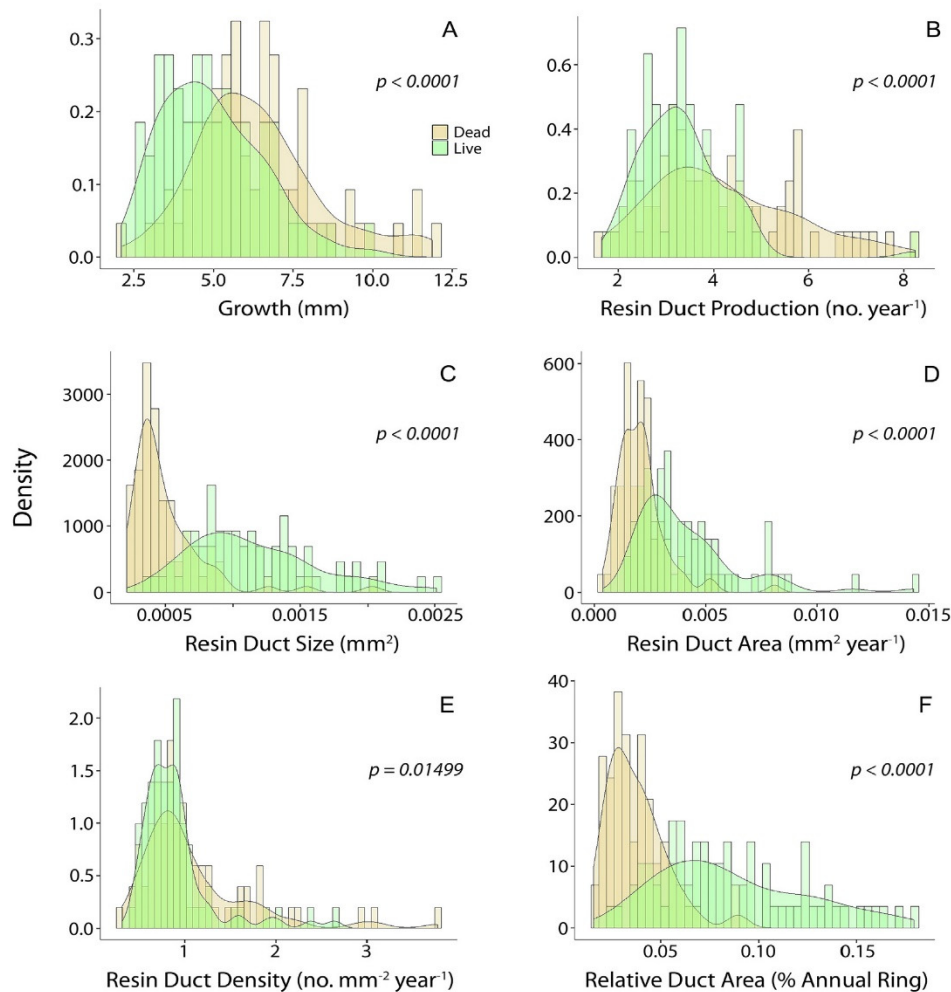


Fig. 6. Kernel density plots for growth and defense metrics across pairs of live and dead whitebark pine. These plots visualize the distribution of samples (depicted as vertical bars) over each continuous variable with a smoothing kernel (smooth lines with shading) applied to assist in visualization of the distributions. Reported p -values are from Wilcoxon's paired t -test comparing each metric across live and dead whitebark pine trees.

bark beetles and blister rust infection (Hood and Sala, 2015). This reduced resin flow in dead trees could be linked to lowered defense and higher mortality despite increased density of ducts, particularly in the years leading up to death (Fig. 8).

Importantly, our results suggest that whitebark pine trees that invest a relatively greater amount of resources into the production of constitutive resin-based defenses have a higher probability of surviving disturbance events. This parallels previous research on other conifers (Kane and Kolb, 2010; Ferrenberg et al., 2014; Hood et al., 2015; Hood et al., 2016). Hood and Sala (2015) found that faster growing ponderosa pine produced fewer but larger resin ducts. Similarly, Hood et al. (2015) showed that ponderosa pine that survived bark beetle attack produced larger resin ducts with greater duct area and relative duct area compared to trees that were killed, while Kane and Kolb (2010) reported that ponderosa pine that survived drought and escaped bark beetle activity produced larger resin ducts with a greater relative duct area than trees that died. Gaylord et al. (2015) found that surviving piñon pine produced fewer annual resin ducts than trees that died but

these ducts were also larger with a greater relative duct area, similar to our findings. Kläy (2011) also found surviving piñon pines to have larger resin ducts with greater relative area.

In contrast to our results, Kane and Kolb (2010) found that ponderosa pine that persisted through stand-level bark beetle activity produced more resin ducts and had a greater density of resin ducts than dead trees with no detectable difference in growth between live and dead pairs. Similarly, Ferrenberg et al. (2014) found that surviving lodgepole and limber pine both produced more resin ducts and had a greater density of resin ducts than dead trees while surviving limber pine also produced smaller resin ducts than their dead counterparts. Additionally, while slower growth appeared to confer defense benefits in our study, Gaylord et al. (2015) found that live trees exhibited faster growth rates than dead trees. Kläy (2011) found that surviving piñon pine produced more resin ducts with no detectable difference in duct density between live and dead trees, whereas we found resin duct density and numbers were comparable for live and dead whitebark pine. However, it is important to note that our live whitebark pine trees

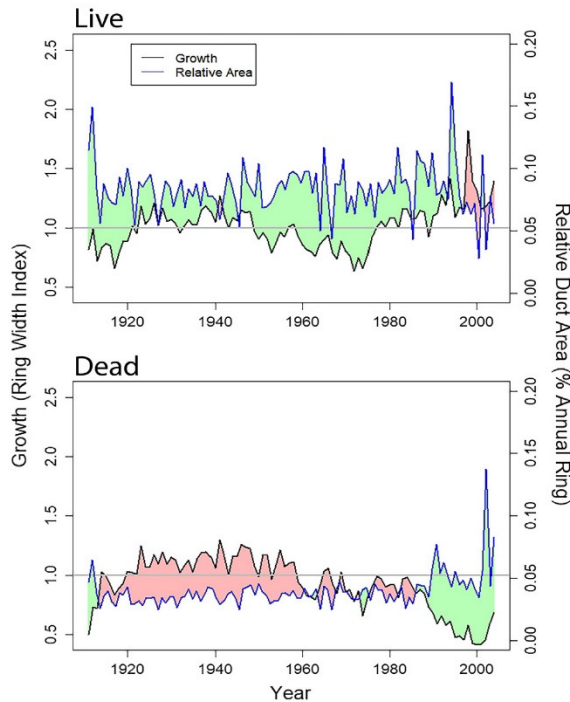


Fig. 7. Time series plots of growth (ring width index) and relative resin duct area (% annual ring) for live (top) and dead (bottom) whitebark pine. The blue line corresponds to growth (values above 1 indicate above average growth and values below 1 indicate below average growth). Green shading suggests a greater investment in resin duct defenses relative to growth (higher % of annual ring dedicated to resin duct structures) while red shading suggests a lower relative investment to growth (lower duct area). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

did not show any direct evidence of active bark beetle attack and thus, our observations do not reflect a change in growth and defense relationships post-selection. Rather, our results suggest a potential role of growth and defense anatomy in relation to host-identification and host-selection, whereby trees that produced larger resin ducts with a greater annual investment in resin duct features (relative to growth) persisted through stand-level bark beetle activity that resulted in high levels of mortality across our study sites.

The positive relationship we found between radial growth and resin

duct production (number of ducts and size of ducts) is consistent with the idea that growth is positively related to duct number (Rigling et al., 2003, Kane and Kolb, 2010, Ferrenberg et al., 2014, Hood and Sala, 2015, Westbrook et al., 2015) and size of resin ducts produced (Ferrenberg et al., 2014, Hood and Sala, 2015). The positive correlation of these defense metrics with increased radial growth suggests factors (e.g. resource availability and climate) that control growth also modulate resin duct structures for both live and dead trees (Fig. S2) (Hood and Sala, 2015, Huang et al., 2019, Mason et al., 2019). The higher relative investment in defense we observed in live trees (increased duct production, size, and area) could reflect a marginal tradeoff with growth as a function of the nutrient-limited, high-elevation environments characteristic to whitebark pine. For instance, when growth is constrained (by resource limitation) photosynthesis continues to produce carbon compounds and this 'carbon surplus' may then be allocated toward resin-based defenses, as suggested by the growth-differentiation balance hypothesis (Stamp, 2003). Alternatively, the live trees may have survived due to more beneficial microsites and/or underlying genetic differences that favor greater investment in resin-based defenses relative to growth, even under resource limitation.

The negative relationship between resin duct density and radial growth suggests that faster growing trees exhibit a lower overall investment of annual carbon into resin duct structures relative to xylem (decreased duct density), despite producing larger resin ducts (Fig. S2) (Kane and Kolb, 2010, Hood and Sala, 2015, Moreira et al., 2015). Our results weakly suggest that slower growing trees invest a proportionally greater amount of annual carbon into resin duct structures than structural carbon, highlighting possible increased value of defending critical tissues in the resource-limited settings typical of whitebark pine forests, as suggested by the resource allocation hypothesis (Endara and Coley, 2011) (Fig. S2). While our study was not designed to explicitly test either the growth-differentiation balance or resource allocation hypotheses directly (Hahn and Maron, 2016), they do provide a valuable framework within which we can evaluate growth and defense tradeoffs. The positive relationship between growth and resin duct production, yet negative relationship between growth and relative duct area, highlight the need to utilize both standardized and non-standardized resin duct metrics proposed by Hood and Sala (2015) to understand how physiological tradeoffs influence survivorship under different selective pressures.

4.1. Influence of climate on growth and defense

A negative relationship was found between winter precipitation and drought conditions (winter z-PDSI) and tree growth and all resin duct characteristics (Fig. 9). This was particularly true for live whitebark pine trees. The strength of these correlations increased post-1960. While the overall correlations are modest, they suggest that years with abundant winter precipitation, and winter seasons preceded by short-

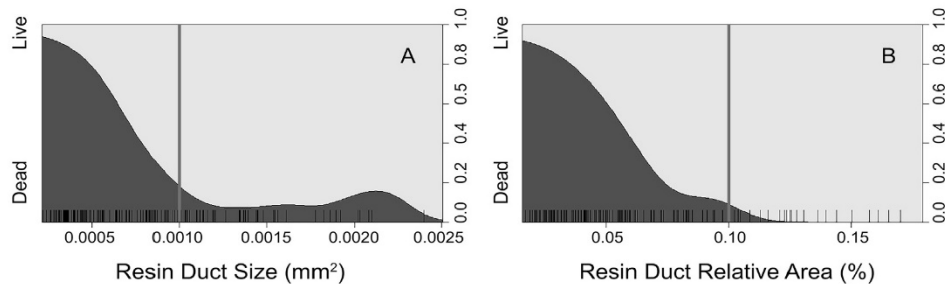


Fig. 8. Conditional density plots describing the probability of mortality in relation to principle resin duct metrics (A) resin duct size; mm^2 and (B) relative resin duct area; % annual ring for 144 whitebark pines (72 live and 72 dead). Light shading reflects live trees while dark shading reflects dead trees. Vertical black bars along the x-axis represents the distribution of samples. See supplementary info for model selection.

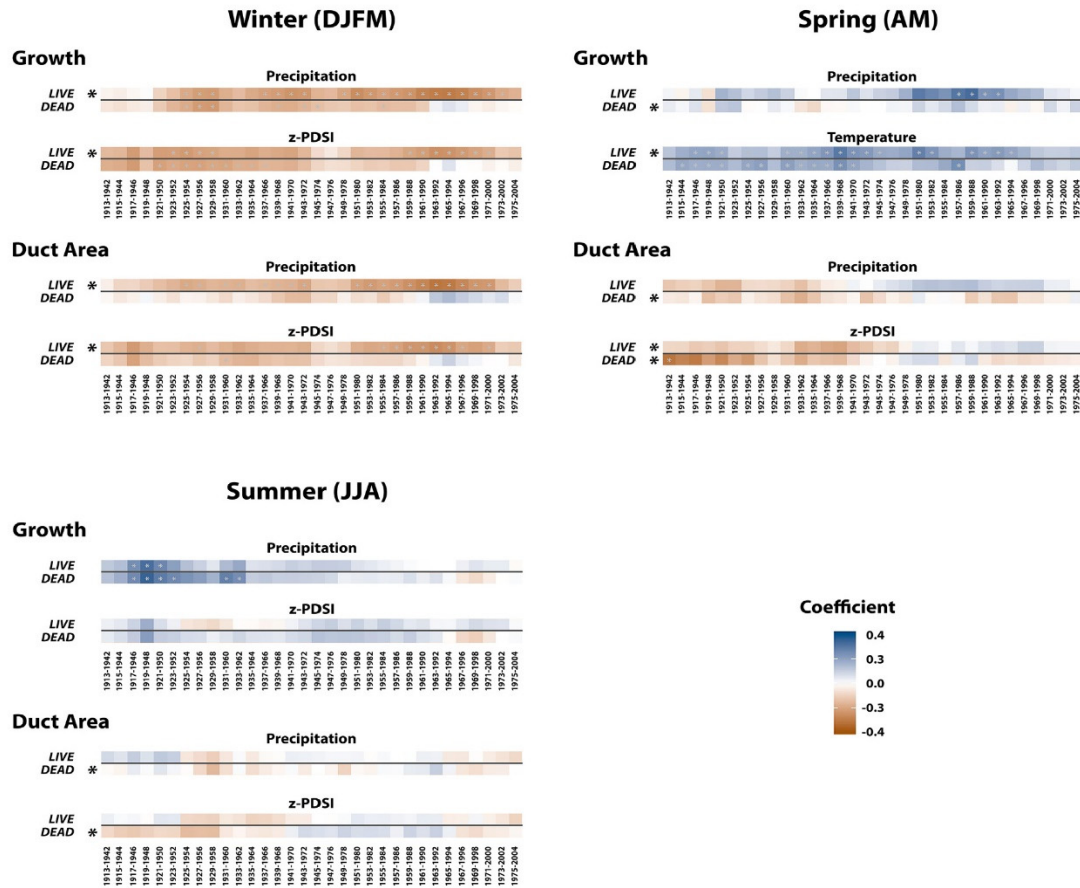


Fig. 9. 30-year moving correlation windows for seasonal climate data (winter/spring/summer). Top panel for each seasonal window: correlations of growth and climate (precipitation/z-PDSI/temperature) for live and dead trees. Bottom panel for each seasonal window: correlations of duct area and climate for live and dead trees. Statistical significance for the full time series (1911–2004) is represented by a ‘*’.

term drought, could predispose whitebark pine to future mortality by reducing their capacity to fix carbon, limiting subsequent investments in both growth and defense. The modest, negative correlations between reduced growth, defense, and winter precipitation could be a result of increased snowpack, which would reduce photosynthetic activity into the growing season, until the snowpack has abated and temperatures have increased sufficiently for photosynthesis (Wilson, 1964; Neuner et al., 1999; Vaganov et al., 1999). When temperatures are low and the soil is frozen, which is more likely at higher elevations, photosynthesis is reduced and trees are at an increased risk of xylem cavitation (Sperry and Robson, 2001; Sparks and Black, 2018). This could limit assimilation by reducing water uptake and nutrient transport, delaying annual growth and production of chemical defenses. These results highlight the need to investigate how snowpack, temperature, and precipitation might influence high-elevation conifers differently than low and mid elevation conifers.

Seasonal changes in precipitation, temperature and short-term drought during the spring and summer months was also weakly correlated with growth and defense metrics for both live and dead whitebark pine, however, these results were more nuanced (Fig. 9; Table S2). The strongest relationship was between spring temperatures and growth (positive correlation) and was most pronounced for live trees. In contrast, spring precipitation and z-PDSI (drought) showed a

weak negative correlation with growth, resin duct production, duct size, and duct area. The strength of these relationships declined post-1960 and even switched to positive, albeit not statistically significant, correlations in live trees in the latter 20th century. Summer precipitation weakly correlated with increased growth in live and dead trees in the early 20th century, however, this relationship further declined in the latter part of the record. These data weakly suggest a transition from positively correlated growth in summer during the early 20th century to positively correlated growth in spring in the latter part of the record – a trend that may be related to shifts in late 20th century climatic conditions. For instance, earlier snowpack melt and decreased snowpack levels observed in the Northern Rocky Mountains over the past century (Pederson et al., 2009; Pederson et al., 2011b; Pederson et al., 2013) could facilitate earlier photosynthesis and carbon acquisition in late winter/early spring.

The decoupling of climate relationships between live and dead trees in the latter 20th century may also result from increasing stress from cumulative impacts of mountain pine beetle and white pine blister rust, whereby infected trees and trees targeted by beetles allocate as many resources as possible to defense (evidenced by increased duct density) despite the potential need to allocate resources to survive droughts and fire (Table S2; Figs. S3, S4). It is likely that a combination of blister rust (introduced to the Flathead region circa 1950) and/or endemic bark

beetle activity (particularly pronounced from the 1960s to 1990s) impacted dead trees. These stressors would have reduced their capacity to fix carbon via photosynthesis due to crown reduction (in the case of blister rust infection) and depleted carbon reserves in the formation of defensive compounds during times of increased beetle pressure. High incidence of blister rust on individuals found in this study indicate white pine blister rust was a significant mortality agent at these sites. These same events could have created favorable growing conditions for the live trees (reduced competition and increased available sunlight) facilitating increased growth and greater allocation of carbon to resin defenses. This is supported by the observed temporal trends in growth and defense metrics (Fig. 5) whereby growth, duct production, duct size and duct area all decreased in dead trees in the latter 20th century while duct density increased. Changes in the relationship between growth and defense and climatic conditions over time also support the idea that multiple disturbances combined to impact whitebark pine individuals at our sites in the late 20th century as we detected a decoupling of growth and defense relationships with climate variables for live and dead trees post-1960 (Figs. S3, S4). The potential shift in response of tree growth and defense in the late 20th century highlights the need for a more thorough investigation of potential physiological responses of plants to dynamic disturbance/climate interactions that have occurred over the past four to five decades.

5. Conclusions

The differences in growth and defense characteristics that we observed between live and dead trees suggests that individuals within whitebark pine stands exhibit genetic variability associated with a range of physiological strategies. In the absence of disturbance, individuals that invest a greater amount of resources towards growth relative to defense experience a fitness benefit allowing individuals to more readily outcompete neighboring vegetation (Herms and Mattson, 1992, Stamp, 2003, Moreira et al., 2014, Moreira et al., 2015, Moreira et al., 2016). Conversely, during periods of heightened insect activity a greater investment in defensive features can increase the probability of survival (Kane and Kolb, 2010, Ferrenberg et al., 2014, Hood and Sala, 2015, Hood et al., 2015, Hood et al., 2016). Within any given stand of whitebark pine there will be individuals with differing physiological strategies (e.g. those that invest more consistently in growth and those with greater regular investments in defense). It is difficult to state which strategies may yield the greatest benefit at any given time, as the interacting disturbances shaping the evolution of whitebark pine are dynamic in both space and time. This supports the well-established premise that maintaining genetic variability in populations is essential for species to persist through varying conditions, especially a species in harsh, highly variable, and disturbance prone alpine environments (Six et al., 2018).

Whitebark pine individuals within both of our sites appear to exhibit differing strategies in the allocation of resources toward growth and defense. Live trees that persisted through 20th century disturbance events produced larger resin ducts with a greater overall annual duct area relative to growth. In contrast, those trees that died invested more into growth, at the expense of defense. Both strategies involve tradeoffs that can confer fitness benefits under different circumstances. As defensive features are energetically expensive to produce and maintain it is difficult to generalize what strategies may be most appropriate under what circumstances. This is an important point when considering contemporary breeding and planting programs, which have been identified as major restoration initiatives in many jurisdictions (Snieszko, 2006, Keane et al., 2012). Within these programs seedlings are traditionally selected for based on growth-related characteristics with little consideration for defensive physiology (White et al., 2007). Our results lend insight into resin duct characteristics that may be beneficial to increasing survivorship and promoting whitebark pine retention, which is particularly important given the sensitivity of whitebark pine to

unforeseeable changes in disturbance regimes.

It is becoming increasingly clear that genetic variability and specific traits that are associated with this variability is often an emergent property of the population of trees within a forest stand or landscape yet the full implications for maintaining this variability under rapidly changing biophysical conditions are perhaps not fully appreciated. This is especially true for long-lived sessile species, which face numerous selective pressures of varying frequency and intensity over their lifetime (de la Mata et al., 2017). The specific suite of interacting factors influencing individuals are unique in space and time and work in concert to increase genetic variability within and across populations (Six et al., 2018). Whitebark pine forests have persisted for thousands of years through dramatic changes to climate. The cumulative selective forces acting upon them, such as insects, pathogens, and abiotic disturbances, have likely resulted in the persistence of a broad suite of physiological strategies with benefits under different conditions (Dmitriew, 2011, Monro and Marshall, 2014, Six et al., 2018). Our results further support the idea that maintaining genetic variability and the traits associated with this variability may be critical for species that experience a complex array of biophysical and biological stressors across space and time. Thus, management prescriptions that focus on favoring genotypes that may be the most fit under one set of conditions may lead to heightened vulnerability under complex and difficult to predict climate-disturbance feedbacks and interactions that forests will experience into the future (de la Mata et al., 2017). This may be especially true for forests in harsh, high-elevation settings. Hence, exhibiting one strategy (growth or defense) for surviving an array of stressors (fire, drought, disease) may undermine the capacity for adaptive response to an uncertain future (Dmitriew, 2011, Monro and Marshall, 2014). This observation highlights the need for forest resource managers to better understand how actions might support or undermine genetic variability in forest systems. Specifically, managers might support efforts that maintain genetic variability by: 1) allowing for natural whitebark regeneration following disturbance, 2) limiting post-disturbance impacts (e.g. salvage logging and/or post-fire treatments that undermine natural regeneration, and 3) avoiding blanket prescriptions that preselect for specific traits that may be advantageous under certain conditions yet detrimental under (often unforeseen) future conditions. For instance, while breeding for whitepine blister rust resistant whitebark pine trees has been recognized as one of the primary objectives for expanding rust-resistant strains across management areas, the focus on this one aspect of whitebark physiology may undermine adaptability to other future stressors. For example, rust-resistance may come at the expense of adequate resin duct defenses or reduced growth, which could predispose future whitebark pine populations to unforeseeable shifts in disturbance dynamics. Ultimately, natural resource managers should be cognizant of the capacity (often not well understood) for the genetic variability in whitebark pine forests to provide adaptive responses to complex disturbance, biophysical and biological interactions, now and into the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2019.117736>.

References

- Abatzoglou, J.T., McEvoy, D.J., Redmond, K.T., 2017. The west wide drought tracker: drought monitoring at fine spatial scales. *Bull. Am. Meteorol. Soc.* 98, 1815–1820.
- Amberson, J., Keville, M., Nelson, C., 2018. Effects of disturbance on tree community dynamics in Whitebark Pine (*Pinus albicaulis* Engelm.) ecosystems. *Forests* 9, 566.
- Arno, S.F., Hoff, R.J. 1989. *Silvics of Whitebark Pine (Pinus albicaulis)*. In: F. S. United States Department of Agriculture, editor. Intermountain Research Station, Ogden, UT.
- Bockino, N.K., Tinker, D.B., 2012. Interactions of white pine blister rust and mountain pine beetle in whitebark pine ecosystems in the Southern Greater Yellowstone Area. *Natural Areas J.* 32, 31–40.
- Bunn, A.G., 2008. A dendrochronology program library in R (dplR). *Dendrochronologia* 26, 115–124.
- Buotte, P.C., Hicke, J.A., Preisler, H.K., Abatzoglou, J.T., Raffa, K.F., Logan, J.A., 2017. Recent and future climate suitability for whitebark pine mortality from mountain pine beetles varies across the Western U.S. *For. Ecol. Manage.* 399, 132–142.
- Cannac, M., Barboni, T., Ferrat, L., Bighelli, A., Castola, V., Costa, J., Trecul, D., Morandini, F., Pasqualini, V., 2009. Oleoresin flow and chemical composition of corsican pine (*Pinus nigra* subsp. *laricio*) in response to prescribed burnings. *For. Ecol. Manage.* 257, 1247–1254.
- Cansler, C.A., McKenzie, D., Halpern, C.B., 2018. Fire enhances the complexity of forest structure in alpine treeline ecotones. *Ecosphere* 9, e02091.
- Carmwath, G.C., Nelson, C.R., 2016. The effect of competition on responses to drought and interannual climate variability of a dominant conifer tree of Western North America. *J. Ecol.* 104, 1421–1431.
- Cole, D. N. 1990. *Recreation in Whitebark Pine Ecosystems: Demand, Problems, and Management Strategies*. Pages 305–309. In: U. S. F. Service, editor. Intermountain Research Station, Ogden, UT.
- Cook, E.R., Kairiukstis, L.A., 1990. *Methods of Dendrochronology*. Springer-Science + Business Media, B. V.
- Cook, E.R., Peters, K., 1997. Calculating unbiased tree-ring indices for the study of climatic and environmental change. *The Holocene* 7, 361–370.
- Daly, C., Neilson, R.P., Phillips, D.L., 1994. A statistical-topographic model for mapping climatological precipitation over mountainous terrain. *J. Appl. Meteorol.* 33, 140–158.
- de la Mata, R., Hood, S.M., Sala, A., 2017. Insect outbreak shifts the direction of selection from fast to slow growth rates in the long-lived conifer *Pinus ponderosa*. *Proc. Natl. Acad. Sci.* 114, 7391–7396.
- Dmitriev, C.M., 2011. The evolution of growth trajectories: what limits growth rate? *Biol. Rev. Camb. Philos. Soc.* 86, 97–116.
- Endara, M.-J., Coley, P.D., 2011. The resource availability hypothesis revisited: a meta-analysis. *Funct. Ecol.* 25, 389–398.
- Ferrenberg, S., Kane, J.M., Langenhan, J.M., 2015. To grow or defend? pine seedlings grow less but induce more defences when a key resource is limited. *Tree Physiol.* 35, 107–111.
- Ferrenberg, S., Kane, J.M., Mitton, J.B., 2014. Resin duct characteristics associated with tree resistance to bark beetles across lodgepole and limber pines. *Oecologia* 174, 1283–1292.
- Ferrenberg, S., Langenhan, J.M., Loskot, S.A., Rozal, L.M., Mitton, J.B., 2017. Resin monoterpene defenses decline within three widespread species of pine (*Pinus*) along a 1530-m elevational gradient. *Ecosphere* 8, e01975.
- Franceschi, V.R., Krokene, P., Christiansen, E., Krekling, T., 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytol.* 167, 353–375.
- Fritts, H.C., 1976. *Tree Rings and Climate*. Academic Press, New York, N.Y.
- Gaylord, M.L., Kolb, T.E., McDowell, N.G., 2015. Mechanisms of Piñon pine mortality after severe drought: a retrospective study of mature trees. *Tree Physiol.* 35, 806–816.
- Gaylord, M.L., Kolb, T.E., Pockman, W.T., Plaut, J.A., Yezzer, E.A., Macalady, A.K., Pangle, R.E., McDowell, N.G., 2013. Drought predisposes piñon-juniper woodlands to insect attacks and mortality. *New Phytol.* 198, 567–578.
- Gaylord, M.L., Kolb, T.E., Wallin, K.F., Wagner, M.R., 2007. Seasonal dynamics of tree growth, physiology, and resin defenses in a Northern Arizona Ponderosa Pine Forest. *Can. J. For. Res.* 37, 1173–1183.
- Geils, B.W., Hummer, K.E., Hunt, R.S., 2010. White pines, ribes, and blister rust: a review and synthesis. *Forest Pathol.* 40, 147–185.
- Goeking, S., Izlar, D., 2018. *Pinus albicaulis* Engelm. (Whitebark Pine) in mixed-species stands throughout its US range: broad-scale indicators of extent and recent decline. *Forests* 9, 131.
- Grissino-Mayer, H.D., 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-Ring Res.* 57, 205–221.
- Guiterman, C.H., Margolis, E.Q., Swetnam, T.W., 2015. Dendroecological methods for reconstructing high-severity fire in pine-oak forests. *Tree-Ring Res.* 71, 67–77.
- Hahn, P.G., Maron, J.L., 2016. A framework for predicting intraspecific variation in plant defense. *Trends Ecol. Evol.* 31, 646–656.
- Hannrup, B., Cahalan, C., Chantre, G., Grabner, M., Karlsson, B., Bayon, I.L., Jones, G.L., Müller, U., Pereira, H., Rodrigues, J.C., Rosner, S., Rozenberg, P., Wilhelmsson, L., Wimmer, R., 2004. Genetic parameters of growth and wood quality traits in *Picea abies*. *Scand. J. For. Res.* 19, 14–29.
- Harms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. *Q. Rev. Biol.* 67, 283–335.
- Heyerdahl, E.K., Loehman, R.A., Falk, D.A., 2014. Mixed-severity fire in lodgepole pine dominated forests: are historical regimes sustainable on Oregon's Pumice Plateau, USA? *Can. J. For. Res.* 44, 593–603.
- Holmes, R.L., Adams, R.K., Fritts, H.C. 1986. *Tree-ring chronologies of Western North America: California, Eastern Oregon and Northern Great Basin; with: Procedures used in the chronology development work including users manuals for computer programs COFECHA and ARSTAN*. Laboratory of Tree-Ring Research, University of Arizona, Tucson, Arizona.
- Hood, S.M., Baker, S., Sala, A., 2016. Fortifying the forest: thinning and burning increase resistance to a bark beetle outbreak and promote forest resilience. *Ecol. Appl.* 26, 1984–2000.
- Hood, S.M., Sala, A., 2015. Ponderosa pine resin defenses and growth: metrics matter. *Tree Physiol.* 35, 1223–1235.
- Hood, S.M., Sala, A., Heyerdahl, E.K., Boutin, M., 2015. Low-severity fire increases tree defense against bark beetle attacks. *Ecology* 96, 1846–1855.
- Huang, J., Hammerbacher, A., Weinhold, A., Reichelt, M., Gleixner, G., Behrendt, T., van Dam, N.M., Sala, A., Gershenzon, J., Trumbore, S., Hartmann, H., 2019. Eyes on the future – evidence for trade-offs between growth, storage and defense in Norway Spruce. *New Phytol.* 222, 144–158.
- Iglesias, V., Krause, T.R., Whitlock, C., 2015. Complex response of white pines to past environmental variability increases understanding of future vulnerability. *PLoS ONE* 10, e0124439.
- Jamieson, M.A., Burkle, L.A., Manson, J.S., Runyon, J.B., Trowbridge, A.M., Zientek, J., 2017. Global change effects on plant-insect interactions: the role of phytochemistry. *Curr. Opin. Insect Sci.* 23, 70–80.
- Kane, J.M., Kolb, T.E., 2010. Importance of resin ducts in reducing ponderosa pine mortality from bark beetle attack. *Oecologia* 164, 601–609.
- Keane, R.E. 2000. *The Importance of Wilderness to Whitebark Pine Research and Management*. Pages 84–92. In: U. S. F. Service, editor. Rocky Mountain Research Station, Ogden, UT.
- Keane, R.E., Arno, S.F., 1993. Rapid decline of whitebark pine in western montana: evidence from 20-year remeasurements. *West. J. Appl. For.* 8, 44–47.
- Keane, R.E., Tomback, D.F., Aubry, C.A., Bower, A.D., Campbell, E.M., Cripps, C.L., Jenkins, M.B., Mahalovich, M.F., Manning, M., McKinney, S.T., Murray, M.P., Perkins, D.L., Reinhart, D.P., Ryan, C., Schoettle, A.W., Smith, C.M. 2012. *A Range-Wide Restoration Strategy for Whitebark Pine (Pinus albicaulis)*. In: U. S. D. o. Agriculture, editor. Forest Service, Rocky Mountain Research Station.
- Keeling, C.I., Bohlmann, J., 2006. Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytol.* 170, 657–675.
- Kendall, K.C., Keane, R.E. 2000. *The Decline of Whitebark Pine*. In: D. F. Tomback, S. F. Arno, and R. E. Keane, editors. Whitebark Pine Communities: Ecology and Restoration. Island Press, Washington, DC, Pages 123–145.
- Kipfmüller, K.F., Salzer, M.W., 2010. Linear trend and climate response of five-needle pines in the western united states related to treeline proximity. *Can. J. For. Res.* 40, 134–142.
- Kipfmüller, K.F., Swetnam, T.W. 2002. *Climate and Mountain Pine Beetle-Induced Tree Mortality in the Selway-Bitterroot Wilderness Area*. Pages 1–41. In: D. o. F. Resources, editor. The University of Idaho, Moscow, ID.
- Kläy, M., 2011. Are Defensive Structures Good Predictors of Tree Mortality Under Drought and Insect Pressure? Institute of Terrestrial Ecosystems, Bern, Germany.
- LaMarche, V.C.J., Hirschboeck, K.K., 1984. Frost rings in trees as records of major volcanic eruptions. *Nature* 307, 121–126.
- Langenheim, J.H., 1994. Higher plant terpenoids: a phytochemical overview of their ecological roles. *J. Chem. Ecol.* 20, 1223–1280.
- Lewinsohn, E., Gijzen, M., Croteau, R., 1991. Defense mechanisms of conifers. *Plant Physiol.* 96, 44–49.
- Macfarlane, W.W., Logan, J.A., Kern, W.R., 2013. An innovative aerial assessment of greater yellowstone ecosystem mountain pine beetle-caused whitebark pine mortality. *Ecol. Appl.* 23, 421–437.
- Maher, C.T., Nelson, C.R., Larson, A.J., Sala, A., 2018. Ecological effects and effectiveness of silvicultural restoration treatments in whitebark pine forests. *For. Ecol. Manage.* 429, 534–548.
- Mason, C.J., Keefover-Ring, K., Villari, C., Klutsch, J.G., Cook, S., Bonello, P., Erbilgin, N., Raffa, K.F., Townsend, P.A., 2019. Anatomical defences against bark beetles relate to degree of historical exposure between species and are allocated independently of chemical defences within trees. *Plant, Cell Environ.* 42, 633–646.
- Mielke, J.L. 1943. *White Pine Blister Rust in Western North America*. In: Yale School of Forestry & Environmental Studies Bulletin Series. School of Forestry and Environmental Studies.

- Millar, C.I., Westfall, R.D., Delany, D.L., 2007. Response of high-elevation limber pine (*Pinus flexilis*) to multiyear droughts and 20th-century warming, Sierra Nevada, California, USA. *Can. J. For. Res.* 37, 2508–2520.
- Mills, L.S., Doak, D.F., 1993. The keystone-species concept in ecology and conservation. *Bioscience* 43, 219–224.
- Monro, K., Marshall, D.J., 2014. Faster is not always better: selection on growth rate fluctuates across life history and environments. *Am. Nat.* 183, 798–809.
- Moreira, X., Mooney, K.A., Rasmann, S., Petry, W.K., Carrillo-Gavilán, A., Zas, R., Sampedro, L., Novotny, V., 2014. Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. *Ecol. Lett.* 17, 537–546.
- Moreira, X., Sampedro, L., Zas, R., Pearse, I.S., 2016. Defensive traits in young pine trees cluster into two divergent syndromes related to early growth rate. *PLoS ONE* 11, e0152537.
- Moreira, X., Zas, R., Solla, A., Sampedro, L., 2015. Differentiation of persistent anatomical defensive structures is costly and determined by nutrient availability and genetic growth-defence constraints. *Tree Physiol.* 35, 112–123.
- Neuner, G., Ambach, D., Aichner, K., 1999. Impact of snow cover on photoinhibition and winter desiccation in evergreen rhododendron ferrugineum leaves during subalpine winter. *Tree Physiol.* 19, 725–732.
- Pederson, G.T., Betancourt, J.L., McCabe, G.J., 2013. Regional patterns and proximal causes of the recent snowpack decline in the Rocky Mountains, U.S. *Geophys. Res. Lett.* 40, 1811–1816.
- Pederson, G.T., Graumlich, L.J., Fagre, D.B., Kipfer, T., Muhlfield, C.C., 2009. A Century of climate and ecosystem change in western montana: what do temperature trends portend? *Clim. Change* 98, 133–154.
- Pederson, G.T., Gray, S.T., Ault, T., Marsh, W., Fagre, D.B., Bunn, A.G., Woodhouse, C.A., Graumlich, L.J., 2011a. Climatic controls on the snowmelt hydrology of the northern rocky mountains. *J. Clim.* 24, 1666–1687.
- Pederson, G.T., Gray, S.T., Woodhouse, C.A., Betancourt, J.L., Fagre, D.B., Littell, J.S., Watson, E., Luckman, B.H., Graumlich, L.J., 2011b. The unusual nature of recent snowpack declines in the North American Cordillera. *Science* 333, 332–335.
- Perrakis, D.D.B., Agee, J.K., Eglitis, A., 2011. Effects of prescribed burning on mortality and resin defenses in old growth ponderosa pine (Crater Lake, Oregon): four years of post-fire monitoring. *Natural Areas J.* 31, 14–25.
- Phillips, M.A., Croteau, R.B., 1999. Resin-based defenses in conifers. *Trends Plant Sci. – Rev.* 4, 1360–1385.
- Powell, E.N., Raffa, K.F., 2011. Fire injury reduces inducible defenses of lodgepole pine against mountain pine beetle. *J. Chem. Ecol.* 37, 1184–1192.
- R_Development_Core_Team. 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rigling, A., Brühlhart, H., Bräker, O.U., Theodor, F., Schweingruber, F.H., 2003. Effects of irrigation on diameter growth and vertical resin duct production in *Pinus sylvestris* L. on Dry Sites in the Central Alps, Switzerland. *Forest Ecol. Manage.* 175, 285–296.
- Rosner, S., Hannrup, B., 2004. Resin canal traits relevant for constitutive resistance of norway spruce against bark beetles: environmental and genetic variability. *For. Ecol. Manage.* 200, 77–87.
- Sala, A., Piper, F., Hoch, G., 2010. Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytol.* 186, 274–281.
- Samman, S., Schwandt, J.W., Wilson, J.L. 2003. Managing for Healthy White Pine Ecosystems in the United States to Reduce the Impacts of White Pine Blister Rust. Page 10. In: U. S. D. o. Agriculture, editor. Forest Service, Missoula, Montana.
- Shanahan, E., Irvine, K.M., Thoma, D., Wilmoth, S., Ray, A., Legg, K., Shovic, H. 2016. Whitebark Pine Mortality Related to White Pine Blister Rust, Mountain Pine Beetle Outbreak, and Water Availability. *Ecosphere* 7.
- Six, D.L., Adams, J., 2007. White pine blister rust severity and selection of individual whitebark pine by the mountain pine beetle (Coleoptera: Curculionidae, Scolytinae). *J. Entomol. Sci.* 42, 345–353.
- Six, D.L., Vergobbi, C., Cutter, M., 2018. Are survivors different? genetic-based selection of trees by mountain pine beetle during a climate change-driven outbreak in a high-elevation pine forest. *Front. Plant Sci.* 9, 993.
- Slack, A.W., Kane, J.M., Knapp, E.E., Sherriff, R.L., 2017. Contrasting impacts of climate and competition on large sugar pine growth and defense in a fire-excluded forest of the Central Sierra Nevada. *Forests* 8, 244.
- Snieszko, R.A., 2006. Resistance breeding against nonnative pathogens in forest trees — current successes in North America. *Can. J. Plant Pathol.* 28.
- Sparks, J.P., Black, R.A., 2018. Winter hydraulic conductivity and xylem cavitation in coniferous trees from upper and lower treeline. *Arct. Antarct. Alp. Res.* 32, 397–403.
- Sperry, J.S., Robson, D.J., 2001. Xylem Cavitation and Freezing in Conifers. *Conifer Cold Hardiness*, pp. 121–136.
- Stamp, N., 2003. Out of the quagmire of plant defense hypotheses. *Q. Rev. Biol.* 78, 23–55.
- Stokes, M.A., Smiley, T.L., 1996. An Introduction to Tree-Ring Dating. The University of Arizona Press, Tucson, AZ.
- Strom, B.L., Goyer, R.A., Ingram, L.L., Boyd, G.D.L., Lott, L.H., 2002. Oleoresin characteristics of progeny of loblolly pines that escaped attack by the southern pine beetle. *For. Ecol. Manage.* 158, 169–178.
- Tomback, D.F., Arno, S.F., Keane, R.E., 2001. Whitebark Pine Communities: Ecology and Restoration. Island Press, Washington, D.C.
- Tomback, D.F., Kendall, K.C., 2001. Biodiversity losses: the downward spiral. In: Tomback, D.F., Arno, S.F., Keane, R.E. (Eds.), *Whitebark Pine Communities: Ecology and Restoration*. Island Press, Washington, DC, pp. 243–262.
- Tomback, D.F., Resler, L.M., Keane, R.E., Pansing, E.R., Andrade, A.J., Wagner, A.C., 2016. Community structure, biodiversity, and ecosystem services in treeline whitebark pine communities: potential impacts from a non-native pathogen. *Forests* 7, 21.
- Tomback, D.F., Sund, S.K., Hoffmann, L.A., 1993. Post-fire regeneration of *Pinus albicaulis*: height-age relationships, age structure, and microsite characteristics. *Can. J. For. Res.* 23, 113–119.
- Trapp, S., Croteau, R., 2001. Defensive resin biosynthesis in conifers. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 52, 689–724.
- Trowbridge, A.M., 2014. Evolutionary ecology of chemically mediated plant-insect interactions. *Ecol. Environ.* 143–176.
- Vaganov, E.A., Hughes, M.K., Kirdyanov, A.V., Schweingruber, F.H., Silkin, P.P., 1999. Influence of snowfall and melt timing on tree growth in Subarctic Eurasia. *Nature* 400, 149–151.
- van de Gevel, S., Larson, E., Grissino-Mayer, H., 2017. Separating trends in whitebark pine radial growth related to Climate and Mountain Pine Beetle Outbreaks in the Northern Rocky Mountains, USA. *Forests* 8, 195.
- Voortech Consulting. 2005. The Tree Ring Measuring Program Project J2X. Voortech Consulting.
- Westbrook, J.W., Walker, A.R., Neves, L.G., Munoz, P., Resende Jr., M.F., Neale, D.B., Wegrzyn, J.L., Huber, D.A., Kirst, M., Davis, J.M., Peter, G.F., 2015. Discovering candidate genes that regulate resin canal number in *Pinus taeda* stems by integrating genetic analysis across environments, ages, and populations. *New Phytol.* 205, 627–641.
- White, T.L., Adams, W.T., Neale, D.B., 2007. Forest Genetics. CABI, Wallingford, UK.
- Wilson, B.F., 1964. The formation of wood in forest trees. In: Zimmermann, M.H. (Ed.), *The Formation of Wood in Forest Trees*. Academic Press, New York, NY, pp. 19–36.
- Wimmer, R., Grabner, M., 1997. Effects of climate on vertical resin duct density and radial growth of norway spruce (*Picea abies*). *Trees* 11, 271–276.
- Zang, C., Biondi, F., 2015. treeclim: an R package for the numerical calibration of proxy-climate relationships. *Ecography* 38, 431–436.

CHAPTER FOUR

GROWTH AND DEFENSE CHARACTERISTICS OF WHITEBARK PINE (*PINUS ALBICAULIS*) AND LODGEPOLE PINE (*PINUS CONTORTA* VAR *LATIFOLIA*) IN A HIGH-ELEVATION, DISTURBANCE-PRONE MIXED-CONIFER FOREST IN NORTHWESTERN MONTANA, USA

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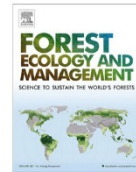
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Growth and defense characteristics of whitebark pine (*Pinus albicaulis*) and lodgepole pine (*Pinus contorta* var *latifolia*) in a high-elevation, disturbance-prone mixed-conifer forest in northwestern Montana, USA

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Bark beetles

ABSTRACT

Recent, widespread tree mortality in the western U.S. resulting from changes in climate, pathogens, insect activity, and forest management practices has led to concerns for many ecologically and culturally important species. Within conifers, resin-based defenses have long been recognized as a primary defense mechanism against a variety of insects and pathogens. Oleoresin produced by trees contain complex mixtures of terpenoids that have numerous insecticidal and fungicidal properties. Research has also identified links between resin duct characteristics and increased probability of survival during bark beetle outbreaks. Whitebark pine (*Pinus albicaulis*) is a culturally significant high elevation species that provides numerous ecological services within subalpine and alpine ecosystems. Whitebark pine has co-evolved with a suite of biotic and abiotic disturbances. Individual trees allocate resources towards growth and resin-based defenses, making it a good candidate species to evaluate growth and defense relationships and tradeoffs. In this study we compared constitutive resin chemistry, tree growth and resin duct anatomy between similarly aged whitebark and lodgepole pine (*P. contorta* var *latifolia*) growing in proximity within a disturbance-prone, mixed-conifer forest in northwestern Montana. These two host species have varying degrees of historical exposure to mountain pine beetle. Our research yields four important findings. First, we did not find evidence of a tradeoff between tree growth and tree defenses (resin duct morphology and resin chemistry). This suggests that trees growing under favorable field conditions can experience high growth rates and still allocate ample resources towards defense. Second, we found that resin ducts and constitutive mono- and sesqui- terpenes were not correlated in lodgepole pine while duct production and area were positively related to constitutive monoterpenes, and duct size and area were positively related to constitutive sesquiterpenes, in whitebark pine. The lack of distinct, consistent relationships between these defensive features suggests that both whitebark and lodgepole pine trees present beetles with numerous, complex combinations of resin-based defenses. Third, based on constitutive terpene profiles, bark beetles are more likely to enter lodgepole pine but more likely to successfully elicit mass attacks in whitebark pine, which agrees with beetle attack and success patterns observed in the field. Fourth, overstory competition, particularly by Engelmann spruce (*Picea engelmannii*), can influence tree defenses, specifically by reducing constitutive terpene concentrations in lodgepole and whitebark pine. Competitive tree interactions could lead to altered bark beetle-conifer interactions as host and nonhost species migrate in response to changing climate. Our results suggest that strategies designed to support whitebark pine populations can benefit from better understanding interactions among growth, competition and physical and chemical defenses in response to multiple disturbance.

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1. Introduction

Recent, large-scale tree mortality in the western U.S. resulting from changes in climate, biotic stressors, and forest management practices has led to concerns for many ecologically and culturally important species (Mattson and Haack 1987, van Mantgem et al. 2004, van Mantgem and Stephenson 2007, Schwandt et al. 2010, Tomback and Achuff 2010, Roy et al. 2014). Given the ecological and economic implications of large-scale mortality events, there is growing interest to better understand physiological strategies employed by trees in response to these shifting disturbance regimes. However, identifying general trends is complicated by the inherent complexity associated with tree defense anatomy and chemistry, both of which vary across populations, individuals, and plant organs (Trowbridge 2014, Jamieson et al. 2017). Large inter- and intra-specific variation in tree physiology and fluctuating disturbance regimes complicates our ability to make generalized assumptions (e.g., that tree mortality can be predicted based on a particular suite of physiological attributes), which hinders development of effective management solutions that account for complex species-specific responses to biotic and abiotic processes.

Within conifers, resin-based defenses have long been recognized as a primary defense mechanism against a variety of insects and pathogens (Lewinsohn et al. 1991, Langenheim 1994, Phillips and Croteau 1999, Trapp and Croteau 2001, Franceschi et al. 2005, Keeling and Bohlmann 2006). Oleoresin produced by trees contains complex mixtures of terpenoids (monoterpenes, sesquiterpenes and diterpenes) (Phillips and Croteau 1999) that have numerous insecticidal and fungicidal properties (Langenheim 1994, Raffa 2014, Trowbridge et al. 2016, Celedon and Bohlmann 2019). Many of these compounds (e.g., monoterpenes) have demonstrated linkages to insect behavior and physiology, from facilitating biosynthesis of communicative pheromones and influencing flight orientation and/or host selection to disrupting the production of crucial enzymes linked to insect growth and biological development (Seybold et al. 2006, Borden et al. 2008, Raffa 2014, Celedon and Bohlmann 2019, Jones et al. 2019).

Resinous compounds are produced, stored, and mobilized within a network of resin duct structures that are connected vertically and horizontally throughout the tree, facilitating rapid mobilization of resins to sites of injury or infection (Hood and Sala 2015, Celedon and Bohlmann 2019). Resin ducts are embedded within annual growth rings (secondary xylem tissues), allowing retrospective quantification of tree carbon allocation between growth and constitutive defenses (Kane and Kolb 2010, Ferrenberg et al. 2014, Ferrenberg et al. 2015, Hood and Sala 2015). Several studies have found relationships between resin duct characteristics (e.g., number of ducts produced, duct size, area, density) and increased probability of survival during bark beetle outbreaks (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood et al. 2015, Hood et al. 2016), drought (Gaylord et al. 2007, Gaylord et al. 2013, Gaylord et al. 2015), and wildfire (Hood et al. 2015, Hood et al. 2016, Slack et al. 2016, Sparks et al. 2017).

Resin duct anatomy is highly variable within and across species and it is unclear as to which growth and defense characteristic(s) may influence survival under biotic and abiotic pressures. For instance, larger resin ducts have been reported in ponderosa pine (*Pinus ponderosa*) (Kane and Kolb 2010, Hood et al. 2015) and whitebark pine (*P. albicaulis*) (Kichas et al. 2020) that persisted through beetle activity, while the opposite (smaller resin ducts) was reported in surviving limber pine (*P. flexilis*) (Ferrenberg et al. 2014). Zhao and Erbilgin (2019) reported larger resin ducts for lodgepole pine (*P. contorta* var. *latifolia*) that persisted through a bark beetle outbreak, while Ferrenberg et al. (2014) did not detect any difference in duct size for surviving lodgepole. There are also inconsistencies in the literature regarding other duct metrics. For instance, Kichas et al. (2020) reported greater duct production and duct density in whitebark pine that died during beetle outbreaks while Kane and Kolb (2010) and Ferrenberg et al. (2014) reported greater production and density in surviving ponderosa and limber pine. Tree

growth is equally complex, with research demonstrating higher growth in surviving trees relative to trees that died from bark beetle attack (Ferrenberg et al. 2014, Hood et al. 2015), while other research has found the opposite (greater relative growth in trees that died compared to trees that survived) (Ferrenberg et al. 2014, Kichas et al. 2020) or no trend at all (Kane and Kolb 2010). Such nuanced and species-specific responses complicate our understanding of how these defensive features function over diverse biogeographic gradients (Ferrenberg et al. 2014, Hood and Sala 2015, Kane et al. 2017, Kichas et al. 2020).

An important component influencing tree survival in the presence of insect pressure is the chemical composition of the resin itself (Table S1) (Duhl et al. 2013, Raffa 2014, Keefover-Ring et al. 2016). While many species of conifers produce similar chemicals (e.g., α -pinene, limonene, β -myrcene), the relative composition and concentration of these compounds vary considerably across taxa as well as with biological development and local environment (Smith 2000, Moreira et al. 2014, Raffa 2014, Moreira et al. 2015, Moreira et al. 2016). This variability leads to diverse and largely unknown interactions with insects and pathogens that can facilitate significant change in forest composition and structure. For instance, mountain pine beetle (*Dendroctonus ponderosae*) uses the host monoterpene ($-$)- α -pinene as a precursor to the synthesis of its aggregation pheromone ($-$)-*trans*-verbenol, (Pitman et al. 1968, Blomquist et al. 2010, Keeling 2016, Chiu et al. 2019) and β -myrcene and δ -3-carene as synergists of flying beetles' attraction to ($-$)-*trans*-verbenol (Seybold et al. 2006, Borden et al. 2008). However, numerous other terpenes exhibit insecticidal and fungicidal properties. For instance, δ -limonene is highly toxic to insects, and the phenolic phenylpropanoid 4-allylanisole (aka estragole) inhibits attraction to pheromones (Hayes and Strom 1994, Joseph et al. 2001, Hofstetter et al. 2005). Some of these compounds (e.g., α -pinene and β -pinene) also serve as indirect defenses by attracting parasitoids of bark colonizers (Mizell et al. 1984, Erbilgin and Raffa 2001, Seybold et al. 2006). Along with monoterpenes, sesquiterpenes represent a significant component of constitutive oleoresin, yet their ecological role(s) in bark beetle-conifer interactions is unknown. Sesquiterpenes have been shown to be highly active in other, unrelated systems (Prasifka et al. 2015, Zhang et al. 2015, Zhang et al. 2018, Zhao et al. 2020), and they may influence resin viscosity (Keefover-Ring et al. 2016). However, there is currently no research demonstrating the effects of sesquiterpenes on bark beetles or their symbionts. Additionally, terpene diversity (a measure of relative composition and abundance of compounds) may play an important role in beetle-conifer interactions (Reid et al. 2017). A higher diversity of compounds could theoretically provide trees with a greater phytochemical arsenal in dealing with a variety of herbivores and pathogens. However, it is unclear if there are any relationships between terpene diversity, resin duct anatomy, and tree growth.

There are many interacting factors that could readily alter the growth and defense strategies of trees. For instance, fire, drought, insect, and pathogen activity have been found to influence defensive features (resin flow, resin chemistry and resin duct anatomy) in conifers (Perakis et al. 2011, Powell and Raffa 2011, Gaylord et al. 2015, Hood et al. 2015, Valor et al. 2017). Competition for resources has long been recognized as an important factor mediating both growth and defense in coniferous tree species (Coomes and Grubb 2000, Larocque et al. 2013) and research has demonstrated increased diameter growth of remaining trees in both natural and managed forest stands when near-tree competition is reduced through various silviculture treatments (Keane et al. 2007, Hood et al. 2016, Tepley et al. 2020). Yet, studies quantifying the effects of competition on conifer defenses (duct anatomy and resin chemistry) are surprisingly limited (Ormeno et al. 2007, Slack et al. 2017). Resin-based defenses are costly to manufacture and maintain, and competition constrains cellular differentiation and biosynthesis of secondary metabolites (Herms and Mattson 1992), altering allocation to growth and defenses. In many ecosystems, biotic and abiotic disturbances drive forest structure and composition, creating a patchwork mosaic of diverse species assemblages in various age classes (Baker

2009). Western U.S. forests are prone to wildfire, insect herbivory and pathogens, which can differentially modulate stand development (Boone et al. 2013, de la Mata et al. 2017, Holtz and Schoettle 2018, Howe et al. 2018). Competition within these forest stands likely influences how trees allocate resources between growth and investment in physical and chemical defenses; however, this is an area of research that is poorly explored. Indeed, we are aware of only three studies that have investigated potential relationships between near-tree competition and the anatomical (Slack et al. 2017) and chemical (Raffa and Berryman 1982, Ormeno et al. 2007) components of resin duct defenses.

Whitebark pine is a critical high-elevation species that provides multiple ecological services within subalpine and alpine ecosystems (Logan et al. 2010, Tomback et al. 2016a, Tomback et al. 2016b, Wagner et al. 2018). However, there is growing concern that whitebark pine may be largely extirpated from its current habitat over the next century due to cumulative impacts of climate change, insect-related mortality, changing fire regimes, increased competition from shade-tolerant species, and the invasive exotic pathogen causing white pine blister rust (*Cronartium ribicola*). These stressors, primarily blister rust, have greatly reduced populations, by as much as 90% in some areas of the northern Rocky Mountains (Keane and Arno 1993, Kendall and Keane 2000, Shanahan et al. 2016, Amberson et al. 2018). In December 2020, the U. S. Fish and Wildlife Service formally proposed whitebark pine as a “threatened” species under the Endangered Species Act, which if approved will require careful and adaptive management to sustain and promote remaining populations. Better understanding the physiological responses of whitebark pine to competition and disturbance could provide useful information for resource managers in developing conservation and restoration efforts. Some work has investigated the physical and chemical defenses of whitebark pines in the Greater Yellowstone Ecosystem (Raffa et al. 2013, Raffa et al. 2017, Mason et al. 2019), but this represents only a portion of its overall range, precluding inference to other forest types across a broader range of environments.

Lodgepole pine (*Pinus contorta* var. *latifolia*) is widely distributed throughout western North America and sometimes co-occurs with whitebark pine and other mountain conifers (Lott and Critchfield 1990). In the Rocky Mountains, lodgepole pine grows in either mixed or pure stands and is fire-adapted. Lodgepole pine has serotinous cones that are held tightly together with resins and are released by heat from fire or persistent hot dry conditions, after which its seeds are distributed by wind (Anderson 2003). Although individual trees can be killed by fire, the prolific release of seed following disturbance provides competitive advantage, resulting in extensive distributions of dense lodgepole pine forests. Lodgepole pine is also the primary host of mountain pine beetle and experiences widespread mortality during outbreaks (Meddens et al. 2012).

We compared growth and resin duct structures between similarly sized lodgepole pine, the most frequent host of mountain pine beetle (Meddens et al. 2012), and whitebark pine, a historically less-exposed host undergoing increased pressure with warming climate (Logan et al. 2010) growing in close proximity within a disturbance-prone, mixed-conifer forest in northwestern Montana. We analyzed tree growth, resin duct morphology, and constitutive phloem chemistry to investigate three questions: 1) Are there correlations among tree growth, resin duct anatomy, and resin chemistry? 2) How do constitutive resin chemistry and growth vs. defense relationships compare between whitebark and lodgepole pine? 3) Does the abundance and diversity of overstory and understory competition influence resin chemistry for either species? For question 1, we predicted H₁) trees with greater growth rates and trees producing more numerous and larger resin ducts would have higher concentrations ($\mu\text{g/g}$ of tissue) and diversity of mono- and sesqui- terpenes. We also predicted H₂) trees with greater relative duct area (% annual ring allocated to resin duct structures) would have greater concentrations and diversity of mono- and sesqui- terpenes. For question 2, we hypothesized H₃) whitebark produce a greater abundance of constitutive monoterpenes

known to positively influence bark beetle behavior (such as α -pinene, β -myrcene, and δ -3-carene) whereas lodgepole produce a greater abundance of compounds known to influence host-recognition (principally β -phellandrene, which has been found to be a primary compound that mountain pine beetle utilize in its selection of host trees (Huber et al. 2000, Miller and Borden 2000)). We also predicted H₄) lodgepole pine has greater relative growth rates than whitebark pine and produces larger resin ducts with a greater annual investment in resin duct structures (increased relative duct area). We suspect that beetle preference for lodgepole pine has selected for more robust constitutive defenses than whitebark pine and that this combination of increased growth and favorable duct anatomy will correspond with a greater abundance and diversity of mono- and sesqui- terpenes compared to whitebark pine. For question 3, we predicted H₅) increased competition (greater abundance and diversity of neighboring overstory and understory vegetation) results in decreased concentrations and diversity of mono- and sesqui- terpenes. We expect that moderately dense, mixed-conifer forest stands of relatively young age comprise a nitrogen-limited system, and as competition increases nutrient availability declines, limiting overall production of primary and secondary metabolites.

2. Methods

2.1. Site description

Data were collected at a high-elevation site on the western slopes of the Mission Range on the Flathead Indian Reservation during the summers of 2016 and 2017 as part of a larger fire-history reconstruction for the Confederated Salish and Kootenai Tribes (CSKT; Fig. 1) (Kichas et al. 2020). The study site is located at approximately latitude: 47.776026, longitude: -113.971389, with an average elevation of 1,916 m. Overstory species include whitebark pine and lodgepole pine, with smaller components of subalpine fir (*Abies lasiocarpa*) and Engelmann spruce (*Picea engelmannii*). Understory species consist primarily of grouse whortleberry (*Vaccinium scoparium*), common huckleberry (*V. membranaceum*), dwarf huckleberry (*V. cespitosum*), bear grass (*Xerophyllum tenax*), smooth woodrush (*Luzula hitchcockii*) and menziesia (*Menziesia ferruginea*). Mean annual temperatures at the site are generally cool, with average temperatures ranging from 1° to 6° C with a 20- to 70-day frost-free period. Annual precipitation averages 140 cm, which occurs primarily as snowfall from November to March. Soils are of the Halloway series, consisting of gravelly silt loam and are moderately drained. Lodgepole pine and whitebark pine are codominant at this site, with lodgepole pine comprising 39% of sampled trees (519 of 1320 trees) and whitebark pine representing 36% of surveyed individuals (476 of 1320 trees). The remaining 25% consists of subalpine fir (187 trees; 14%), Engelmann spruce (126 trees; 10%), western larch (*Larix occidentalis*; 9 trees; <1%) and Douglas-fir (*Pseudotsuga menziesii*; 4 trees; <1%). Mortality is also prevalent, with approximately 86% of whitebark trees and 45% of lodgepole pine trees sampled for the fire history study being dead with evidence of bark beetle activity (such as blue staining in retrieved increment cores and/or bark beetle galleries on the bole of sampled individuals). Evidence of disturbance legacies, including wildfires and insect / pathogen outbreaks are common across the sample plots. The broader region has a recent history of documented bark beetle activity in the 1930s, 1960s–1980s and 2002–2009 (Arno and Hoff 1989, Kipfmuehler and Swetnam 2002, Buotte et al. 2017, van de Gevel et al. 2017). This portion of the Mission Range is also characterized by mixed- and lethal-severity fire regimes, resulting in a complex mosaic of establishment (CSKT 2000, 2007). White pine blister rust is evident, having been introduced to this region of the Rocky Mountains circa 1950 (Samman et al. 2003, Geils et al. 2010).

2.2. Field sampling

As part of the CSKT fire-history reconstruction project, a 200 m grid

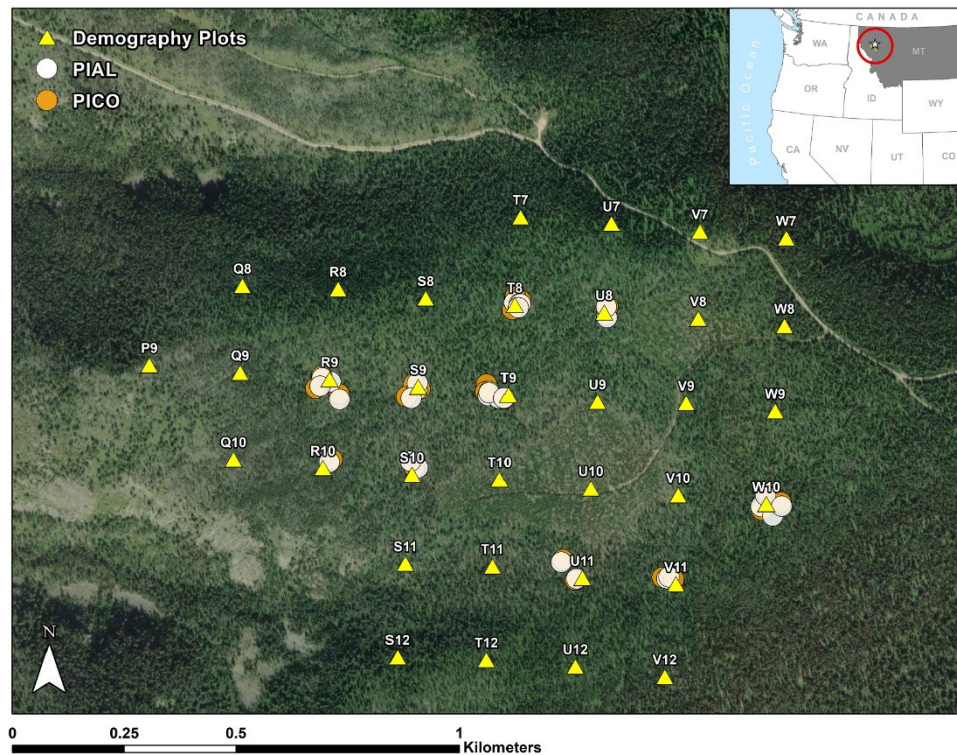


Fig. 1. Map of sampled trees: 30 whitebark pine (white) and 30 lodgepole pine (orange) from a high elevation, mixed-conifer stand in the Mission Range (~4.5 km east of Flathead Lake) on the Flathead Indian Reservation in northwestern Montana. Yellow triangles indicate permanent demography plots that were established in 2016 as part of a larger fire-history study for the Confederated Salish and Kootenai Tribes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was overlain across the study area using a combination of remote sensing and field surveying for optimal placement (Fig. 1). This gridded sampling design captured much of the topographic and vegetative variability across the site, providing for an accurate assessment of disturbance legacies and microsite differences in forest structure and stand composition. Within the 200 m grid, individual plots (hereafter “macroplots”) were located 200 m apart with a total of 34 plots, covering approximately 88 ha (Fig. 1).

Each macroplot was spatially referenced and plot centers were permanently marked and tagged. From the macroplot center, four 10 m wide, 100 m long belt transects were established in each cardinal direction (north, south, east, and west; Fig. S1). The closest ten trees within each 100 m transect were sampled (to a maximum distance of 100 m) with a diameter threshold of 15 cm diameter at breast height (DBH) (Heyerdahl et al., 2014). Data collected for each tree at each macroplot included species, condition (live or dead), DBH, canopy base height, crown class (dominant, codominant, intermediate, or suppressed), tree height, and evidence of fire. We collected increment cores from 40 sample trees per macroplot near the base of each tree (at or below 15 cm from the root collar) using Haglöf 4.3 mm diameter increment borers. A 25 m² circular microplot was also established around plot center to estimate percent cover of understory species (Fig. S1).

2.3. Field sampling – Terpene composition

From the larger sample of stand-level demographic data, we identified 30 pairs of live whitebark and lodgepole pines for this analysis. To

control for microsite variability and visible differences in ontogeny we paired trees based on size (± 3 cm DBH) and distance (< 15 m apart). In June 2018, individual trees were relocated in the field, across the previously established macroplots. To assess the potential influence of overstory competition on constitutive resin chemistry, we recorded additional information, including species and DBH for all live and dead trees (≥ 1 cm DBH) within five meters of each subject and for all trees (≥ 5 cm DBH) to a distance of ten meters (Fig. S2). For understory competition, we identified species and estimated percent cover within a 5.6 m diameter microplot around each subject tree. We collected phloem tissue of approximately 1.5 cm width and 5 cm length for all 60 individuals. Tissue samples were immediately placed in glass vials on dry ice and were transported to Montana State University for storage in a -80° C freezer within 24 h of collection. After the phloem tissues were collected, we retrieved one additional increment core at DBH using 4.3 mm diameter borers within approximately 5 cm from the phloem sampling location.

2.4. Increment core analysis

Increment cores were air-dried and processed using standard dendrochronological techniques (Stokes and Smiley 1996). We measured ring widths to the nearest 0.001 mm using a Velmex measuring stage interfaced with the recording software Measure J2X (Voortech Consulting 2005). We crossdated tree ring series visually and graphically using ring-width patterns and frost rings (LaMarche and Hirschboeck 1984). Crossdating was facilitated using site-specific chronologies from ring-width measurements, which were compiled as

part of a previous study (Kichas et al. 2020) and statistically checked using COFECHA (Holmes et al. 1986, Grissino-Mayer 2001).

Individual tree cores were scanned at high resolution (1200 d.p.i.) on an Epson V550 flatbed scanner and resin duct features were measured using the program ImageJ (version 1.46r, National Institutes of Health, Bethesda, MD, USA). All resin ducts were measured to the nearest $1 \times 10^{-7} \text{ mm}^2$ using the ellipse tool and the calendar year in which they formed was documented. For defense metrics we followed the methods outlined by Hood and Sala (2015) and collected five measurements, including three non-standardized measures of resin duct size (mean size of all resin ducts per annual ring; mm^2), duct production (number of ducts year^{-1}), and total duct area (sum of resin duct size: $\text{mm}^2 \text{ year}^{-1}$), and two measures standardized to tree growth, duct density (number of resin ducts $\text{mm}^{-2} \text{ year}^{-1}$) and relative duct area (% annual ring).

2.5. Analysis of monoterpenes and sesquiterpenes

Following the methods of Trowbridge et al. (2019), phloem tissues were finely chopped (exact weights between ~ 0.2 and 0.3 g) and placed into 2-mL glass vials along with 1 mL of GC-grade n-hexane (Fisher Scientific) containing 0.1 $\mu\text{L mL}^{-1}$ (+)-fenchone (Sigma-Aldrich) as an internal standard. Samples were then agitated for 24 h, after which 100 μL of the solution from each sample was transferred into micro-inserts in small mouth clear GC vials and capped with PTFE liners (Alltech Associates, Deerfield, IL). Chemical analysis was then performed using gas chromatography-mass spectrometry (GC-MS). Monoterpenes and sesquiterpenes were identified by comparing retention times of known standards and mass spectra using the NIST library and MSD Chemstation software. Concentrations for each compound were calculated using five-point calibration curves with injections of known amounts of pure standards and the internal standard, fenchone. When pure standards were unavailable, standard curves for (-)- α -pinene, terpinolene, bornyl acetate, and caryophyllene were used for monoterpenes, oxygenated monoterpenes, monoterpene esters and sesquiterpenes, respectively. For unidentified compounds, putative NIST IDs were used when match quality was greater than 80.

2.6. Data analysis

All analyses were conducted using R (v. 3.5.0) (R Development Core Team 2008). We used Wilcoxon paired *t*-tests, linear regression, correlation, and multivariate analyses of variance to investigate relationships in growth and defense anatomy, as well as constitutive resin chemistry, across lodgepole and whitebark pine pairs. We evaluated significance using one-tailed tests for *a priori* expectations described in the Introduction (e.g., higher concentrations of terpenes, greater duct size, greater duct production, etc.) and two-tailed tests for all other tests using $\alpha = 0.05$.

It is currently unclear how long resin ducts in conifers remain biologically active and contribute to constitutive and induced resin chemistry (Trapp and Croteau 2001). As such, we measured growth and defense metrics over the full record (1914–2018) as well as over the most recent 20-years (1998–2018), and 5-years (2013–2018) prior to sampling. We applied a Bonferroni correction factor ($\alpha = 0.05/3 = 0.017$) for all growth periods to account for multiple comparisons across similar growth intervals. From the raw tree ring width data we used the *dplR* package (v. 1.7.1) (Bunn 2008) to calculate cross-sectional basal area increments (BAI; $\text{mm}^2 \text{ year}^{-1}$) as an approximate indicator of volumetric growth over time (Biondi and Qeadan 2008).

For mono- and sesqui- terpenes, we standardized concentrations by phloem mass (g) to calculate absolute concentration ($\mu\text{g} / \text{g}$ phloem) for each compound. We then summed the concentrations for all identified compounds to obtain total concentration (41 identified monoterpenes and 39 identified sesquiterpenes). For individual compounds, we calculated the relative concentration of each as percent of the total concentration. A one-way Welch's ANOVA was used to test for a species

effect on total mono- and sesqui- terpenes, followed by a Tukey's honest significant difference test (HSD) *post-hoc* to identify pairwise differences between species. Due to non-normally distributed data, mono- and sesqui- terpene amounts were either square root or log-transformed for these analyses. Mono- and sesqui- terpene diversity was calculated as the Shannon diversity index (H'), a measure of within-sample diversity. We also used non-metric multidimensional scaling (NMDS) to explore mono- and sesqui- terpene dissimilarity between the two species (Kenkel and Orlóci 1986, Dixon 2003). Dissimilarity for the NMDS ordination was calculated using the *betadisper* function in the *vegan* package (v. 2.5–6) in R (Dixon 2003).

To assess the role of competition we summed basal area (m^2) for each species of competitor and for all trees sampled around each focal tree to a maximum distance of 10 m (Fig. S2). From these data we calculated stand density index (SDI) (Reineke 1933) for each tree and developed generalized linear models to assess whether local competition and/or canopy position, influence constitutive levels of mono- and sesqui- terpenes and/or growth and resin duct anatomy. All models were compared using Akaike information criterion (AIC) (Burnham et al. 2010).

3. Results

3.1. Relationship between constitutive resin chemistry and resin ducts

Whitebark and lodgepole pines at our study site were similar in terms of size (diameter and height), age, and growth (Table 1). In general, tree size, tree growth, and resin duct properties correlated poorly with constitutive mono- and sesqui- terpenes in both species. However, across the full time-series (1914–2018) resin duct area was positively correlated with monoterpenes ($r = 0.44$, $p = 0.0152$) and sesquiterpenes ($r = 0.44$, $p = 0.0150$) in whitebark pine only (Table 2). This held true for whitebark pine duct area and sesquiterpenes over the 20-years prior to sampling ($r = 0.48$, $p = 0.0079$) but not over the 5-years prior to sampling (Table S2). Whitebark pine resin duct size also tended to be correlated positively with sesquiterpenes across the full record ($r = 0.41$, $p = 0.0127$; Table 2) and over the 20-years prior to sampling ($r = 0.45$, $p = 0.0069$) but not over the 5-years prior to sampling (Table S2).

Resin duct production was positively correlated with monoterpenes in whitebark pine ($r = 0.43$, $p = 0.0095$) across the full time-series but showed no relationship in lodgepole pine (Table 2). We did not detect relationships between constitutive mono- and sesqui- terpenes and resin duct density, relative duct area, growth (BAI), or tree size (DBH) for either species (Table 2).

3.2. Chemical composition of constitutive resin in trees

Whitebark pine contained 31% greater abundance ($\mu\text{g} / \text{g}$ of sample tissue) of constitutive monoterpenes ($p = 0.0193$; Fig. 2A) and 68%

Table 1

Mensuration data (mean $\pm$ S.E.) for 30 whitebark pine (*P. albicaulis*) and 30 lodgepole pine (*P. contorta* var. *latifolia*) sampled at a high elevation montane site on the Flathead Indian Reservation in northwestern Montana.

	<i>Pinus albicaulis</i>	<i>Pinus contorta</i>
Age (years)	92.2 (± 2.11)	88.6 (± 1.78)
DBH (cm)	24.2 (± 0.81)	24.5 (± 0.84)
Height (m)	20.2 (± 1.31)	21 (± 1.3)
Live Crown Ratio (%)	73 (± 0.02)	74 (± 0.02)
Aspect ($^\circ$)	188.5 (± 13.02)	177.1 (± 12.48)
BAI ($\text{cm}^2 \text{ year}^{-1}$)	523.7 (± 50.11)	486.7 (± 42.55)
Duct Production (no. year^{-1})	3.2 (± 0.16)	2.9 (± 0.1)
Duct Size (mm^2)	0.021 (± 0.001)	0.024 (± 0.001)
Duct Area ($\text{mm}^2 \text{ year}^{-1}$)	0.038 (± 0.002)	0.039 (± 0.002)
Duct Density (no. $\text{mm}^{-2} \text{ year}^{-1}$)	0.85 (± 0.1)	0.78 (± 0.08)
Relative Duct Area (% annual ring)	0.92 (± 0.05)	1.03 (± 0.06)

Note: DBH = diameter at breast height; BAI = basal area increment

Table 2

Spearman correlation coefficients of mono- and sesqui- terpene concentrations ($\mu\text{g} / \text{g}$ fresh weight) with tree ring growth and resin duct anatomical measurements in whitebark (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*). Correlations were conducted on metrics averaged over the full time series (1914–2018).

	Monoterpenes		Sesquiterpenes	
	<i>Pinus albicaulis</i>	<i>Pinus contorta</i>	<i>Pinus albicaulis</i>	<i>Pinus contorta</i>
DBH	0.13	0.19	0.28	0.21
Growth (BAI)	0.18	0.24	0.24	0.1
Duct Production	0.43	–0.1	0.23	–0.18
Duct Size	0.26	0.34	0.41	0.18
Duct Area	0.44	0.28	0.44	0.06
Duct Density	0.35	–0.18	–0.06	–0.2
Relative Duct Area	0.33	–0.13	0.003	–0.22

Note: Bold values indicate significant correlations ($p < 0.017$). DBH = diameter at breast height; BAI = basal area increment.

greater abundance of constitutive sesquiterpenes ($p < 0.0001$; Fig. 2B) compared to lodgepole pine. The diversity (H') of constitutive monoterpenes did not differ significantly between whitebark and lodgepole pine (Fig. S3A). Unlike monoterpenes, constitutive sesquiterpene diversity was greater in whitebark pine compared to lodgepole pine ($p < 0.0001$; Fig. S3B). Generally, mono- and sesqui- terpene diversity was unrelated to tree growth and resin duct anatomy. However, in whitebark pine, monoterpene diversity was positively related to resin duct production over the full 104-year record ($r = 0.43$, $p = 0.0092$; Table S3).

We identified 22 monoterpenes that differed considerably across the two species (Fig. 3A, Table S4). Specifically, we found 5.96x greater (–)- α -pinene, 8.2x greater (+)- α -pinene, 4.5x greater β -myrcene, and 2.5x greater (+)- β -pinene in whitebark pine (Table S4). Whitebark also had greater quantities of β -ocimene (92.8x higher), γ -terpinene (3.4x higher), anisole (9.9x higher), α -longipinene (7.7x higher), ρ -cymene (36.2x higher), cis- β -terpineol (5x higher), 3-carene (3x higher), and two unidentified monoterpenes: MTP 6 (6x higher) and MTP 9 (4.3x higher; Table S4). In contrast, lodgepole pine exhibited greater concentrations of α -phellandrene (6.8x higher), β -phellandrene (1.4x higher), dill ether (32.4x higher), (+)-linalool (1.8x higher), and an unidentified monoterpene (MTP 3; 1.7x higher). There were also more nuanced differences regarding some compounds. For instance, lodgepole pine contained greater concentrations of (–)-sabinene (4.8x higher), while whitebark contained 15.7x more of the stereoisomer (+)-sabinene (Table S4). Similarly, whitebark contained 4.8x more of (–)- δ -limonene, while lodgepole contained 5.7x more of the stereoisomer (+)- δ -limonene (Fig. 3A, Table S4). Lodgepole pine also produced more of the phenolic phenylpropanoid 4-allylanisole (10.1x higher)

than whitebark pine (Fig. 3A, Table S4).

We identified 33 sesquiterpenes that were significantly different between whitebark and lodgepole pine (Fig. 3B, Table S4). Whitebark pine contained greater quantities (raw concentrations and percent composition) for 73% of these, while lodgepole pine contained greater quantities of α -cadinol (1.5x higher), and seven unknown sesquiterpenes (SQT 4, SQT 6, SQT 8, SQT 9, SQT 11, SQT 14, SQT 15) (Fig. 3B, Table S4).

3.3. Relationships between tree growth and resin ducts in whitebark and lodgepole pine

Overall, the variables describing growth and resin duct properties were similar between the two species (Table 1). Tree size (DBH), height, and growth (BAI) were similar as was resin duct production, duct area, duct density, and relative duct area. However, across the full time series (1914–2018) lodgepole pines produced 10% larger resin ducts on average relative to whitebark pine ($p = 0.0033$; Fig. 4).

Unstandardized resin duct metrics (size, area, and production) correlated positively with growth (BAI) for both species, while standardized duct metrics (duct density and relative duct area) correlated negatively with growth. Specifically, resin duct production showed a strong positive correlation with growth for whitebark pine ($r = 0.59$, $p = 0.0007$; Fig. S4A) but not for lodgepole pine (Table 3). Resin duct size and growth were positively correlated for both whitebark pine ($r = 0.52$, $p = 0.0034$) and lodgepole pine ($r = 0.56$, $p = 0.0014$; Fig. S4B). Similarly, resin duct area and growth were strongly, positively correlated for whitebark ($r = 0.71$, $p < 0.00001$) and lodgepole ($r = 0.55$, $p = 0.0016$; Table 3, Fig. S4C). In contrast, resin duct density and growth were negatively correlated for whitebark pine ($r = -0.36$, $p = 0.0539$) and lodgepole pine ($r = -0.55$, $p = 0.0019$; Table 3, Figure S4D). Relative duct area was also negatively correlated with growth for whitebark pine ($r = -0.6$, $p = 0.0005$) and lodgepole pine ($r = -0.61$, $p = 0.0004$; Table 3, Fig. S4E).

Tree size (DBH) was positively correlated with growth (BAI) for both lodgepole ($r = 0.69$, $p < 0.0001$) and whitebark pine ($r = 0.77$, $p < 0.0001$; Table 3). In whitebark pine, tree size was also positively correlated with resin duct production ($r = 0.39$, $p = 0.0352$), duct size ($r = 0.46$, $p = 0.0098$), and duct area ($r = 0.56$, $p = 0.0014$; Table 3). Tree size was negatively correlated with resin duct density in whitebark pine ($r = -0.37$, $p = 0.04215$) and relative duct area in lodgepole ($r = -0.45$, $p = 0.01179$) and whitebark pine ($r = -0.58$, $p = 0.0008$; Table 3).

3.4. Relationship of overstory competition and constitutive resin chemistry

Stand density index (SDI) was not correlated with tree size, tree growth, nor resin duct metrics for both species (Table 4). SDI was also

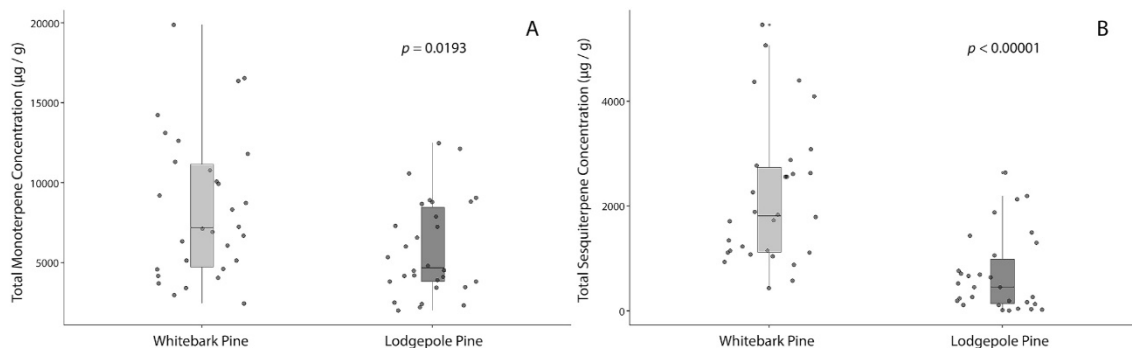


Fig. 2. Comparison of total constitutive concentrations ($\mu\text{g} / \text{g}$ fresh weight) of monoterpenes (A) and sesquiterpenes (B) across 30 whitebark pine (*P. albicaulis*) and 30 lodgepole pine (*P. contorta*).

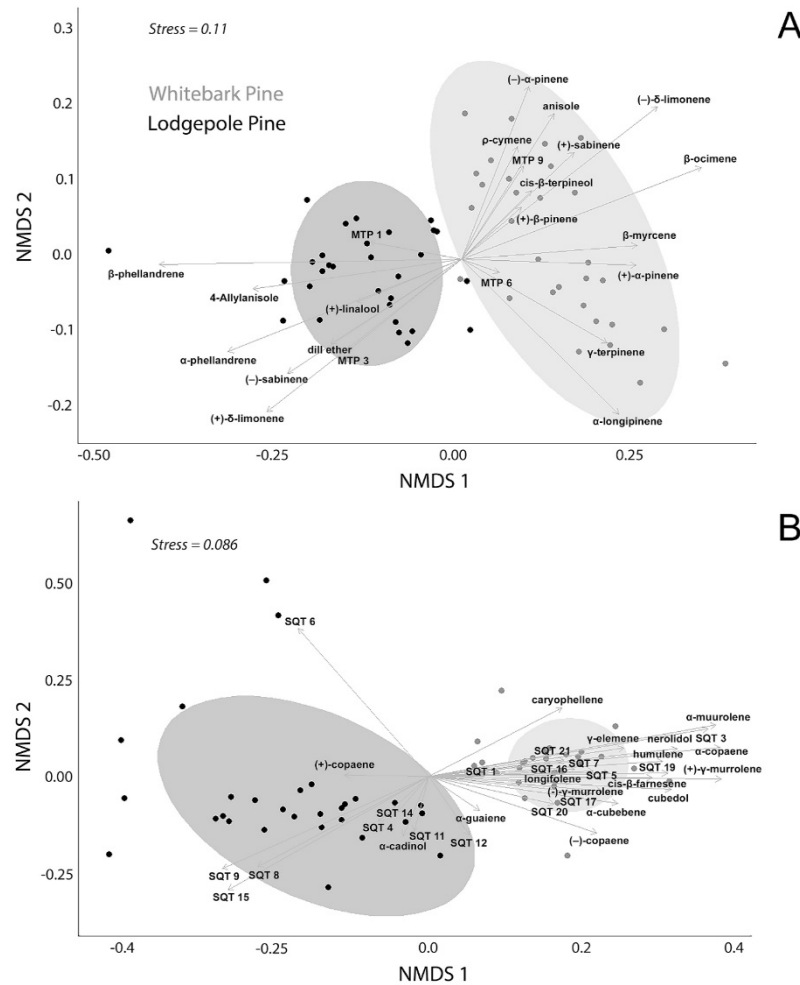


Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plot of the first and second dimensions for the A) monoterpene and B) sesquiterpene profiles of 30 whitebark pine and 30 lodgepole pine trees. Ellipses represent a 99% confidence interval for each species. Important individual monoterpenes are overlain with the relative magnitude of the arrows corresponding to their respective importance in the ordination.

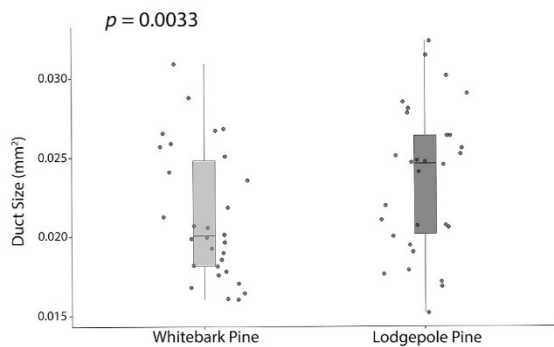


Fig. 4. Comparison of resin duct size across the full time series (1914–2018).

not correlated with constitutive mono- nor sesqui- terpenes for lodgepole pine (Table 4). However, in whitebark pine there was a significant negative correlation between monoterpenes and SDI ($r = -0.38$, $p = 0.0179$; Table 4), indicating monoterpene concentrations declined with increasing stand density.

Lodgepole pine basal area comprised approximately 55% of near-tree competition (average DBH = 24.8 cm $\pm$ 0.77), followed by whitebark pine (25%, average DBH = 15.3 cm $\pm$ 0.8), subalpine fir (12%, average DBH = 14.5 cm $\pm$ 1), Engelmann spruce (5%, average DBH = 19.3 cm $\pm$ 1.9), and Douglas-fir (3%, average DBH = 22.7 cm $\pm$ 4.7). Engelmann spruce was consistently in the top performing models predicting constitutive monoterpenes as a function of near-tree competition (Table 5). In addition, constitutive mono- and sesqui- terpene concentrations in whitebark pine were negatively affected by near-tree competition with Engelmann spruce ($p = 0.0227$) while no relationships were found for lodgepole pine (Table S5). Generally, constitutive levels of monoterpenes in whitebark pine decreased with increasing Engelmann spruce basal area.

Table 3

Spearman correlation coefficients of tree ring growth and resin duct anatomical measurements of *Pinus contorta* and *Pinus albicaulis*. Correlations were conducted on metrics averaged over the full time series (1914–2018).

<i>Pinus albicaulis</i>	DBH	Growth (BAI)	Duct Production	Duct Size	Duct Area	Duct Density	Relative Duct Area
Growth (BAI)	0.77						
Duct Production	0.39	0.59					
Duct Size	0.46	0.52	0.11				
Duct Area	0.56	0.71	0.63	0.84			
Duct Density	-0.37	-0.36	0.36	-0.48	-0.17		
Relative Duct Area	-0.58	-0.6	0.01	-0.34	-0.24	-0.85	
<i>Pinus contorta</i>							
Growth (BAI)	0.69						
Duct Production	0.12	0.08					
Duct Size	0.25	0.56	-0.06				
Duct Area	0.24	0.55	0.36	0.9			
Duct Density	-0.35	-0.55	0.25	-0.62	-0.45		
Relative Duct Area	-0.45	-0.61	0.14	-0.32	-0.22	0.87	

Note: Bold values indicate significant correlations ($p < 0.05$). DBH = diameter at breast height; BAI = basal area increment.

Table 4

Spearman correlation coefficients of stand density (Reineke's Stand Density Index; SDI), tree size (DBH), tree ring growth (BAI), and resin duct anatomical measurements for whitebark (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*). Correlations were conducted on metrics averaged over the full time series (1914–2018).

	Stand Density Index (SDI)	
	<i>Pinus albicaulis</i>	<i>Pinus contorta</i>
Monoterpene concentration	-0.38	0.09
Sesquiterpene concentration	-0.29	-0.24
Monoterpene Diversity (H')	-0.26	0.16
Sesquiterpene Diversity (H')	-0.24	-0.34
DBH	-0.05	-0.09
Growth (BAI)	-0.05	-0.28
Duct Production	0.01	-0.05
Duct Size	-0.17	-0.33
Duct Area	-0.15	-0.33
Duct Density	-0.05	0.35
Relative Duct Area	-0.22	0.28

Note: Bold values indicate significant correlations ($p < 0.05$). DBH = diameter at breast height; BAI = basal area increment.

Table 5

Generalized linear models predicting constitutive monoterpenes as a function of overstory competition. Log likelihood reflects the probability of the data given the model, while K represents the relative number of sample parameters for each model. Top five models are shown as well as intercept-only model for reference. (†) indicates a positive coefficient in the model while (‡) indicates a negative coefficient.

Model	AICc	ΔAIC	LogL	K
1. SPC (†) + PIEN (‡) + PSME (‡) + PICO (†) + [PIEN * SPC] (‡) + [PICO * SPC] (‡)	1159.6	0.00	-570.39	6
2. SPC (†) + PIEN (‡) + PSME (‡) + [PIEN * SPC] (‡)	1160.7	1.12	-573.57	4
3. SPC (†) + PIEN (‡) + [PIEN * SPC] (‡)	1161.3	1.69	-575.09	3
4. SPC (†) + PIEN (‡) + PSME (‡) + PICO (†) + [PIEN * SPC] (‡) + [PSME * SPC] (‡) + [PICO * SPC] (‡)	1161.3	1.75	-569.87	7
5. SPC (†) + PIEN (‡) + PSME (‡) + PICO (†) + ABLA (‡) + [PIEN * SPC] (‡) + [PICO * SPC] (‡)	1161.8	2.21	-570.10	7
6. Intercept-Only Model	1168.8	9.2	-582.28	1

Abbreviations: SPC (species), PIEN (Engelmann spruce competitors), PSME (Douglas-fir competitors), PICO (lodgepole pine competitors), ABLA (subalpine fir competitors).

4. Discussion

Our 104-year record comparing growth and defense characteristics within and between whitebark and lodgepole pine yields four important findings. First, we did not find evidence of a tradeoff between tree growth and tree defenses (resin duct morphology and resin chemistry; Table 2, S2). This suggests that trees growing under favorable field conditions can experience high growth rates and be well defended. Second, we found that resin duct size, production, and area are not related to constitutive mono- and sesqui- terpene concentrations in lodgepole pine, whereas resin duct production and area were positively related to constitutive monoterpenes, and resin duct size and area were positively related to constitutive sesquiterpenes, in whitebark pine (Table 2, S2). The lack of distinct, universal relationships between these defensive features presents beetles with numerous and unpredictable combinations of resin-based defenses, particularly from its historical host, lodgepole pine (Mason et al. 2019, Howe et al. 2020). Third, based on constitutive terpene profiles, bark beetles are more likely to enter lodgepole pine but more likely to successfully elicit mass attacks in whitebark pine, which agrees with beetle attack and success patterns in the field (Fig. 3) (Bentz et al. 2015). Fourth, overstory competition, particularly by Engelmann spruce, can influence tree defenses, specifically constitutive terpene concentrations (Table 5, S5). Competitive tree interactions could lead to altered bark beetle-conifer interactions as host and nonhost species migrate in response to changing climate. This is particularly relevant within montane environments, where climate-related tree species migration could occur more rapidly (elevational range shifts over hundreds of meters) compared to flatter regions (latitudinal range shifts over hundreds of kilometers).

Overall, relationships between growth and defense in lodgepole and whitebark pine were relatively similar (Table 3). Counter to our hypothesis (H_1), we did not find evidence to suggest that trees producing more and larger resin ducts also have higher concentrations and diversity of mono- and sesqui- terpenes. In contrast, whitebark pine trees produced smaller resin duct structures than lodgepole pine (Fig. 4) but also had greater constitutive levels of mono- and sesqui- terpenes (Fig. 2). These findings differ from results in the Greater Yellowstone Ecosystem reporting greater duct production and greater levels of constitutive monoterpenes and diterpenes in lodgepole pine than whitebark pine but are similar in both showing higher sesquiterpenes in whitebark pine (Fig. S3) (Raffa et al. 2013, Raffa et al. 2017, Mason et al. 2019). In addition, resin duct area positively correlated with constitutive mono- and sesqui- terpenes for whitebark pine, but not in lodgepole pine, suggesting that whitebark pines with increased resin duct area also have greater concentrations of constitutive mono- and sesqui- terpenes (Table 2, S2). This is supported by research demonstrating that, in some species, increased resin duct area is positively related to resin flow (Hood and Sala 2015, Yi et al. 2021). Duct area also correlated strongly

with tree size (DBH) and growth (BAI) in whitebark pine (Table 3), which could assist managers in the identification of candidate trees for silvicultural treatments and/or seed collection efforts (Table 3). Counter to our hypothesis (H₂) relative duct area was not related to constitutive mono- and sesqui- terpene concentrations for either species (Table 2). Lodgepole pine had greater relative duct area than whitebark pine but lower levels of constitutive mono- and sesqui- terpenes (Fig. 2). In addition, sesquiterpene diversity was lower in lodgepole pine than whitebark pine while we did not detect significant difference in monoterpene diversity between the two species (Fig. S3).

Interestingly, we did not detect significant tradeoffs in the allocation of resources between tree growth and investment in resin duct structures and constitutive mono- and sesqui- terpenes. Rather, we found generally positive relationships between tree size and growth versus unstandardized resin duct metrics and constitutive mono- and sesqui- terpenes (Table 2, S2). This likely reflects the highly variable site quality of natural forest stands rather than an absence of within-plant allocation posited by *Hermes and Mattson (1992)*. That is, within-plant allocation patterns do not necessarily scale up to between-plant inverse relationships (and may even be reversed) at the landscape scale (*Howe et al. 2020*). This view has some support, as *Waring and Pitman (1985)*, *Bleiker et al. (2005)*, *Knapp et al. (2013)*, *Williams et al. (2018)* and *Keen et al. (2020)* found that fast-growing trees were most likely to survive bark beetle outbreaks. However, other research has demonstrated the opposite, whereby slower growing trees were more likely to persist through increased beetle activity (*de la Mata et al. 2017*, *Reed and Hood 2020*). Investment in plant growth and defense is nuanced and changes across geographic environments and microclimates, as well as with plant ontogeny, stand dynamics and seasonality. Given the numerous physiological mechanisms modulating tree growth and the expression of defensive features, more research is needed to better understand if and how these relationships differ across broader ecological and temporal scales.

We interpret the lack of clear relationships among tree growth, resin duct morphology and resin chemistry as part of complex evolutionary processes shaping bark beetle-host interactions. While mountain pine beetles have evolved to exploit chemical signatures in the identification and colonization of individuals, trees present bark beetles with innumerable permutations of physical and chemical defenses (*Mason et al. 2019*). A beetle cannot readily predict resin duct architecture during host identification and colonization. Thus, trees with well-developed resin duct defenses have an advantage of enduring bark beetle attack. This is supported by research demonstrating the importance of resin duct anatomy in surviving beetle activity for a variety of conifers (*Vázquez-González et al. 2020*). For instance, *Kichas et al. (2020)* found that whitebark pine that persisted through stand-level beetle activity produced fewer but larger resin ducts with greater resin duct area and greater relative duct area than whitebark pine that died. Similar results have been reported for lodgepole pine (*Ferrenberg et al. 2014*, *Zhao and Erbilgin 2019*), ponderosa pine (*Kane and Kolb 2010*, *Hood et al. 2015*), limber pine (*Ferrenberg et al. 2014*, *Bentz et al. 2017*), maritime pine (*P. pinaster*) (*Zas et al. 2014*), foxtail pine (*P. balfouriana*) and Great Basin bristlecone pine (*P. flexilis*) (*Bentz et al. 2017*). Likewise, the composition and quantities of resin chemistry, both constitutive and induced, can mediate tree survival from bark beetles (*Raffa et al. 2005*, *Zhao et al. 2011*, *Erbilgin et al. 2017*). There is also evidence to suggest that insect activity can shape growth and resin-based defenses directly in lodgepole pine (*Zhao et al. 2019*) and ponderosa pine (*de la Mata et al. 2017*). However, the myriad interacting factors influencing tree defenses complicates our ability to generalize relationships across species or make assumptions about resin duct characteristics and beetle interactions. More work is needed to understand the many biotic and abiotic processes modulating tree growth and development of resin-based defenses, specifically relating to climate, stand dynamics, and disturbance histories.

4.1. Constitutive resin chemistry

We found clear species-level differences in the composition of mono- and sesqui- terpenes between whitebark and lodgepole pine (Fig. 3, Table S4). These differences relate to mountain pine beetle behavior and attack success and largely agree with other research (*Raffa et al. 2013*, *Raffa et al. 2017*). In support of H₃, whitebark pine produced greater quantities of compounds known to enhance beetle success, including (–)- α -pinene, which beetles use as precursor for the biosynthesis of their aggregation pheromone, (–)-*trans*-verbenol (*Blomquist et al. 2010*, *Keeling 2016*). Whitebark also produced more β -myrcene, which mountain pine beetles utilize as synergists for pheromone attraction (*Borden et al. 2008*). While lodgepole pine produced less of these compounds we found significantly greater levels of α -phellandrene and β -phellandrene, which mountain pine beetles exploit for host recognition. Lodgepole pine also produced more compounds that inhibit beetle fitness, including (+)- δ -limonene, which is highly toxic to mountain pine beetles, and 4-allylanisole (aka estragole), which inhibits attraction to pheromones (*Hayes and Strom 1994*). Thus, despite having fewer exploitable compounds for pheromone production and enhancement, the increased concentration of compounds important for host recognition likely results in mountain pine beetles identifying lodgepole pines for colonization over co-occurring whitebark pine under endemic beetle activity. However, under mass attack and outbreak scenarios, beetles are more likely to succeed when colonizing whitebark pine trees. As beetle activity is projected to increase with increasing temperatures, particularly at higher elevations, the implications for whitebark pine mortality are concerning and may warrant management actions to mitigate potential loss of this important species.

We found some evidence for our hypothesis (H₄) as lodgepole pine produced larger resin ducts with greater relative duct area compared to whitebark pine; however, counter to our hypothesis, lodgepole pine had lower levels of constitutive terpenes (Figs. 2, 4). We do not interpret the lack of relationship between resin duct anatomy and constitutive resin chemistry in lodgepole pine as an indication that lodgepole pine are any less defended against bark beetles than whitebark pine. There are many factors, such as climate, soil quality, tree size / age, stand dynamics, and disturbance histories, that can influence tree physiology and defense anatomy in conifers. For instance, the chemical composition of oleoresin can change dramatically when trees are exposed to fungi vectored by bark beetles (*Keefover-Ring et al. 2016*). Notably, our results only pertain to constitutive chemical defenses. While there is a growing body of literature investigating constitutive and inducible defenses, more work is needed to better understand the relationship of constitutive and inducible defenses within and across species, as well as ecological implications of these relationships (*Howe et al. 2020*). In addition, the potential ecological roles of sesquiterpenes in beetle-conifer-microbial interactions are unknown. Sesquiterpenes can play a role in cold tolerance in some plants (*Zhao et al. 2020*), which is consistent with their higher abundance in the high elevation whitebark pine, but again such a function has not been tested in pines. More work is needed to understand how these compounds vary across species and environments and what functions they may serve in conifer defense.

4.2. Influence of competition on tree defense

Trees sampled for this study were relatively young (90 year mean age) (*Reed et al. 2018*) and growing in a moderately dense lodgepole pine-dominant, mixed-conifer forest stand in the northern Rocky Mountains. We hypothesized that competition in this setting would reduce nutrient availability, limiting the overall capacity for trees to produce primary and secondary metabolites. We found some support for this hypothesis (H₅), as stand density (SDI) was negatively correlated with constitutive monoterpene concentrations for whitebark pine (Table 4). Also, the frequency of relationships having a negative although statistically insignificant relationships suggests that additional

studies into whether increased competition reduces growth and defense in whitebark and lodgepole pine is warranted. Future studies providing greater spatial and temporal resolution and more explicitly directed design may reveal ecologically meaningful insights into the role of local competition.

We did not detect notable influence of inter- or intra- specific competition on constitutive terpene abundance or diversity (Table S5). However, we did find evidence that Engelmann spruce interacts with whitebark pine, resulting in decreased monoterpene concentrations with increasing Engelmann spruce presence (Table 5, S5). This trend was observed in whitebark pine despite Engelmann spruce occupying a small percentage of local competition (5%). In addition, Engelmann spruce was a comparatively strong predictor of constitutive monoterpenes in whitebark pine compared to other competitors (Table 5). This merits further investigation because as climate warms and different tree species migrate along elevational and/or latitudinal gradients they may increasingly encounter species, such as Engelmann spruce, that could negatively influence their defenses. This could have important implications for management and conservation efforts, particularly given projected increases in both temperature and mountain pine beetle activity in the western U.S. over the next century (Bentz et al. 2016, Buotte et al. 2016, 2017).

Whether these effects are attributable to the microsites where Engelmann spruce resides or to more nuanced direct and indirect effects of Engelmann spruce ecology (e.g., changes in soil characteristics through litter deposition, root exudates, and unique microbial assemblages) remains unclear. It is also possible that other tree-level factors, such as tree height and canopy vigor, could influence these relationships, as taller trees with more photosynthetically active canopies may be superior competitors for light and limiting nutrients. While we only assessed diameter and species information for competitor trees, we recommend that future studies investigating the role of competition on conifer defenses also consider other aspects of tree growth and function. Understanding the degree to which competition and/or differences in site characteristics influence constitutive and induced resin-based defenses will enable managers to modify prescriptions to better achieve desired objectives.

4.3. Conclusions

We found differences in whitebark and lodgepole pine growth and physical / chemical defenses that have important implications for bark beetle behavior and forest management. Importantly, we did not detect tradeoffs between tree growth and constitutive resin-based defenses, indicating that trees at this site are both growing fast and well defended. Second, tree growth, resin duct architecture, and resin chemistry are largely unrelated. The lack of relationships between these different factors presents beetles with many permutations of resin-based defenses that can address the multitude of contexts under which attacks occur. Third, whitebark pine contains greater levels of constitutive compounds known to enhance bark beetle behavior and success, while lodgepole pine contains greater concentrations of compounds known to increase host recognition. Lastly, our record suggests that interspecific competition, particularly with Engelmann spruce, can influence constitutive resin chemistry. This may in turn increase susceptibility to mountain pine beetle, yielding unexpected challenges in whitebark pine conservation and restoration efforts.

These results have several important implications. From the perspective of a bark beetle, whitebark pine appear more chemically conducive for reproductive success while lodgepole pine are more identifiable as potential hosts. Under endemic scenarios mountain pine beetle persist in low numbers, colonizing stressed individuals with depleted defense systems. However, during outbreak conditions, beetle populations can overwhelm defenses of vigorous, well-defended trees. As such, even the healthiest whitebark pine growing within this mixed-conifer setting may be vulnerable to beetle-induced mortality. However,

conifers have co-evolved with insects and have developed complex defensive strategies in response to these pressures. Resin duct architecture can facilitate oleoresin production and mobilization in ways that are not readily perceptible to beetles. The lack of clear relationships between these various defensive features reflects the complexity of the biophysical relationships connecting them. In addition, local competition and species-specific interactions may further influence growth and defense characteristics, leading to unpredictable outcomes as species migrate in response to a warming climate.

Changing forest conditions are confronting managers with increasingly limited options for preserving and promoting whitebark pine populations. Many management prescriptions rely on superficial performance indicators (primarily growth) that fail to account for the important role of physical and chemical defenses in moderating disturbance interactions. Few studies have investigated patterns of growth and defense using multiple proxies (resin duct anatomy and resin chemistry), so there is a need to expand this research to a broader range of species and environments.

CRediT authorship contribution statement

Nickolas E. Kichas: Conceptualization, Investigation, Formal analysis, Methodology, Visualization, Writing - original draft, Funding acquisition. **Amy M. Trowbridge:** Conceptualization, Investigation, Formal analysis, Methodology, Resources, Writing - review & editing. **Kenneth F. Raffa:** Investigation, Supervision, Writing - review & editing. **Shealyn C. Malone:** Formal analysis, Investigation, Writing - review & editing. **Sharon M. Hood:** Conceptualization, Investigation, Methodology, Writing - review & editing. **Richard G. Everett:** Project administration, Resources. **Gregory T. Pederson:** Resources, Writing - review & editing. **David B. McWethy:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2021.119286>.

References

- Amberson, J., Keville, M., Nelson, C., 2018. Effects of Disturbance on Tree Community Dynamics in Whitebark Pine (*Pinus albicaulis* Engelm.) Ecosystems. *Forests* 9 (566).
- Anderson, M.D., 2003. *Pinus contorta* var. *latifolia*. Fire Effects Information System. U.S. D.A. Forest Service, Fire Sciences Laboratory.
- Arno, S.F., Hoff, R.J., 1989. Silvics of Whitebark Pine (*Pinus albicaulis*). In: F. S. United States Department of Agriculture, (Ed.) *Lattermountain Research Station*, Ogden, UT.

- Baker, W.L., 2009. Fire Ecology in Rocky Mountain Landscapes, 1 edition. Island Press, Washington, D.C.
- Bentz, B.J., Boone, C., Raffa, K.F., 2015. Tree Response and Mountain Pine Beetle Attack Preference, Reproduction and Emergence Timing in Mixed Whitebark and Lodgepole Pine Stands. *Agric. For. Entomol.* 17, 421–432.
- Bentz, B.J., Duncan, J.P., Powell, J.A., 2016. Elevational Shifts in Thermal Suitability for Mountain Pine Beetle Population Growth in a Changing Climate. *Forestry* 89, 271–283.
- Bentz, B.J., Hood, S.M., Hansen, E.M., Vandygriff, J.C., Mock, K.E., 2017. Defense Traits in the Long Lived Great Basin Bristlecone Pine and Resistance to the Native Herbivore Mountain Pine Beetle. *New Phytol.* 213, 611–624.
- Biondi, F., Qeadan, F., 2008. A Theory-Driven Approach to Tree-Ring Standardization: Defining the Biological Trend from Expected Basal Area Increment. *Tree-Ring Research* 64, 81–96.
- Bleiker, K.P., Staffan Lindgren, B., MacLachlan, L.E., 2005. Resistance of Fast- and Slow-Growing Subalpine Fir to Pheromone-Induced Attack by Western Balsam Bark Beetle (Coleoptera: Scolytinae). *Agric. For. Entomol.* 7, 237–244.
- Blomquist, G.J., Figueroa-Teran, R., Aw, M., Song, M., Gorzalski, A., Abbott, N.L., Chang, E., Tittiger, C., 2010. Pheromone Production in Bark Beetles. *Insect Biochem. Mol. Biol.* 40, 699–712.
- Boone, C.K., Keefover-Ring, K., Mapes, A.C., Adams, A.S., Bohlmann, J., Raffa, K.F., 2013. Bacteria Associated with a Tree-Killing Insect Reduce Concentrations of Plant Defense Compounds. *J. Chem. Ecol.* 39, 1003–1006.
- Borden, J.H., Pureswaran, D.S., Lafontaine, J.P., 2008. Synergistic Blends of Monoterpenes for Aggregation Pheromones of the Mountain Pine Beetle (Coleoptera: Curculionidae). *J. Econ. Entomol.* 101, 1266–1275.
- Bunn, A.G., 2008. A Dendrochronology Program Library in R (dplR). *Dendrochronologia* 26, 115–124.
- Buotte, P.C., Hicke, J.A., Preisler, H.K., Abatzoglou, J.T., Raffa, K.F., Logan, J.A., 2016. Climate Influences on Whitebark Pine Mortality from Mountain Pine Beetle in the Greater Yellowstone Ecosystem. *Ecol. Appl.* 26, 2507–2524.
- Buotte, P.C., Hicke, J.A., Preisler, H.K., Abatzoglou, J.T., Raffa, K.F., Logan, J.A., 2017. Recent and Future Climate Suitability for Whitebark Pine Mortality from Mountain Pine Beetles Varies Across the Western U.S. *For. Ecol. Manage.* 399, 132–142.
- Burnham, K.P., Anderson, D.R., Huyvaert, K.P., 2010. AIC Model Selection and Multimodel Inference in Behavioral Ecology: Some Background, Observations, and Comparisons. *Behav. Ecol. Sociobiol.* 65, 23–35.
- Celedon, J.M., Bohlmann, J., 2019. Oleoresin Defenses in Conifers: Chemical Diversity, Terpene Synthases and Limitations of Oleoresin Defense Under Climate Change. *New Phytol.*
- Chiu, C.C., Keeling, C.I., Bohlmann, J., 2019. The Cytochrome P450 CYP6DE1 Catalyzes the Conversion of Alpha-Pinene into the Mountain Pine Beetle Aggregation Pheromone Trans Verbenol. *Science Reports* 9, 1477.
- Coomes, D.A., Grubb, P.J., 2000. Impacts of Root Competition in Forests and Woodlands: A Theoretical Framework and Review of Experiments. *Ecol. Monogr.* 70, 171–207.
- CSKT, 2000. Flathead Indian Reservation Forest Management Plan. In: *Forestry*, T., (Ed.) Confederated Salish and Kootenai Tribes, Pablo, MT, p. 304.
- CSKT, 2007. Flathead Indian Reservation Fire Management Plan. In: *Management*, D.O. F., (Ed.) Confederated Salish and Kootenai Tribes, Ronan, MT, p. 115.
- de la Mata, R., Hood, S.M., Sala, A., 2017. Insect Outbreak Shifts the Direction of Selection from Fast to Slow Growth Rates in the Long-Lived Conifer *Pinus ponderosa*. *Proc. Natl. Acad. Sci.* 114, 7391–7396.
- Dixon, P., 2003. VEGAN, a Package of R Functions for Community Ecology. *J. Veg. Sci.* 14, 927–930.
- Duhl, T.R., Gochis, D., Guenther, A.B., Ferrenberg, S., Pendall, E., 2013. Emissions of BVOC from Lodgepole Pine in Response to Mountain Pine Beetle Attack in High and Low Mortality Forest Stands. *Biogeosciences* 10, 483–499.
- Erbilgin, N., Cale, J.A., Hussain, A., Ishangulyeva, G., Klutsch, J.G., Najjar, A., Zhao, S., 2017. Weathering the Storm: How Lodgepole Pine Trees Survive Mountain Pine Beetle Outbreaks. *Oecologia* 184, 469–478.
- Erbilgin, N., Raffa, K.F., 2001. Modulation of Predator Attraction to Pheromones of Two Prey Species by Stereochemistry of Plant Volatiles. *Oecologia* 127, 444–453.
- Ferrenberg, S., Kane, J.M., Langenhan, J.M., 2015. To Grow or Defend? Pine Seedlings Grow Less But Induce More Defences When a Key Resource is Limited. *Tree Physiol.* 35, 107–111.
- Ferrenberg, S., Kane, J.M., Mitton, J.B., 2014. Resin Duct Characteristics Associated with Tree Resistance to Bark Beetles Across Lodgepole and Limber Pines. *Oecologia* 174, 1283–1292.
- Franceschi, V.R., Krokene, P., Christiansen, E., Krekling, T., 2005. Anatomical and Chemical Defenses of Conifer Bark Against Bark Beetles and Other Pests. *New Phytol.* 167, 353–375.
- Gaylord, M.L., Kolb, T.E., McDowell, N.G., 2015. Mechanisms of Piñon Pine Mortality after Severe Drought: A Retrospective Study of Mature Trees. *Tree Physiol.* 35, 806–816.
- Gaylord, M.L., Kolb, T.E., Pockman, W.T., Plant, J.A., Yezzer, E.A., Macalady, A.K., Pangle, R.E., McDowell, N.G., 2013. Drought Predisposes Pinon-Juniper Woodlands to Insect Attacks and Mortality. *New Phytol.* 198, 567–578.
- Gaylord, M.L., Kolb, T.E., Wallin, K.F., Wagner, M.R., 2007. Seasonal Dynamics of Tree Growth, Physiology, and Resin Defenses in a Northern Arizona Ponderosa Pine Forest. *Can. J. For. Res.* 37, 1173–1183.
- Geils, B.W., Hummer, K.E., Hunt, R.S., 2010. White Pines, Ribes, and Blister Rust: A Review and Synthesis. *Forest Pathol.* 40, 147–185.
- Grissino-Mayer, I.D., 2001. Evaluating Crossdating Accuracy: A Manual and Tutorial for the Computer Program COFECHA. *Tree-Ring Research* 57, 205–221.
- Hayes, J.L., Strom, B.L., 1994. 4-Allylanisole as an Inhibitor of Bark Beetle (Coleoptera: Scolytidae) Aggregation. *J. Econ. Entomol.* 87, 1586–1594.
- Hermes, D.A., Mattson, W.J., 1992. The Dilemma of Plants: To Grow or Defend. *Q. Rev. Biol.* 67, 283–335.
- Hofstetter, R.W., Mahfouz, J.B., Klepzig, K.D., Ayres, M.P., 2005. Effects of Tree Phytochemistry on the Interactions among Endophytic Fungi Associated with the Southern Pine Beetle. *J. Chem. Ecol.* 31, 539–560.
- Holmes, R.L., Adams, R.K., Fritts, H.C., 1986. Tree-ring chronologies of Western North America: California, Eastern Oregon and Northern Great Basin; with: Procedures used in the chronology development work including users manuals for computer programs COFECHA and ARSTAN. Laboratory of Tree-Ring Research, University of Arizona, Tucson, Arizona.
- Holtz, C.T., Schoettle, A.W., 2018. Is Resistance to Mountain Pine Beetle Associated with Genetic Resistance to White Pine Blister Rust in Limber Pine? *Forests* 9, 595.
- Hood, S.M., Baker, S., Sala, A., 2016. Fortifying the Forest: Thinning and Burning Increase Resistance to a Bark Beetle Outbreak and Promote Forest Resilience. *Ecol. Appl.* 26, 1984–2000.
- Hood, S.M., Sala, A., 2015. Ponderosa Pine Resin Defenses and Growth: Metrics Matter. *Tree Physiol.* 35, 1223–1235.
- Hood, S.M., Sala, A., Heyerdahl, E.K., Boutin, M., 2015. Low-Severity Fire Increases Tree Defense Against Bark Beetle Attacks. *Ecology* 96, 1846–1855.
- Howe, M., Keefover-Ring, K., Raffa, K.F., 2018. Pine Engravers Carry Bacterial Communities Whose Members Reduce Concentrations of Host Monoterpenes With Variable Degrees of Redundancy, Specificity, and Capability. *Environ. Entomol.* 47, 638–645.
- Howe, M., Mason, C.J., Gratton, C., Keefover-Ring, K., Wallin, K., Yanchuk, A., Zhu, J., Raffa, K.F., 2020. Relationships Between Conifer Constitutive and Inducible Defenses Against Bark Beetles Change Across Levels of Biological and Ecological Scale. *Oikos*.
- Huber, D.P.W., Gries, R., Borden, J.H., Pierce Jr., H.D., 2000. A Survey of Antennal Responses by Five Species of Coniferophagous Bark Beetles (Coleoptera: Scolytidae) to Bark Volatiles of Six Species of Angiosperm Trees. *Chemoecology* 10, 103–113.
- Jamieson, M.A., Burkle, L.A., Manson, J.S., Runyon, J.B., Trowbridge, A.M., Zientek, J., 2017. Global Change Effects on Plant Insect Interactions: The Role of Phytochemistry. *Curr. Opin. Insect Sci.* 23, 70–80.
- Jones, K.L., Shagelski, V.A., Marculis, N.G., Wijerathna, A.N., Evenden, M.L., 2019. Factors Influencing Dispersal by Flight in Bark Beetles (Coleoptera: Curculionidae: Scolytinae): From Genes to Landscapes. *Can. J. For. Res.* 49, 1024–1041.
- Joseph, G., Kelsey, R.G., Peck, R.W., Niwa, C.G., 2001. Response of Some Scolytids and their Predators to Ethanol and 4-allylanisole in Pine Forests of Central Oregon. *J. Chem. Ecol.* 27, 697–715.
- Kane, J.M., Kolb, T.E., 2010. Importance of Resin Ducts in Reducing Ponderosa Pine Mortality from Bark Beetle Attack. *Oecologia* 164, 601–609.
- Kane, J.M., Varner, J.M., Metz, M.R., van Mantgem, P.J., 2017. Characterizing Interactions Between Fire and Other Disturbances and Their Impacts on Tree Mortality in Western U.S. Forests. *Forest Ecology and Management* 405, 188–199.
- Keane, R.E., Arno, S.F., 1993. Rapid Decline of Whitebark Pine in Western Montana: Evidence from 20 Year Remeasurements. *West. J. Appl. For.* 8, 44–47.
- Keane, R.E., Gray, K.L., Dickinson, L.J., 2007. Whitebark pine diameter growth response to removal of competition. In: *Agriculture, U.S.D.O., (Ed.) U.S. Forest Service, Rocky Mountain Research Station*, pp. 1–9.
- Keefover-Ring, K., Trowbridge, A., Mason, C.J., Raffa, K.F., 2016. Rapid Induction of Multiple Terpenoid Groups by Ponderosa Pine in Response to Bark Beetle Associated Fungi. *J. Chem. Ecol.* 42, 1–12.
- Keeling, C.I., 2016. Bark Beetle Research in the Postgenomic Era. In: Tittiger, C., Blomquist, G. (Eds.), *Pine Bark Beetles - Advances in Insect Physiology*. Simon Fraser University, Burnaby, BC, Canada, pp. 265–294.
- Keeling, C.I., Bohlmann, J., 2006. Genes, Enzymes and Chemicals of Terpenoid Diversity in the Constitutive and Induced Defence of Conifers Against Insects and Pathogens. *New Phytol.* 170, 657–675.
- Keen, R.M., Voelker, S.L., Bentz, B.J., Wang, S.-Y.-S., Ferrell, R., 2020. Stronger Influence of Growth Rate than Severity of Drought Stress on Mortality of Large Ponderosa Pines during the 2012–2015 California Drought. *Oecologia* 194, 359–370.
- Kendall, K.C., Keane, R.E., 2000. The Decline of Whitebark Pine. In: Tomback, D.F., Arno, S.F., Keane, R.E. (Eds.), *Whitebark Pine Communities: Ecology and Restoration*. Island Press, Washington, DC, pp. 123–145.
- Kenkel, N.C., Orlóci, L., 1986. Applying Metric and Nonmetric Multidimensional Scaling to Ecological Studies: Some New Results. *Ecology* 67, 919–928.
- Kichas, N.E., Hood, S.M., Pederson, G.T., Everett, R.G., McWethy, D.B., 2020. Whitebark Pine (*Pinus albicaulis*) Growth and Defense in Response to Mountain Pine Beetle Outbreaks. *For. Ecol. Manage.* 457.
- Kipfmüller, K.F., Swetnam, T.W., 2002. Climate and mountain pine beetle-induced tree mortality in the selway-bitterroot wilderness area. In: *Resources, D.O.F., (Ed.) The University of Idaho, Moscow, ID*, pp. 1–41.
- Knapp, P.A., Soule, P.T., Maxwell, J.T., 2013. Mountain Pine Beetle Selectivity in Old Growth Ponderosa Pine Forests, Montana, USA. *Ecol. Evol.* 3, 1141–1148.
- LaMarche, V.C.J., Hirschboeck, K.K., 1984. Frost Rings in Trees as Records of Major Volcanic Eruptions. *Nature* 307, 121–126.
- Langenheim, J.H., 1994. Higher Plant Terpenoids: A Phytocentric Overview of their Ecological Roles. *J. Chem. Ecol.* 20, 1223–1280.
- Larocque, G.R., Luckai, N., Adhikary, S.N., Groot, A., Bell, F.W., Sharma, M., 2013. Competition Theory — Science and Application in Mixed Forest Stands: Review of Experimental and Modelling Methods and Suggestions for Future Research. *Environmental Reviews* 21, 71–84.
- Lewinsohn, E., Gijzen, M., Croteau, R., 1991. Defense Mechanisms of Conifers. *Plant Physiol.* 96, 44–49.
- Logan, J.A., Macfarlane, W.W., Willcox, L., 2010. Whitebark Pine Vulnerability to Climate Driven Mountain Pine Beetle Disturbance in the Greater Yellowstone Ecosystem. *Ecol. Appl.* 20, 895–902.

- Lotan, J.E., Critchfield, W.B., 1990. *Pinus contorta* Dougl. ex. Loud. - Lodgepole Pine. U.S.D.A Forest Service, Washington, D.C.
- Mason, C.J., Keefover-Ring, K., Villari, C., Klutsch, J.G., Cook, S., Bonello, P., Erbilgin, N., Raffa, K.F., Townsend, P.A., 2019. Anatomical Defences against Bark Beetles Relate to Degree of Historical Exposure between Species and are Allocated Independently of Chemical Defences within Trees. *Plant, Cell Environ.* 42, 633–646.
- Mattson, W.J., Haack, R.A., 1987. The Role of Drought in Outbreaks of Plant-Eating Insects. *Bioscience* 37, 110–118.
- Meddens, A.J., Hicke, J.A., Ferguson, C.A., 2012. Spatiotemporal Patterns of Observed Bark Beetle-Caused Tree Mortality in British Columbia and the Western United States. *Ecol. Appl.* 22, 1876–1891.
- Miller, D.R., Borden, J.H., 2000. Dose-Dependent and Species-Specific Responses of Pine Bark Beetles (Coleoptera: Scolytidae) to Monoterpenes in Association with Pheromones. *Can. Entomol.* 132, 183–195.
- Mizell 3rd, R.F., Frazier, J.L., Nebeker, T.E., 1984. Response of the Clerid Predator *Thanosinus dubius* (F.) to Bark Beetle Pheromones and Tree Volatiles in a Wind Tunnel. *J. Chem. Ecol.* 10, 177–187.
- Moreira, X., Mooney, K.A., Rasmann, S., Petry, W.K., Carrillo-Gavilán, A., Zas, R., Sampedro, L., Novotny, V., 2014. Trade-Offs Between Constitutive and Induced Defences Drive Geographical and Climatic Clines in Pine Chemical Defences. *Ecol. Lett.* 17, 537–546.
- Moreira, X., Sampedro, L., Zas, R., Pearce, I.S., 2016. Defensive Traits in Young Pine Trees Cluster into Two Divergent Syndromes Related to Early Growth Rate. *PLoS ONE* 11, e0152537.
- Moreira, X., Zas, R., Solla, A., Sampedro, L., 2015. Differentiation of Persistent Anatomical Defensive Structures is Costly and Determined by Nutrient Availability and Genetic Growth-Defence Constraints. *Tree Physiol.* 35, 112–123.
- Ormeno, E., Fernandez, C., Mevy, J.-P., 2007. Plant Coexistence Alters Terpene Emission and Content of Mediterranean Species. *Phytochemistry* 68, 840–852.
- Perrakis, D.D.B., Agee, J.K., Eglitis, A., 2011. Effects of Prescribed Burning on Mortality and Resin Defenses in Old Growth Ponderosa Pine (Crater Lake, Oregon): Four Years of Post-Fire Monitoring. *Natural Areas Journal* 31, 14–25.
- Phillips, M.A., Croteau, R.B., 1999. Resin Based Defenses in Conifers. *Trends in Plant Science - Reviews* 4, 1360–1385.
- Pitman, G.B., Vité, J.P., Kinzer, G.W., Fentiman, A.F., 1968. Bark Beetle Attractants: Trans-verbenol isolated from *Dendroctonus*. *Nature* 218, 168–169.
- Powell, E.N., Raffa, K.F., 2011. Fire Injury Reduces Inducible Defenses of Lodgepole Pine against Mountain Pine Beetle. *J. Chem. Ecol.* 37, 1184–1192.
- Prasifka, J.R., Spring, O., Conrad, J.R., Cook, L.W., Palmquist, D.E., Foley, M.E., 2015. Sesquiterpene lactone composition of wild and cultivated sunflowers and biological activity against an insect pest. *J. Agric. Food Chem.* 63, 4042–4049.
- R Development Core Team, 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Raffa, K.F., 2014. Terpenes Tell Different Tales at Different Scales: Glimpses into the Chemical Ecology of Conifer - Bark Beetle - Microbial Interactions. *J. Chem. Ecol.* 40, 1–20.
- Raffa, K.F., Aukema, B.H., Erbilgin, N., Klepzig, K.D., Wallin, K.F., 2005. Interactions Among Conifer Terpenoids and Bark Beetles Across Multiple Levels of Scale: An Attempt to Understand Links Between Population Patterns and Physiological Processes. In: Romeo, J.T. (Ed.), *Chemical Ecology and Phytochemistry of Forest Ecosystems*. Elsevier, pp. 79–118.
- Raffa, K.F., Berryman, A.A., 1982. Accumulation of Monoterpenes and Associated Volatiles Following Inoculation of Grand Fir with a Fungus Transmitted by the Fir Engraver, *Scolytus ventralis* (Coleoptera: Scolytidae). *Can. Entomol.* 114, 797–810.
- Raffa, K.F., Mason, C.J., Bonello, P., Cook, S., Erbilgin, N., Keefover-Ring, K., Klutsch, J.G., Villari, C., Townsend, P.A., 2017. Defence Syndromes In Lodgepole - Whitebark Pine Ecosystems Relate To Degree Of Historical Exposure To Mountain Pine Beetles. *Plant, Cell Environ.* 40, 1791–1806.
- Raffa, K.F., Powell, E.N., Townsend, P.A., 2013. Temperature Driven Range Expansion of an Irruptive Insect Heightened by Weakly Coevolved Plant Defenses. *Proc. Natl. Acad. Sci.* 110, 2193–2198.
- Reed, C.C., Ballantyne, A.P., Cooper, L.A., Sala, A., 2018. Limited Evidence for CO₂-Related Growth Enhancement in Northern Rocky Mountain Lodgepole Pine Populations Across Climate Gradients. *Glob. Change Biol.*
- Reed, C.C., Hood, S.M., 2020. Few Generalizable Patterns of Tree-Level Mortality During Extreme Drought and Concurrent Bark Beetle Outbreaks. *Sci. Total Environ.* 750, 141306.
- Reid, M.L., Sekhon, J.K., LaFramboise, L.M., 2017. Toxicity of Monoterpene Structure, Diversity and Concentration to Mountain Pine Beetles, *Dendroctonus ponderosae*: Beetle Traits Matter More. *J. Chem. Ecol.* 43, 351–361.
- Reineke, L.H., 1933. Perfecting a Stand-Density Index for Even-Aged Forests. *Journal of Agricultural Research* 46, 627–638.
- Roy, B.A., Alexander, H.M., Davidson, J., Campbell, F.T., Burdon, J.J., Snieszko, R., Brasier, C., 2014. Increasing Forest Loss Worldwide from Invasive Pests Requires New Trade Regulations. *Front. Ecol. Environ.* 12, 457–465.
- Samman, S., Schwandt, J.W., Wilson, J.L., 2003. Managing for healthy white pine ecosystems in the United States to reduce the impacts of white pine blister rust. In: *Agriculture, U.S.D.o., (Ed.), Forest Service, Missoula, Montana*, pp. 10.
- Schwandt, J.W., Lockman, L.B., Klejman, J.T., Muir, J.A., 2010. Current Health Issues and Management Strategies for White Pines in the Western United States and Canada. *Forest Pathol.* 40, 226–250.
- Seybold, S.J., Huber, D.P.W., Lee, J.C., Graves, A.D., Bohlmann, J., 2006. Pine Monoterpenes and Pine Bark Beetles: A Marriage of Convenience for Defense and Chemical Communication. *Phytochem. Rev.* 5, 143–178.
- Shanahan, E., Irvine, K.M., Thoma, D., Wilmoth, S., Ray, A., Legg, K., Shovic, H., 2016. Whitebark Pine Mortality Related to White Pine Blister Rust, Mountain Pine Beetle Outbreak, and Water Availability. *Ecosphere* 7.
- Slack, A.W., Kane, J.M., Knapp, E.E., Sherriff, R.L., 2017. Contrasting Impacts of Climate and Competition on Large Sugar Pine Growth and Defense in a Fire-Excluded Forest of the Central Sierra Nevada. *Forests* 8, 244.
- Slack, A.W., Zeibig-Kichas, N.E., Kane, J.M., Varner, J.M., 2016. Contingent Resistance in Longleaf Pine (*Pinus palustris*) Growth and Defense 10 Years Following Smoldering Fires. *For. Ecol. Manage.* 364, 130–138.
- Smith, R.H., 2000. Xylem Monoterpenes of Pines: Distribution, Variation, Genetics, Function. In: Service, U.S.D.A.F. (Ed.), *Pacific Southwest Research Station*. Albany, California, pp. 1–454.
- Sparks, A.M., Smith, A.M.S., Tallhelm, A.F., Kolden, C.A., Yedinak, K.M., Johnson, D.M., 2017. Impacts of Fire Radiative Flux on Mature *Pinus ponderosa* Growth and Vulnerability to Secondary Mortality Agents. *International Journal of Wildland Fire* 26.
- Stokes, M.A., Smiley, T.L., 1996. An Introduction to Tree-Ring Dating. The University of Arizona Press, Tucson, AZ.
- Tepley, A.J., Hood, S.M., Keyes, C.R., Sala, A., 2020. Forest Restoration Treatments in a Ponderosa Pine Forest Enhance Physiological Activity and Growth Under Climatic Stress. *Ecological Applications*.
- Tomback, D.F., Achuff, P., 2010. Blister Rust and Western Forest Biodiversity: Ecology, Values and Outlook for White Pines. *Forest Pathol.* 40, 186–225.
- Tomback, D.F., Blakeslee, S.C., Wagner, A.C., Wunder, M.B., Resler, L.M., Pyatt, J.C., Diaz, S., 2016a. Whitebark Pine Facilitation at Treeline: Potential Interactions for Disruption by an Invasive Pathogen. *Ecol. Evol.* 6, 5144–5157.
- Tomback, D.F., Resler, L.M., Keane, R.E., Pansing, E.R., Andrade, A.J., Wagner, A.C., 2016b. Community Structure, Biodiversity, and Ecosystem Services in Treeline Whitebark Pine Communities: Potential Impacts from a Non-Native Pathogen. *Forests* 7, 21.
- Trapp, S., Croteau, R., 2001. Defensive Resin Biosynthesis in Conifers. *Annual Review of Plant Physiology and Plant Molecular Biology* 52, 689–724.
- Trowbridge, A.M., 2014. Evolutionary Ecology of Chemically Mediated Plant Insect Interactions. *Ecology and the Environment*, pp. 143–176.
- Trowbridge, A.M., Bowers, M.D., Monson, R.K., 2016. Conifer Monoterpene Chemistry During an Outbreak Enhances Consumption and Immune Response of an Eruptive Folivore. *J. Chem. Ecol.* 42, 1281–1292.
- Trowbridge, A.M., Stoy, P.C., Adams, H.D., Law, D.J., Breshears, D.D., Helmig, D., Monson, R.K., 2019. Drought Supersedes Warming in Determining Volatile and Tissue Defenses of Piñon Pine (*Pinus edulis*). *Environ. Res. Lett.* 14.
- Valor, T., Ormeno, E., Casals, P., 2017. Temporal Effects of Prescribed Burning on Terpene Production in Mediterranean Pines. *Tree Physiol.* 37, 1622–1636.
- van de Gevel, S., Larson, E., Grissino Mayer, H., 2017. Separating Trends in Whitebark Pine Radial Growth Related to Climate and Mountain Pine Beetle Outbreaks in the Northern Rocky Mountains, USA. *Forests* 8, 195.
- van Mantgem, P.J., Stephenson, N.L., 2007. Apparent Climatically Induced Increase of Tree Mortality Rates in a Temperate Forest. *Ecol. Lett.* 10, 909–916.
- van Mantgem, P.J., Stephenson, N.L., Keifer, M., Keeley, J., 2004. Effects of an Introduced Pathogen and Fire Exclusion on the Demography of Sugar Pine. *Ecol. Appl.* 14, 1590–1602.
- Vázquez-González, C., Zas, R., Erbilgin, N., Ferrenberg, S., Rozas, V., Sampedro, L., 2020. Resin Ducts as Resistance Traits in Conifers: Linking Dendrochronology and Resin-Based Defenses. *Tree Physiol.*
- Consulting, Voortech, 2005. The Tree Ring Measuring Program Project J2X. Voortech Consulting.
- Wagner, A., Tomback, D., Resler, L., Pansing, E., 2018. Whitebark Pine Prevalence and Ecological Function in Treeline Communities of the Greater Yellowstone Ecosystem, U.S.A.: Potential Disruption by White Pine Blister Rust. *Forests* 9 (635).
- Waring, R.H., Pitman, G.B., 1985. Modifying Lodgepole Pine Stands to Change Susceptibility to Mountain Pine Beetle Attack. *Ecology* 66, 889–897.
- Williams, H., Hood, S., Keyes, C., Egan, J., Negrón, J., 2018. Subwatershed-Level Lodgepole Pine Attributes Associated with a Mountain Pine Beetle Outbreak. *Forests* 9.
- Yi, M., Jia, T., Dong, L., Zhang, L., Leng, C., Liu, S., Lai, M., 2021. Resin Yield in *Pinus elliottii* Engelm. is Related to the Resin Flow Rate, Resin Components and Resin Duct Characteristics at Three Locations in Southern China. *Ind. Crops Prod.* 160.
- Zas, R., Moreira, X., Ramos, M., Lima, M.R.M., Nunes da Silva, M., Solla, A., Vasconcelos, M.W., Sampedro, L., 2014. Intraspecific Variation of Anatomical and Chemical Defensive Traits in Maritime Pine (*Pinus pinaster*) as Factors in Susceptibility to the Pinewood Nematode (*Bursaphelenchus xylophilus*). *Trees* 29, 663–673.
- Zhang, C. Y., Luo, L., Xia, J., Song, Y. N., Zhang, L. J., Zhang, M., Rahman, K., Ye, Y., Zhang, H., Zhu, J.-Y., 2018. Sesquiterpenes and Lignans from the Flower Buds of *Daphne genkwa* and their Nitric Oxide Inhibitory Activities. *Nat. Prod. Res.* 32, 2893–2899.
- Zhang, Y., Li, Z.-X., Yu, X.-D., Fan, J., Pickett, J.A., Jones, H.D., Zhou, J.-J., Birkett, M.A., Caulfield, J., Napier, J.A., Zhao, G.-Y., Cheng, X.-G., Shi, Y., Bruce, T.J., Xia, L.-Q., 2015. Molecular Characterization of Two Isoforms of a Farnesyl Pyrophosphate Synthase Gene in Wheat and their Roles in Sesquiterpene Synthesis and Inducible Defence against Aphid Infestation. *New Phytol.* 206, 1101–1115.
- Zhao, M., Zhang, N., Gao, T., Jin, J., Jing, T., Wang, J., Wu, Y., Wan, X., Schwab, W., Song, C., 2020. Sesquiterpene Glucosylation Mediated by Glucosyltransferase UG191Q2 is Involved in the Modulation of Cold Stress Tolerance in Tea Plants. *New Phytol.* 226, 362–372.

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Zhao, S., Erbilgin, N., 2019. Larger Resin Ducts Are Linked to the Survival of Lodgepole Pine Trees During Mountain Pine Beetle Outbreak. *Front. Plant Sci.* 10, 1459.

Zhao, S., Klutsch, J.G., Cale, J.A., Erbilgin, N., 2019. Mountain Pine Beetle Outbreak Enhanced Resin Duct Defenses of Lodgepole Pine Trees. *For. Ecol. Manage.* 441, 271–279.

Zhao, Y., Zhou, Y., Jiang, H., Li, X., Gan, D., Peng, X., Zhu, S., Cheng, B., 2011. Systematic Analysis of Sequences and Expression Patterns of Drought-Responsive Members of the Hb-Zip Gene Family in Maize. *PLoS ONE* 6, e28488.

CHAPTER FIVE

INCREASED WHITEBARK PINE (*PINUS ALBICAULIS*) GROWTH AND DEFENSE
UNDER A WARMER AND REGIONALLY DRIER CLIMATE

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Increased Whitebark Pine (*Pinus albicaulis*) Growth and Defense under a Warmer and Regionally Drier Climate

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Key Points: (140 characters max)

- High-elevation whitebark pine trees show increased growth and defense in response to regional warming and drying.
- Regional drought, reduced snowpack, dry soils, and increased atmospheric vapor pressure deficit drive production of anatomical resin defenses.
- Resin duct size and area are positively associated with annual average temperature while duct production is controlled by regional drought.

30 **Abstract**

31 Tree defense characteristics play a crucial role in modulating conifer-bark beetle interactions.
 32 While there is a growing body of literature investigating factors mediating tree growth and resin-
 33 based defenses in conifers, there is a lack of uniformity in the methods used to evaluate the
 34 relative role of geometric size-age relationships versus climate and other factors in driving
 35 anatomical changes in trees. We: 1) evaluated the relationship between biological trends in resin
 36 duct anatomy with climatic variability; and 2) determined if whitebark pine growth and resin
 37 duct morphology is constrained by climate and/or regional drought conditions. We found that
 38 high-elevation whitebark pine trees experienced increased growth and defense under warmer and
 39 regionally drier conditions, with climate variables explaining a substantive proportion of
 40 variation (10-25%) in tree diameter growth and resin duct anatomy. Our results suggest that
 41 whitebark pine growth and defense was historically limited by shorter growing seasons in high-
 42 elevation subalpine environments.

43 **Plain Language Summary**

44 Long-term drying trends, increased fire activity, disease prevalence, and insect outbreaks
 45 throughout the western U.S. highlight a need to understand how trees are responding to changing
 46 climatic and ecosystem conditions. In many conifers, oleoresin produced by trees is the primary
 47 defense against insects and pathogens and is stored and mobilized through a network of resin
 48 duct structures distributed throughout the tree stem. Pine trees persisting through bark beetle
 49 outbreaks often have distinct resin duct characteristics compared to trees that died. However, the
 50 degree to which climate influences resin duct morphology is poorly understood, particularly for
 51 high-elevation species. In this study our objective was to evaluate relationships between climate,
 52 tree growth, and resin duct anatomy for whitebark pine, a high-elevation conifer of management
 53 concern in the northern Rocky Mountains, USA. Importantly, removing age-growth related
 54 trends in resin duct data was critical before evaluating climate-resin duct temporal relationships.
 55 Whitebark pine at our study sites increased growth and defense (producing more and larger resin
 56 ducts with greater area) under regional warming, reduced snowpack, drier soils, and increased
 57 vapor pressure deficit.

58 **1. Introduction**

59 Widespread tree mortality associated with changing climate and disturbance regimes
 60 predisposes many western U.S. forests to future, unpredictable changes in forest stand structure,
 61 function and species composition (Allen et al., 2015; Allen et al., 2010; Smith et al., 2015; van
 62 Mantgem et al., 2009). Long-term drying trends coupled with increased fire activity, pathogen
 63 activity, and insect outbreaks all highlight a need to understand physiological responses of trees
 64 to abiotic and biotic stressors (Bentz et al., 2016; Dudley et al., 2021; Jenkins et al., 2014;
 65 Stephens et al., 2013). Currently, the majority of research linking drought- and disturbance-
 66 related mortality centers on lower-elevation, moisture-limited forest systems (Das et al., 2013;
 67 McDowell et al., 2008; Stephenson et al., 2019; van Mantgem & Stephenson, 2007; Zhang et al.,
 68 2017) with little attention to higher-elevation, energy-limited systems (i.e., systems limited by
 69 growing season mean temperature and length). There is a critical need to evaluate how climate
 70 trends and severe drought that are causing widespread tree mortality in moisture-limited low-
 71 elevation systems will affect cooler, short-growing season subalpine forests in the western U.S.
 72 More specifically, there is a need to understand how tree growth and defense strategies in
 73 energy-limited systems are consistent with or differ from moisture-limited systems. This is

particularly important for informing ecosystem models that are often parameterized based on data from lower elevation forest taxa.

Whitebark pine (*Pinus albicaulis*) is an ecologically and culturally important high-elevation conifer with a bifurcated distribution. The western populations range from the southern Sierra Nevada mountains in California (36° N) north through the Coast and Cascade ranges of Oregon and Washington into west-central British Columbia (55° N). The eastern distribution tracks the northern Rocky Mountains, extending from Colorado poleward through Idaho and Montana into western Alberta (Arno & Hoff, 1989; Fryer, 2002; Little, 1971). Whitebark pine has been recognized as a foundation species in high-elevation montane ecosystems due to its importance in promoting snowpack accumulation and retention, vegetative establishment, and as a critical food source for a variety of wildlife (Tomback et al., 2001). However, extensive whitebark pine mortality due to the introduction of a highly aggressive exotic pathogen white pine blister rust (*Cronartium ribicola*) and extensive mountain pine beetle (*Dendroctonus ponderosae*) outbreaks caused by shifting climate, along with future projections of continued population declines due to warming (Chang et al., 2014), led the U.S. Fish & Wildlife Service to list whitebark pine as “threatened” under the Endangered Species Act in December 2020. Managers tasked with supporting and maintaining the viability of whitebark pine into the future need information on how whitebark forests respond to climate and disturbance synergies across biophysical gradients throughout its range.

In whitebark pine, and other conifers, defense characteristics play a crucial role in moderating bark-beetle interactions, with trees persisting through bark beetle outbreaks often exhibiting distinct resin duct characteristics (Ferrenberg et al., 2014; Hood & Sala, 2015; Hood et al., 2015; Kane & Kolb, 2010; Kichas et al., 2020). While there is a growing body of literature investigating tree growth and resin-based defenses in conifers, there is a lack of uniformity in methods employed to disentangle the anatomical changes related to geometric size-age relationships versus climate and other factors across studies (Hood & Sala, 2015; Vázquez-González et al., 2020). Because mature trees typically experience increased mortality relative to smaller, younger trees (Audley et al., 2020; Boone et al., 2011; Safranyik & Carroll, 2006), it is important to remove the influence of geometric size-age relationships before investigating climate-resin duct relationships. Though various approaches have been applied, only two studies have utilized traditional dendroclimatological detrending methods and these show inconsistent results, with one study reporting marginal influence of climate on interannual duct production in *P. pinaster* (Vázquez-González et al., 2021) and the other reporting significant relationships between resin duct density, temperature, and precipitation in *Picea abies* (Wimmer & Grabner, 1997). Problems with the application of these methods may partially stem from the fact that they have only been employed on temporally limited (< 50 year) datasets dominated by young trees. Longer time periods outside of the juvenile growth curve are optimal for estimating and separating geometric growth curves from climate driven multi-decadal patterns and long-term trends in resin duct anatomy in long-lived tree species.

Based largely on data from lower elevation forest taxa, species distribution models project that increased warming and regional drying will amplify whitebark pine mortality through direct and indirect effects of drought, decreased tree resilience, and increased susceptibility to insects and pathogens (Chang et al., 2014; Millar & Delany, 2019; Wong & Daniels, 2017). However, while several studies have investigated climate-growth relationships in conifers there are exceedingly limited data on how climate influences resin duct morphology, which is important

for understanding growth-defense interactions and within-tree resource allocation patterns. Here we present an evaluation of high-elevation whitebark pine growth and defense in response to climate variability across two U.S. northern Rocky Mountain sites spanning 1924 to 2015. Our objectives were: 1) to evaluate relationships between age- and growth-related trends in resin duct morphology (duct production, size, area, density, relative area) with climatic variability (i.e., seasonal and annual temperature and moisture); and to 2) determine if whitebark pine growth and resin duct morphology is constrained by changing climatic conditions including warming temperatures and regional drought. In contrast to previous research from drier, moisture-limited systems we expected to find increased tree growth and defense (i.e., increased resin duct production, size, area) in our energy-limited systems with increased warming and regional drying (Harold C. Fritts, 1971; Wettstein et al., 2011).

2. Materials and Methods

2.1 Site Description

Data for this study were collected across two high-elevation (1,905 – 2,120 m a.s.l.) whitebark pine sites on the Flathead Indian Reservation as part of a larger fire history reconstruction project for the Confederated Salish and Kootenai Tribes (CSKT). We selected sites where whitebark pine was an important component of the canopy (> 15 % canopy cover). As part of the CSKT fire history project, a 200 m grid was overlain across each site using a combination of remote sensing and field surveying for optimal placement (supporting information Figure S1). Within the 200 m grid, individual plots were located 200 m apart along cardinal directions, with a total of 38 plots for the 3LK site (covering approximately 100 ha) and 34 plots for the BLD site (covering approximately 88 ha) (Kichas et al., 2020). For this study, we utilized tree-ring data from 58 live whitebark pine trees at the 3LK site and 54 live whitebark pine at the BLD site for a total of 112 trees. The average size (diameter at breast height) of whitebark pine trees sampled was 21.2 cm ($\pm$ 0.57 cm), with an average age of 102 years ($\pm$ 1.5 years). Increment cores collected from live whitebark pine trees were processed using standard dendrochronological techniques (supporting information Text S1).

2.2 Data Analysis

All analyses were conducted using the statistical package R (v. 3.5.0) (R Development Core Team, 2008). We used several multivariate analyses including Principal Component Analysis, correlation, and regression to examine relationships between radial growth, resin duct morphology and climate. We first investigated the potential influence of local fire activity on whitebark pine growth and resin duct morphology. We utilized spatially explicit 20th century wildfire records to determine dates of fire events adjacent to our study sites. Results from superposed epoch analysis indicate no significant differences in growth or defense in response to regional fire activity. We then assessed the relationships of tree growth and resin duct anatomy to climatic conditions. We measured growth and defense metrics over the full record (1908-2015) but limited our climate analysis to a period of 1924-2015 to both increase our sample depth (n = 80 trees at 1924) and reduce inclusion of growth and defense data over the earliest part of the record when juvenile growth vigor may obscure climate and drought relationships.

For climate analyses, tree growth and resin duct data were conservatively and interactively detrended using the *dplr* package (version 1.7.2) (Bunn, 2008) with the goal of removing geometric age-related growth trends while retaining as much decadal+ scale variability as

possible. Each tree-ring and resin duct series was individually fit with either a negative exponential curve (79.7%), Hegershoff curve (1.6%), Friedman super smoother (5.1%), or a spline with a 0.5 frequency response at 75% the series length to remove the geometric growth trend (13.6%) (H. C. Fritts, 1976). The resultant series were then standardized by dividing by the fitted trend line, transformed into unitless ratio (index), and then combined into a composite chronology by calculating the weighted mean across all samples (supporting information Figure S2, Data Sets S1, S2) (Cook & Peters, 1997). We utilized a rotated principal components analysis (PCA) to assess within-sample variability and rule out other potential external factors (e.g., site aspect, soils, genetics, etc.) than climate that could influence variability within the detrended dataset. We found no significant sub-sample variability outside of the mean response, indicating the detrended tree-ring and resin duct chronologies exhibit variability controlled by a common factor and hence are suitable for climate analyses (supporting information Figure S3).

Estimates of local hydroclimate within the study areas were obtained at a spatial resolution of 4 km from the Wolock and McCabe (2018) monthly water-balance model (MWBM) output (Data available: <https://doi.org/10.5066/F71V5CWN>). The MWBM uses precipitation and temperature input data from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) (Daly et al., 2008) to estimate monthly evapotranspiration (potential and actual), runoff, snowfall, and soil moisture storage. We opted to use this version of the PRISM-based water balance dataset because (1) instrumental weather data were not available at these study sites (Abatzoglou et al., 2017; Daly et al., 1994; Kipfmüller & Salzer, 2010), (2) its accuracy has already been evaluated at watershed scales across the contiguous U.S. (McCabe & Wolock, 2011), and (3) both PRISM and the MWBM are commonly utilized dataset for many dendroecological and climatic studies, particularly in areas with complex topography (Cansler et al., 2018; Carnwath & Nelson, 2016; Ferrenberg et al., 2017; Goeking & Izlar, 2018; Guiterman et al., 2015; Hood et al., 2015; Kipfmüller & Salzer, 2010; Martin et al., 2020; Martin et al., 2019; Millar et al., 2007; Pederson et al., 2013).

To obtain the best representation of climatic conditions affecting trees in our study we averaged climate data from the nearest four grid points for each site. We grouped monthly climate data into seasonal and annual windows to evaluate potential responses of trees to seasonal variability. We also included climate data from the year prior to growth to account for lagged influences of climate on tree growth and duct morphology. Monthly climate variables obtained for this analysis included temperature, precipitation, and vapor pressure deficit (from the PRISM Climate Group, Oregon State University: <http://prism.oregonstate.edu>), snowfall, soil moisture storage, and potential and actual evapotranspiration (from the MWBM), as well as Palmer Drought-Severity Indices (PDSI / z-PDSI) from the western Montana climate division in the WestWide Drought Tracker product (Abatzoglou et al., 2017) (Data available: <https://wrcc.dri.edu/wwdt/>), and April 1 snow-water equivalence (Pederson, Gray, Woodhouse, et al., 2011) (Data available: <https://www.ncei.noaa.gov/access/paleo-search/study/11205>).

To determine climate variables best associated with tree growth and resin duct morphology, we performed regression subset selection using the *LEAPS* package (version 3.1) (Lumley, 2020). Mallow's C_p , the coefficient of determination (R^2), and Bayesian information criterion (BIC) were used to inform the most parsimonious selection of predictor climate variables, and in addition, top models were run through a forward-backward stepwise procedure to help eliminate overfitting issues. Variables were allowed to enter the model with an alpha-to-enter significance level $\alpha_E = 0.15$ and removed from the model with an alpha-to-remove significance level $\alpha_R = 0.1$.

We also partitioned climate variables into upper and lower quartiles to evaluate potential differences in whitebark pine growth and resin duct morphology across the extremes of the respective climate distributions (e.g., cooler and wetter versus warmer and drier) (supporting information Figure S4).

3. Results and Discussion

3.1 Growth and defense increase with regional warming and drying

Initial assessments of relationships between tree size, growth, and resin duct morphology indicates the need to first remove the biological influence of early vigorous tree growth from tree anatomical defense metrics using dendrochronological detrending methods prior to performing climate analyses (supporting information Figure S2, Data Sets S1, S2). These results also provide evidence showing age as a primary control on resin defenses with greater duct production and area over the juvenile growth years (DeAngelis et al., 1986), which supports field observations that young or juvenile trees typically experience lower mortality during insect outbreaks compared to larger, more mature trees (Audley et al., 2020; Boone et al., 2011; Safranyik & Carroll, 2006). Additionally, the relationship between raw (non-detrended) data shows positive or neutral relationships between whitebark pine tree size (diameter at breast height), radial growth, and resin duct morphology (supporting information Table S1). Traditional, “conservative” biological growth curve detrending methods (e.g., negative exponential curves, negative to zero slope regression lines, and rigid splines) designed to retain decadal- to centennial-scale variability and long-term trends in tree-ring records were successfully utilized in producing detrended, standardized indices of growth and defense suitable for use in climate analyses (supporting information Figure S2).

In assessments of climate relationships to the detrended growth and defense metrics we compared upper and lower quartiles of climate variables related to extreme warm and dry, or cool and wet conditions, (warm season [May-Sep] average temperature, annual average Palmer-Drought Severity Index, cool-season [Oct-Apr] total snowfall, and warm season [May-Sep] maximum vapor pressure deficit [VPD]) against whitebark pine growth and defense. We find that reduced snowpack, drier soils, and increased vapor pressure deficit, collectively drives increased production of anatomical resin defenses (Figure 1). Significant differences in tree growth, resin duct production, duct size, and duct area for the lower and upper quartiles of these climate variables indicates more favorable growing conditions and/or growing season length allowed for both increased growth and defense in these trees.

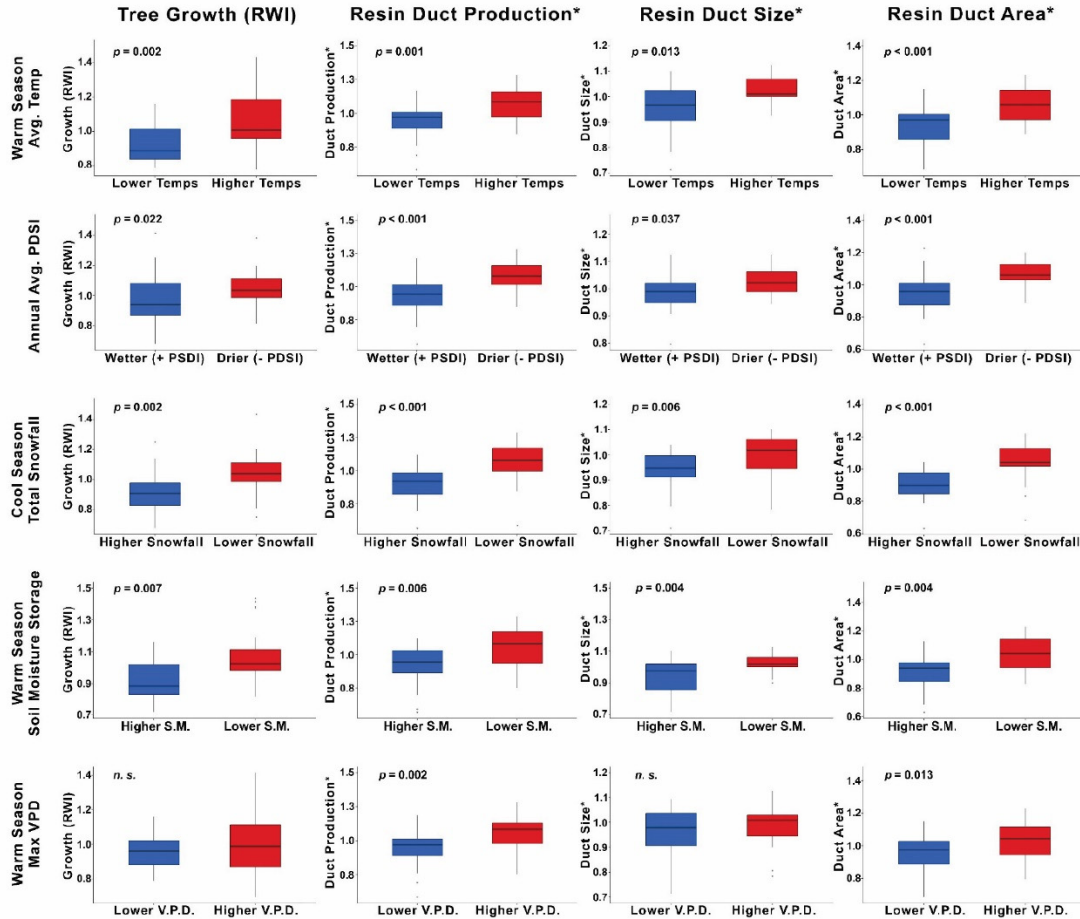


Figure 1. Comparison of detrended whitebark pine tree growth and resin duct morphology across the upper and lower quartiles of reported climate variables: warm season (May-Sep) average temperature ($^{\circ}\text{C}$), annual average Palmer-Drought Severity Index (PDSI), cool season (Oct-Apr) total snowfall (mm), and warm season (May-Sep) maximum vapor pressure deficit (VPD) from 1924-2015.

Unlike many lower-elevation moisture-limited forests, high-elevation whitebark pine trees in the U.S. northern Rockies have experienced increased growth and produced more and larger resin ducts with a greater total duct area under warmer and regionally drier conditions (Figure 1). As documented with previous research, the drier, lower-elevation forests experience recent warming and drying as a stressor, resulting in decreased growth and defense. In contrast, in our high-elevation systems warming and drying appear to release the physical constraints of snowpack on tree growth and the generally cool-wet conditions that limit photosynthetic activity and carbon accumulation. Increased warming in these alpine systems results in earlier snowmelt and ground thaw which increases biologically available water and provides temperatures favorable for xylogenesis and nutrient exchange (Chhin et al., 2008; Danby & Hik, 2007). Hence, decreased precipitation (and presumably increased insolation due to less cloud cover) facilitate increased spring and autumn photosynthetic activity, promoting greater carbon sequestration annually (Goldblum & Rigg, 2005; Körner, 1998).

We propose that a larger reservoir of annual carbohydrates, fueled by an extended growing season, allows these high-elevation trees to simultaneously allocate resources towards both tree growth and resin duct defenses, with no discernible growth-defense tradeoffs (Figure 1, supporting information Table S1). While similar patterns of increased tree growth under warmer and regionally drier conditions have been reported for other conifers in high-elevation, energy-limited systems throughout western North America (Graumlich & Brubaker, 1986; Kemp-Jennings et al., 2021; Miyamoto et al., 2010; Peterson et al., 2002; Salzer et al., 2009), these results are the first to also show enhanced resin duct defenses.

3.2 Whitebark pine growth and resin duct morphology have unique climate responses

Climate explains a substantial proportion of variation in whitebark pine tree growth and resin duct morphology, with R^2 values of models ranging from 10 to 25% (Table 1). Annual average temperature is closely tied to resin duct size and duct area, while summer (Jun-Aug) mean temperature is positively associated with tree growth and resin duct density (Table 1, Figure 2). Annual average PDSI is also positively associated with tree growth, with increased growth occurring under regionally drier (more negative) PDSI values (Table 1, Figure 2). Similarly, average summer PDSI and warm season (May-Sep) maximum vapor pressure deficit are positively associated with resin duct production (Table 1, Figure 2).

The combination of resin duct characteristics collectively influences a tree's ability to produce, store, and mobilize oleoresin. In applying this perspective, that a tree's resin-based defenses are in fact a sum-of-its-parts, key climate variables (mean annual temperature, warm season maximum vapor pressure deficit, and summer PDSI) collectively explain a considerable (~ 49%) proportion of variability in resin duct anatomy (duct production, duct size, and duct area) (Table 1; Figure 2) after the primary control (size-age relationship) has been removed through conservative detrending. Together, our findings suggest that these high-elevation trees are responding to climatic controls in nuanced and complex ways by actively adjusting components of tree growth and resin duct morphology in response to changing environmental conditions. While duct anatomy is important for tree survival during bark beetle outbreaks, resin chemistry and resin viscosity are also critical components modulating tree-insect-pathogen interactions (Kichas et al., 2021). Sampling temporal variation in resin chemistry, viscosity, and flow is not possible in a retrospective analysis in this study; however, relatively small increases in resin duct area would lead to a greater capacity to deliver oleoresin to insect attack sites, as resin flow is proportional to the fourth power of resin duct diameter in accordance with Hagen-Poiseuille's law (Schopmeyer et al., 1954; Vázquez-González et al., 2020; Yi et al., 2021). In addition, increasing temperatures correlate with reduced resin viscosity and increased resin flow in some species, which may further enhance resin mobilization in whitebark pine under warmer conditions (Wermelinger et al., 2021; Zas et al., 2020).

Table 1. Top climate models predicting tree growth (RWI), detrended duct production / size / area, and standardized duct metrics (duct density and relative duct area) as determined by best subsets regression. Models were developed through a forward-backward stepwise procedure to remove highly correlated variables. Arrows reflect the positive (↑) or negative (↓) influence of climate variables on whitebark pine growth and defense metrics.

Model	Coefficient	S.E.	Pr > t	F	DF	R ²	Adj. R ²	p-value
Growth* ~ (↑) Summer Avg. Temp + (↓) Annual Avg. PDSI + (↑) Summer z-PDSI	0.076 -0.028 0.062	0.019 0.009 0.017	< 0.001 0.002 < 0.001	7.7	88	0.21	0.18	< 0.001
Duct Production* ~ (↑) Warm VPD Max + (↓) Summer PDSI	0.023 -0.014	0.009 0.007	0.013 0.043	11	89	0.21	0.19	< 0.001
Duct Size* ~ (↑) Annual Avg. Temp	0.028	0.008	0.001	11	90	0.11	0.1	0.001
Duct Area* ~ (↑) Annual Avg. Temp	0.061	0.014	< 0.001	18	90	0.17	0.16	< 0.001
Duct Density ~ (↑) Summer Avg. Temp + (↓) Fall PDSI + (↑) Annual Total PPT	0.044 -0.053 0.001	0.021 0.014 0.000	0.038 < 0.001 0.003	9.5	88	0.25	0.22	< 0.001
Relative Duct Area ~ (↓) Fall PDSI + (↑) Annual Total PPT	-0.052 0.001	0.015 0.000	< 0.001 < 0.001	7.2	89	0.14	0.12	0.001

Within these high-elevation, energy-limited systems, warmer temperatures and regionally drier conditions are associated with increased tree growth, duct production, duct size, and duct area (Figure 2). Ultimately, these systems experience warming and regional drought as a release from cold and wet conditions that limit photosynthetic activity. As these high-elevation ecosystems continue to experience increased warming, energy-limited whitebark pine trees appear poised to be better defended against insects and pathogens via increased vigor and enhanced resin duct anatomy (Table 1; Figures 1, 2). These metrics of resin-based defensive structures are important characteristics influencing tree mortality to insect-pathogen complexes (Hood et al., 2015; Kane & Kolb, 2010; Kichas et al., 2020). However, the benefit of enhanced growth and defense in response to warming and drying conditions is likely a non-linear relationship with critical thresholds at which further warming and drying would eventually result in these systems transitioning into moisture-limited systems and their subsequent physiological responses (Pedlar et al., 2021). Also, since climate-controlled production of defense structures is a secondary response relative to size-age primary control, there are likely thresholds of tree size beyond which the additional production of defensive structures during drought offers limited additional benefit.

Additionally, many other factors that could interact to influence tree mortality that may obscure these relationships. For instance, projected enhanced thermal suitability for mountain pine beetle might amplify outbreaks, potentially overwhelming even well-defended trees (Bentz et al., 2016; Boone et al., 2011; Reed & Hood, 2020). White pine blister rust can also cause extensive mortality in healthy, vigorous trees, however the extent to which this pathogen affects resin duct formation and resin flow is unknown as is the degree to which resin-based defenses relate to the success of white pine blister rust infection. While our study was not explicitly designed to test for these interactions there was evidence for both mountain pine beetle- and white pine blister rust-caused mortality across each of our study sites, with the majority (~ 82%) of whitebark pine trees having been killed prior to our initial sampling effort (574 out of 701 whitebark pine trees were dead at the time of sampling). White pine blister rust migrated into this

broader region of the northern Rocky Mountains circa 1950 (Geils et al., 2010; Samman et al., 2003). The area has also been affected by numerous large-scale bark beetle outbreaks, occurring in the 1930s, 1960s–1980s and most recently 2000s–2010s (Arno & Hoff, 1989; Buotte et al., 2017; Gibson et al., 2008; Kipfmüller & Swetnam, 2002; Lestina et al., 2019). Live whitebark pine trees sampled for this study have persisted through these recent disturbance events, yet it is unknown how future disturbance synergies may push these systems past a threshold at which regional warming and drying may inhibit rather than enhance growth and defense responses. More research is needed to investigate relationships between exposure to white pine blister rust and insect-pathogen complexes on resin duct development and within-tree resource allocation patterns.

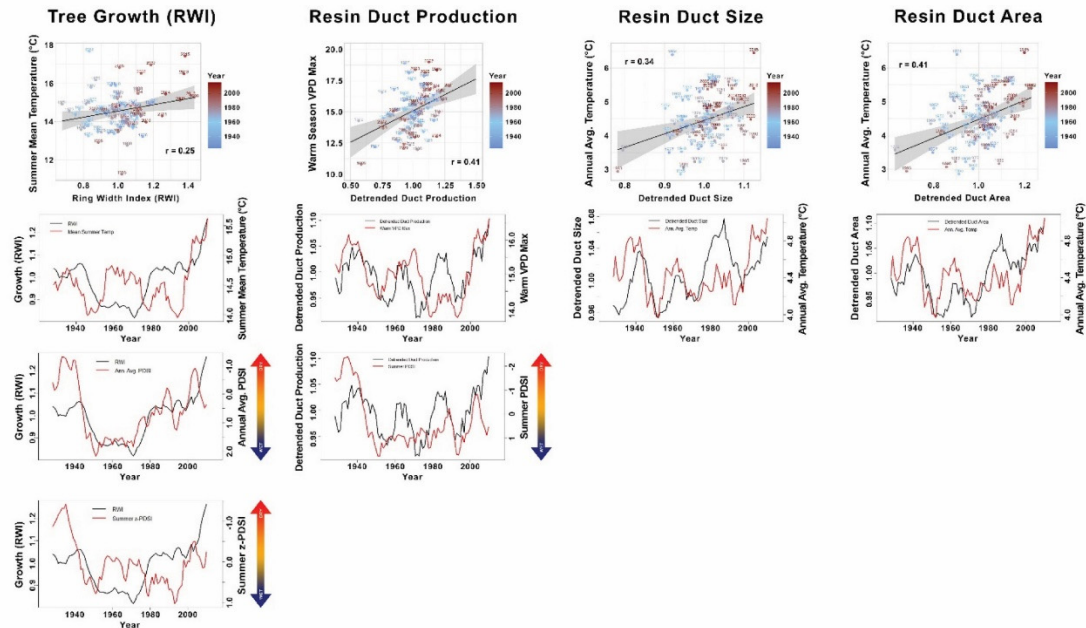


Figure 2. Scatterplot and time series relationships (10-year moving averages) for whitebark pine tree growth, detrended resin duct metrics that were not standardized to growth (duct production, duct size, duct area), and the primary climate variables derived from best-subsets regression from 1924–2015 (Table 1). The plotted 10-year moving averages between variables highlight covariation at decadal-scales.

4. Conclusions

We found that high-elevation whitebark pine trees in the northern Rocky Mountains have experienced increased growth and defense under warming temperatures, reduced snowpack, drier soils and increased vapor pressure deficit. In response to these changing conditions, these alpine forest ecosystems are essentially experiencing a release from conditions that historically limited photosynthetic activity. Contrary to previous research conducted in moisture-limited systems, our results show that regional warming and drying trends may enhance both growth and defense in energy-limited subalpine forests. This relationship highlights an important distinction between high-elevation systems and lower elevation systems - where drought stress, as reflected by traditional climate variables and indices, beneficially translates to a longer-growing season. An

extended growing season in these high-elevation systems promotes accumulation of structural and non-structural carbon and, ultimately, increased growth and defense structures in trees. Of critical importance, we expect this positive relationship to cross a threshold at which even energy-limited high-elevation systems experience drought-stress and limited growth and defense as warming continues. This further emphasizes how the relationships with tree growth, resin ducts, and climate will vary in complex ways, within and across species, as well as over topographic gradients (Ackerman & Goldblum, 2021; Mäkinen et al., 2002; Miyamoto et al., 2010). Future studies should investigate these patterns and consider climate-resin duct relationships across elevational transects to assess if and how these relationships change across important biophysical gradients and investigate at what point the benefits of regional warming and drying in these systems will become liabilities for alpine forests.

Open Research

Data Availability Statement

The whitebark pine tree growth and resin duct time series data used for climate analyses in the study (supporting information Data Sets S1, S2) (Kichas et al., 2022) are currently being archived in the International Tree-Ring Data Bank [ITRDB] (<https://www.ncei.noaa.gov/products/paleoclimatology/tree-ring>). For climate data, monthly Water-Balance Model output for snowfall, soil moisture storage, and potential/actual evapotranspiration used in this paper are from Wolock and McCabe (2018) and were obtained on 10 October 2020 via their website (<https://doi.org/10.5066/F71V5CWN>). Monthly climate variables for temperature, precipitation, and vapor pressure deficit are from the PRISM Climate Group, Oregon State University (Daly et al., 2008) and were obtained 15 October 2020 via their website (<http://prism.oregonstate.edu>). Palmer Drought-Severity Indices (PDSI / z-PDSI) are from Abatzoglou et al. (2017) and were obtained 18 October 2020 via their website (<https://wrcc.dri.edu/wwdt/>). April 1 snow-water equivalence data are from (Pederson, Gray, Ault, et al., 2011) and were obtained 21 October 2020 via their website (<https://www.ncei.noaa.gov/access/paleo-search/study/11205>). All analyses were conducted using the statistical package R (v. 3.5.0) (R Development Core Team, 2008).

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400 **CRedit authorship contribution statement**

401 **Nickolas E. Kichas**: Conceptualization, Investigation, Data production, Formal analysis,
 402 Methodology, Visualization, Writing - original draft, Funding acquisition. **Gregory T.**
 403 **Pederson**: Conceptualization, Investigation, Formal analysis, Methodology, Resources, Writing
 404 - review & editing. **Sharon M. Hood**: Investigation, Methodology, Writing - review & editing.
 405 **Richard G. Everett**: Project administration, Resources. **David B. McWethy**: Methodology,
 406 Supervision, Funding acquisition, Writing - review & editing.

407 **References**

- 408 Abatzoglou, J. T., McEvoy, D. J., & Redmond, K. T. (2017). The West Wide Drought Tracker:
 409 Drought Monitoring at Fine Spatial Scales. *Bulletin of the American Meteorological*
 410 *Society*, 98(9), 1815-1820. doi:10.1175/bams-d-16-0193.1
- 411 Ackerman, F., & Goldblum, D. (2021). Lodgepole Pine and Interior Spruce Radial Growth
 412 Response to Climate and Topography in the Canadian Rocky Mountains, Alberta.
 413 *Canadian Journal of Forest Research*, 1-16. doi:10.1139/cjfr-2019-0305
- 414 Allen, C. D., Breshears, D. D., & McDowell, N. G. (2015). On Underestimation of Global
 415 Vulnerability to Tree Mortality and Forest Die-Off from Hotter Drought in the
 416 Anthropocene. *Ecosphere*, 6(8), 129. doi:10.1890/ES15-00203.1
- 417 Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., . . .
 418 Cobb, N. (2010). A Global Overview of Drought and Heat-Induced Tree Mortality
 419 Reveals Emerging Climate Change Risks for Forests. *Forest Ecology and Management*,
 420 259(4), 660-684. doi:10.1016/j.foreco.2009.09.001
- 421 Arno, S. F., & Hoff, R. J. (1989). *Silvics of Whitebark Pine (Pinus albicaulis)*. (GTR-INT-253).
 422 Ogden, UT: Intermountain Research Station
- 423 Audley, J. P., Fettig, C. J., Steven Munson, A., Runyon, J. B., Mortenson, L. A., Steed, B. E., . . .
 424 Negrón, J. F. (2020). Impacts of Mountain Pine Beetle Outbreaks on Lodgepole Pine
 425 Forests in the Intermountain West, U.S., 2004–2019. *Forest Ecology and Management*,
 426 475. doi:10.1016/j.foreco.2020.118403
- 427 Bentz, B. J., Duncan, J. P., & Powell, J. A. (2016). Elevational Shifts in Thermal Suitability for
 428 Mountain Pine Beetle Population Growth in a Changing Climate. *Forestry*, 89(3), 271-
 429 283. doi:10.1093/forestry/cpv054
- 430 Boone, C. K., Aukema, B. H., Bohlmann, J., Carroll, A. L., & Raffa, K. F. (2011). Efficacy of
 431 Tree Defense Physiology Varies with Bark Beetle Population Density: A Basis for
 432 Positive Feedback in Eruptive Species. *Canadian Journal of Forest Research*, 41(6),
 433 1174-1188. doi:10.1139/x11-041
- 434 Bunn, A. G. (2008). A Dendrochronology Program Library in R (dplR). *Dendrochronologia*,
 435 26(2), 115-124. doi:10.1016/j.dendro.2008.01.002
- 436 Buotte, P. C., Hicke, J. A., Preisler, H. K., Abatzoglou, J. T., Raffa, K. F., & Logan, J. A. (2017).
 437 Recent and Future Climate Suitability for Whitebark Pine Mortality from Mountain Pine
 438 Beetles Varies Across the Western U.S. *Forest Ecology and Management*, 399, 132-142.
 439 doi:10.1016/j.foreco.2017.05.032
- 440 Cansler, C. A., McKenzie, D., & Halpern, C. B. (2018). Fire Enhances the Complexity of Forest
 441 Structure in Alpine Treeline Ecotones. *Ecosphere*, 9(2), e02091. doi:10.1002/ecs2.2091
- 442 Carnwath, G. C., & Nelson, C. R. (2016). The Effect of Competition on Responses to Drought
 443 and Interannual Climate Variability of a Dominant Conifer Tree of Western North
 444 America. *Journal of Ecology*, 104(5), 1421-1431. doi:10.1111/1365-2745.12604

- Chang, T., Hansen, A. J., & Piekielek, N. (2014). Patterns and Variability of Projected Bioclimatic Habitat for *Pinus albicaulis* in the Greater Yellowstone Area. *PLoS One*, 9(11), e111669. doi:10.1371/journal.pone.0111669
- Chhin, S., Hogg, E. H., Lieffers, V. J., & Huang, S. (2008). Influences of Climate on the Radial Growth of Lodgepole Pine in Alberta. *Botany*, 86(2), 167-178. doi:10.1139/b07-120
- Cook, E. R., & Peters, K. (1997). Calculating Unbiased Tree-Ring Indices for the Study of Climatic and Environmental Change. *The Holocene*, 7(3), 361-370. doi:10.1177/095968369700700314
- Daly, C., Halbleib, M., Smith, J. I., Gibson, W. P., Doggett, M. K., Taylor, G. H., . . . Pasteris, P. (2008). Physiographically Sensitive Mapping of Climatological Temperature and Precipitation across the Conterminous United States. *International Journal of Climatology*, 28(15), 2031-2064. doi:10.1002/joc.1688
- Daly, C., Neilson, R. P., & Phillips, D. L. (1994). A Statistical-Topographic Model for Mapping Climatological Precipitation over Mountainous Terrain. *Journal of Applied Meteorology*, 33, 140-158.
- Danby, R. K., & Hik, D. S. (2007). Responses of White Spruce (*Picea glauca*) to Experimental Warming at a Subarctic Alpine Treeline. *Global Change Biology*, 13(2), 437-451. doi:10.1111/j.1365-2486.2006.01302.x
- Das, A. J., Stephenson, N. L., Flint, A. L., Das, T., & van Mantgem, P. J. (2013). Climatic Correlates of Tree Mortality in Water- and Energy-Limited Forests. *PLoS One*, 8(7), e69917. doi:10.1371/journal.pone.0069917
- DeAngelis, J. D., Nebeker, T. E., & Hodges, J. D. (1986). Influence of Tree Age and Growth Rate on the Radial Resin Duct System in Loblolly Pine (*Pinus taeda*). *Canadian Journal of Botany*, 64(5), 1046-1049. doi:10.1139/b86-142
- Dudney, J., Willing, C. E., Das, A. J., Latimer, A. M., Nesmith, J. C. B., & Battles, J. J. (2021). Nonlinear Shifts in Infectious Rust Disease Due to Climate Change. *Nature Communications*, 12(1), 5102. doi:10.1038/s41467-021-25182-6
- Ferrenberg, S., Kane, J. M., & Mitton, J. B. (2014). Resin Duct Characteristics Associated with Tree Resistance to Bark Beetles Across Lodgepole and Limber Pines. *Oecologia*, 174(4), 1283-1292. doi:10.1007/s00442-013-2841-2
- Ferrenberg, S., Langenhan, J. M., Loskot, S. A., Rozal, L. M., & Mitton, J. B. (2017). Resin Monoterpene Defenses Decline within Three Widespread Species of Pine (*Pinus*) along a 1530-m Elevational Gradient. *Ecosphere*, 8(10), e01975. doi:10.1002/ecs2.1975
- Fritts, H. C. (1971). Dendroclimatology and Dendroecology. *Quaternary Research*, 1(4), 419-449. doi:10.1016/0033-5894(71)90057-3
- Fritts, H. C. (1976). *Tree Rings and Climate*. New York, N.Y.: Academic Press.
- Fryer, J. L. (2002). *Pinus albicaulis*. In *Fire Effects Information System*. Rocky Mountain Research Station: U.S. Department of Agriculture.
- Geils, B. W., Hummer, K. E., & Hunt, R. S. (2010). White Pines, Ribes, and Blister Rust: A Review and Synthesis. *Forest Pathology*, 40(3-4), 147-185. doi:10.1111/j.1439-0329.2010.00654.x
- Gibson, K., Skov, K., Jorgensen, C., Smith, S., Kegley, S., & Witcosky, J. (2008). *Mountain Pine Beetle Impacts in High-Elevation Five-Needle Pines: Current Trends and Challenges*. (R1-08-020). Missoula, MT: U.S. Forest Service

- Goeking, S., & Izlar, D. (2018). *Pinus albicaulis* Engelm. (Whitebark Pine) in Mixed-Species Stands throughout Its US Range: Broad-Scale Indicators of Extent and Recent Decline. *Forests*, 9(3), 131. doi:10.3390/f9030131
- Goldblum, D., & Rigg, L. S. (2005). Tree Growth Response to Climate Change at the Deciduous-Boreal Forest Ecotone, Ontario, Canada. *Canadian Journal of Forest Research*, 35(11), 2709-2718. doi:10.1139/x05-185
- Graumlich, L. J., & Brubaker, L. B. (1986). Reconstruction of Annual Temperature (1590–1979) for Longmire, Washington, Derived from Tree Rings. *Quaternary Research*, 25(2), 223-234. doi:10.1016/0033-5894(86)90059-1
- Guiterman, C. H., Margolis, E. Q., & Swetnam, T. W. (2015). Dendroecological Methods For Reconstructing High-Severity Fire In Pine-Oak Forests. *Tree-Ring Research*, 71(2), 67-77. doi:10.3959/1536-1098-71.2.67
- Hood, S. M., & Sala, A. (2015). Ponderosa Pine Resin Defenses and Growth: Metrics Matter. *Tree Physiology*, 35(11), 1223-1235. doi:10.1093/treephys/tpv098
- Hood, S. M., Sala, A., Heyerdahl, E. K., & Boutin, M. (2015). Low-Severity Fire Increases Tree Defense Against Bark Beetle Attacks. *Ecology*, 96(7), 1846–1855.
- Jenkins, M. J., Runyon, J. B., Fettig, C. J., Page, W. G., & Bentz, B. J. (2014). Interactions among the Mountain Pine Beetle, Fires, and Fuels. *Forest Science*, 60(3), 489-501. doi:10.5849/forsci.13-017
- Kane, J. M., & Kolb, T. E. (2010). Importance of Resin Ducts in Reducing Ponderosa Pine Mortality from Bark Beetle Attack. *Oecologia*, 164(3), 601-609. doi:10.1007/s00442-010-1683-4
- Kemp-Jennings, S., Peterson, D. L., & Bunn, A. G. (2021). Temporal Variability in Climate-Growth Response of Mountain Hemlock at Treeline in Washington and Oregon. *Northwest Science*, 94(3-4). doi:10.3955/046.094.0306
- Kichas, N. E., Hood, S. M., Pederson, G. T., Everett, R. G., & McWethy, D. B. (2020). Whitebark Pine (*Pinus albicaulis*) Growth and Defense in Response to Mountain Pine Beetle Outbreaks. *Forest Ecology and Management*, 457. doi:10.1016/j.foreco.2019.117736
- Kichas, N. E., Pederson, G. T., Hood, S. M., Everett, R. G., & McWethy, D. B. (2022). *Time Series Data for Raw and Detrended Whitebark Pine Tree Growth and Defense Metrics* [Dataset]. International Tree-Ring Data Bank. Retrieved from: forthcoming
- Kichas, N. E., Trowbridge, A. M., Raffa, K. F., Malone, S. C., Hood, S. M., Everett, R. G., . . . Pederson, G. T. (2021). Growth and Defense Characteristics of Whitebark Pine (*Pinus albicaulis*) and Lodgepole Pine (*Pinus contorta* var *latifolia*) in a High-Elevation, Disturbance-Prone Mixed-Conifer Forest in northwestern Montana, USA. *Forest Ecology and Management*, 493. doi:10.1016/j.foreco.2021.119286
- Kipfmüller, K. F., & Salzer, M. W. (2010). Linear Trend and Climate Response of Five-Needle Pines in the Western United States Related to Treeline Proximity. *Canadian Journal of Forest Research*, 40(1), 134-142. doi:10.1139/x09-187
- Kipfmüller, K. F., & Swetnam, T. W. (2002). *Climate and Mountain Pine Beetle-Induced Tree Mortality in the Selway-Bitterroot Wilderness Area*. (RMRS-99611-RJVA). Moscow, ID: The University of Idaho
- Körner, C. (1998). A Re-Assessment of High Elevation Treeline Positions and Their Explanation. *Oecologia*, 115(4), 445-459. doi:10.1007/s004420050540

- Lestina, J. T., Egan, J. M., Harley, G. L., Jenne, J. L., Kaiden, J. D., & Sontag, S. (2019). *Broad-level Reconstruction of Mountain Pine Beetle Outbreaks from 1999-2015 across the Northern Region*. Missoula, MT: U.S. Forest Service, Northern Region
- Little, E. L. (1971). *Atlas of United States Trees. Volume 1 Conifers and Important Hardwoods*. Washington, D.C.: U.S. Forest Service
- Lumley, T. (2020). LEAPS - Regression Subset Selection (Version 3.1).
- Mäkinen, H., Nöjd, P., Kahle, H.-P., Neumann, U., Tveite, B., Mielikäinen, K., . . . Spiecker, H. (2002). Radial Growth Variation of Norway Spruce (*Picea abies* (L.) Karst.) Across Latitudinal and Altitudinal Gradients in Central and Northern Europe. *Forest Ecology and Management*, 171(3), 243-259. doi:10.1016/s0378-1127(01)00786-1
- Martin, J. T., Pederson, G. T., Woodhouse, C. A., Cook, E. R., McCabe, G. J., Anchukaitis, K. J., . . . King, J. (2020). Increased Drought Severity Tracks Warming in the United States' Largest River Basin. *Proceedings of the National Academy of Science*, 117(21), 11328-11336. doi:10.1073/pnas.1916208117
- Martin, J. T., Pederson, G. T., Woodhouse, C. A., Cook, E. R., McCabe, G. J., Wise, E. K., . . . King, J. (2019). 1200 years of Upper Missouri River Streamflow Reconstructed from Tree Rings. *Quaternary Science Reviews*, 224. doi:10.1016/j.quascirev.2019.105971
- McCabe, G. J., & Wolock, D. M. (2011). Independent Effects of Temperature and Precipitation on Modeled Runoff in the Conterminous United States. *Water Resources Research*, 47(11). doi:10.1029/2011wr010630
- McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., . . . Yezpez, E. A. (2008). Mechanisms of Plant Survival and Mortality During Drought: Why do Some Plants Survive While Others Succumb to Drought? *New Phytologist*, 178(4), 719-739. doi:10.1111/j.1469-8137.2008.02436.x
- Millar, C. I., & Delany, D. L. (2019). Interaction between Mountain Pine Beetle-Caused Tree Mortality and Fire Behavior in Subalpine Whitebark Pine Forests, Eastern Sierra Nevada, CA; Retrospective Observations. *Forest Ecology and Management*, 447, 195-202. doi:10.1016/j.foreco.2019.05.052
- Millar, C. I., Westfall, R. D., & Delany, D. L. (2007). Response of High-Elevation Limber Pine (*Pinus flexilis*) to Multiyear Droughts and 20th-Century Warming, Sierra Nevada, California, USA. *Canadian Journal of Forest Research*, 37(12), 2508-2520. doi:10.1139/x07-097
- Miyamoto, Y., Griesbauer, H. P., & Scott Green, D. (2010). Growth Responses of Three Coexisting Conifer Species to Climate Across Wide Geographic and Climate Ranges in Yukon and British Columbia. *Forest Ecology and Management*, 259(3), 514-523. doi:10.1016/j.foreco.2009.11.008
- Pederson, G. T., Betancourt, J. L., & McCabe, G. J. (2013). Regional Patterns and Proximal Causes of the Recent Snowpack Decline in the Rocky Mountains, U.S. *Geophysical Research Letters*, 40(9), 1811-1816. doi:10.1002/grl.50424
- Pederson, G. T., Gray, S. T., Ault, T., Marsh, W., Fagre, D. B., Bunn, A. G., . . . Graumlich, L. J. (2011). Climatic Controls on the Snowmelt Hydrology of the Northern Rocky Mountains. *Journal of Climate*, 24(6), 1666-1687. doi:10.1175/2010jcli3729.1
- Pederson, G. T., Gray, S. T., Woodhouse, C. A., Betancourt, J. L., Fagre, D. B., Littell, J. S., . . . Graumlich, L. J. (2011). The Unusual Nature of Recent Snowpack Declines in the North American Cordillera. *Science*, 333(6040), 332-335. doi:10.1126/science.1201570

- 579 Pedlar, J. H., McKenney, D. W., Lu, P., & Thomson, A. (2021). Response of Northern
 580 Populations of Black Spruce and Jack Pine to Southward Seed Transfers: Implications for
 581 Climate Change. *Atmosphere*, 12(10). doi:10.3390/atmos12101363
- 582 Peterson, D. W., Peterson, D. L., & Ettl, G. J. (2002). Growth Responses of Subalpine Fir to
 583 Climatic Variability in the Pacific Northwest. *Canadian Journal of Forest Research*,
 584 32(9), 1503-1517.
- 585 R Development Core Team. (2008). R: A Language and Environment for Statistical Computing
 586 (Version 3.31). Vienna, Austria: R Foundation for Statistical Computing.
- 587 Reed, C. C., & Hood, S. M. (2020). Few Generalizable Patterns of Tree-Level Mortality During
 588 Extreme Drought and Concurrent Bark Beetle Outbreaks. *Science of the Total*
 589 *Environment*, 750, 141306. doi:10.1016/j.scitotenv.2020.141306
- 590 Safranyik, L., & Carroll, A. L. (2006). *The Mountain Pine Beetle: A Synthesis of Biology,*
 591 *Management, and Impacts on Lodgepole Pine* Victoria, British Columbia: Natural
 592 Resources Canada
- 593 Salzer, M. W., Hughes, M. K., Bunn, A. G., & Kipfmüller, K. F. (2009). Recent Unprecedented
 594 Tree-Ring Growth in Bristlecone Pine at the Highest Elevations and Possible Causes.
 595 *Proceedings of the National Academy of Science*, 106(48), 20348-20353.
 596 doi:10.1073/pnas.0903029106
- 597 Samman, S., Schwandt, J. W., & Wilson, J. L. (2003). *Managing for Healthy White Pine*
 598 *Ecosystems in the United States to Reduce the Impacts of White Pine Blister Rust*. (R1-
 599 03-118). Missoula, Montana: Forest Service
- 600 Schopmeyer, C. S., Mergen, F., & Evans, T. C. (1954). Applicability of Poiseuille's Law to
 601 Exudation of Oleoresin from Wounds on Slash Pine. *Plant Physiol*, 29(1), 82-87.
 602 doi:10.1104/pp.29.1.82
- 603 Smith, J. M., Paritsis, J., Veblen, T. T., & Chapman, T. B. (2015). Permanent Forest Plots Show
 604 Accelerating Tree Mortality in Subalpine Forests of the Colorado Front Range from 1982
 605 to 2013. *Forest Ecology and Management*, 341, 8-17. doi:10.1016/j.foreco.2014.12.031
- 606 Stephens, S. L., Agee, J. K., Fulé, P. Z., North, M. P., Romme, W. H., Swetnam, T. W., &
 607 Turner, M. G. (2013). Managing Forests and Fire in Changing Climates. *Science*,
 608 342(6154), 41-42. doi:10.1126/science.1240294
- 609 Stephenson, N. L., Das, A. J., Ampersee, N. J., Bulaon, B. M., Yee, J. L., & Edwards, D. (2019).
 610 Which Trees Die During Drought? The Key Role of Insect Host-Tree Selection. *Journal*
 611 *of Ecology*. doi:10.1111/1365-2745.13176
- 612 Tomback, D. F., Arno, S. F., & Keane, R. E. (2001). *Whitebark Pine Communities: Ecology and*
 613 *Restoration*. Washington, D.C.: Island Press.
- 614 van Mantgem, P. J., & Stephenson, N. L. (2007). Apparent Climatically Induced Increase of Tree
 615 Mortality Rates in a Temperate Forest. *Ecology Letters*, 10(10), 909-916.
 616 doi:10.1111/j.1461-0248.2007.01080.x
- 617 van Mantgem, P. J., Stephenson, N. L., Byrne, J. C., Daniels, L. D., Franklin, J. F., Fulé, P. Z., . .
 618 . Veblen, T. T. (2009). Widespread Increase of Tree Mortality Rates in the Western
 619 United States. *Science*, 323(5913), 521-524. doi:10.1126/science.1165000
- 620 Vázquez-González, C., Sampedro, L., Rozas, V., Voltas, J., & Zas, R. (2021). Population
 621 Differentiation in Climate Sensitivity of Resin Duct Formation during Growth
 622 Resumption in *Pinus pinaster*. *Dendrochronologia*, 67.
 623 doi:10.1016/j.dendro.2021.125839

- Vázquez-González, C., Zas, R., Erbilgin, N., Ferrenberg, S., Rozas, V., & Sampedro, L. (2020). Resin Ducts as Resistance Traits in Conifers: Linking Dendrochronology and Resin-Based Defenses. *Tree Physiology*. doi:10.1093/treephys/tpaa064
- Wermelinger, B., Rigling, A., Schneider Mathis, D., Kenis, M., & Gossner, M. M. (2021). Climate Change Effects on Trophic Interactions of Bark Beetles in Inner Alpine Scots Pine Forests. *Forests*, 12(2). doi:10.3390/f12020136
- Wettstein, J. J., Littell, J. S., Wallace, J. M., & Gedalof, Z. e. (2011). Coherent Region-, Species-, and Frequency-Dependent Local Climate Signals in Northern Hemisphere Tree-Ring Widths. *Journal of Climate*, 24(23), 5998-6012. doi:10.1175/2011jcli3822.1
- Wimmer, R., & Grabner, M. (1997). Effects of Climate on Vertical Resin Duct Density and Radial Growth of Norway Spruce (*Picea abies*). *Trees*, 11, 271-276.
- Wolock, D. M., & McCabe, G. J. (2018). *Water Balance Model Inputs and Outputs for the Conterminous United States, 1900-2015*.
- Wong, C. M., & Daniels, L. D. (2017). Novel Forest Decline Triggered by Multiple Interactions among Climate, an Introduced Pathogen and Bark Beetles. *Global Chang Biology*, 23(5), 1926-1941. doi:10.1111/gcb.13554
- Yi, M., Jia, T., Dong, L., Zhang, L., Leng, C., Liu, S., & Lai, M. (2021). Resin Yield in *Pinus elliottii* Engelm. is Related to the Resin Flow Rate, Resin Components and Resin Duct Characteristics at Three Locations in Southern China. *Industrial Crops and Products*, 160. doi:10.1016/j.indcrop.2020.113141
- Zas, R., Touza, R., Sampedro, L., Lario, F. J., Bustingorri, G., & Lema, M. (2020). Variation in Resin Flow Among Maritime Pine Populations: Relationship with Growth Potential and Climatic Responses. *Forest Ecology and Management*, 474. doi:10.1016/j.foreco.2020.118351
- Zhang, Q., Shao, M., Jia, X., & Wei, X. (2017). Relationship of Climatic and Forest Factors to Drought- and Heat-Induced Tree Mortality. *PLoS One*, 12(1), e0169770. doi:10.1371/journal.pone.0169770

CONCLUSIONS

In response to widespread mortality, there is an urgent need to develop strategies for maintaining distributions of whitebark pine throughout its contemporary range (Keane et al. 2012, Goeking and Izlar 2018, Goeking et al. 2018). For instance, scientists have developed genetic strains resistant to white pine blister rust and are monitoring planting efforts to better understand how these strains perform in natural settings over time (Kim et al. 2003, Bower and Aitken 2008, Liu et al. 2016, Liu et al. 2017a). These are important efforts that will greatly assist managers in retaining and promoting whitebark pine within the complex environments where it is found. However, seedling growth is often the primary characteristic selected for within these breeding programs and the preferential selection for these two traits (growth and rust resistance) likely fails to account for diverse defensive strategies (both physical and chemical defenses) that differ considerably across genotypes (Bower and Aitken 2008). For example, in our manuscript investigating stand dynamics within whitebark pine populations, we found that trees that have persisted through recent stand-level mountain pine beetle activity and white pine blister rust infection produced anatomically larger resin ducts with a greater annual investment in these resin duct features (Kichas et al. 2020). However, these trees did so at the expense of growth, exhibiting lower overall growth rates compared to trees that died. This suggests a complex trade-off between the allocation of resources to growth and defense, however, such trade-offs are poorly understood within natural ecosystems.

Changes in the composition and structure of whitebark pine stands, resulting from fire suppression, increased competition from shade-tolerant species, and shifting insect and pathogen interactions all highlight a critical need to better understand the evolutionary tradeoffs between

the investment of resources into growth and defense in whitebark pine. Developing a better understanding of how these physiological properties differ within and across whitebark pine populations is critical for establishing successful strategies for restoration. This is especially true given the dynamic nature of mixed-conifer stands within the northern Rocky Mountains. Our results lend insight into different growth and defense strategies, and specifically resin duct characteristics and resin chemistry that may be beneficial to increasing survivorship and promoting whitebark pine establishment and persistence within natural, disturbance-prone mixed-conifer ecosystems. In addition, our research provides novel insight on whitebark pine growth and defense characteristics utilizing data from a region of the northern Rocky Mountains where the species is currently underrepresented in the academic literature. There are six key points that can be drawn from this work:

- 1) Whitebark pine trees that have persisted through recent stand-level disturbance produced fewer but larger resin duct structures with greater relative duct area compared to trees that died.
- 2) There are important differences in the chemical composition of constitutive resin between whitebark and lodgepole pine that generally support field observations. Under endemic scenarios, mountain pine beetle preferentially select lodgepole pine, while under eruptive outbreak scenarios, mountain pine beetle are successful in colonizing higher-elevation whitebark pine trees.
- 3) There are complex relationships between tree growth, resin duct anatomy and constitutive resin chemistry that present beetles with many permutations of resin-based defenses.

- 4) Competition, particularly with Engelmann spruce (*Picea engelmannii*) can influence constitutive resin chemistry in whitebark pine.
- 5) Whitebark pine in subalpine settings of the northern Rocky Mountains are exhibiting increased growth as well as increased defense under warmer and regionally drier conditions.

Evidence from this research suggests whitebark pine employs a complex array of physiological strategies in response to changing disturbance and climatic conditions. The broader study region from which our trees were sampled has been affected by numerous large-scale bark beetle outbreaks, occurring in the 1930s, 1960s-1980s, and most recently 2000s-2010s. There was strong evidence for both mountain pine beetle and white pine blister rust mortality across each of our study sites, with the majority (~ 82%) of whitebark pine trees having been killed prior to our initial sampling effort. In this context, producing larger resin duct structures and investing a greater proportion of annual carbon into resin-based defenses is advantageous in persisting through this biotic stressor. However, under different environmental pressures investment in resin ducts at the expense of growth could be disadvantageous. For instance, during periods of endemic beetle activity stand competition could reflect a greater stress and select for individuals that can more efficiently allocate resources to height and radial growth, at the expense of energetically demanding resin-based defenses. Climate can further modulate growth and resin duct morphology and adds another layer of complexity to the evolutionary filtering of this unique species. Whitebark pine in the northern Rocky Mountains have evolved with these environmental stressors over hundreds of thousands of years and exhibit high phenotypic plasticity. As such, it is essential to maintain as much stand-level genetic variability

as possible in order to buffer against future unpredictable changes in climate and disturbance regimes that may negatively impact this species.

Managers tasked with preserving and promoting whitebark pine populations are presented with increasingly limited options for achieving successful outcomes. Many management prescriptions rely on surficial performance indicators (primarily growth) that may fail to account for the important role of physical and chemical defenses in moderating disturbance interactions. Few studies have investigated patterns of growth and defense using multiple proxies (resin duct anatomy and resin chemistry) and our work highlights a need to expand research to a broader range of species and environments.

REFERENCES CITED

- Allen, C. D., A. K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D. D. Breshears, E. H. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J.-H. Lim, G. Allard, S. W. Running, A. Semerci, and N. Cobb. 2010. A Global Overview of Drought and Heat-Induced Tree Mortality Reveals Emerging Climate Change Risks for Forests. *Forest Ecology and Management* **259**:660-684.
- Arno, S. F., and R. J. Hoff. 1989. Silvics of Whitebark Pine (*Pinus albicaulis*). in F. S. United States Department of Agriculture, editor. Intermountain Research Station, Ogden, UT.
- Baier, P., E. Führer, T. Kirisits, and S. Rosner. 2002. Defence Reactions of Norway Spruce against Bark Beetles and the Associated Fungus *Ceratocystis polonica* in Secondary Pure and Mixed Species Stands. *Forest Ecology and Management* **159**:73-86.
- Baker, W. L. 2009. *Fire Ecology in Rocky Mountain Landscapes*. 1 edition. Island Press, Washington, D.C.
- Bannan, M. W. 1936. Vertical Resin Ducts in the Secondary Wood of the Abietineae. *The New Phytologist* **35**:11-46.
- Barton, K. E., and J. Koricheva. 2010. The Ontogeny of Plant Defense and Herbivory: Characterizing General Patterns Using Meta-Analysis. *The American Naturalist* **175**:481-493.
- Beaver, R. A. 1989. Insect-Fungus Relationships in the Bark and Ambrosia Beetles. Pages 121-143 *Insect-fungus Interactions*.
- Bell, A. A. 1981. Biochemical Mechanisms of Disease Resistance. *Annual Review of Plant Physiology* **32**:21-81.
- Bentz, B. J., S. M. Hood, E. M. Hansen, J. C. Vandygriff, and K. E. Mock. 2017. Defense Traits in the Long-Lived Great Basin Bristlecone Pine and Resistance to the Native Herbivore Mountain Pine Beetle. *New Phytologist* **213**:611-624.
- Bentz, B. J., J. Régnière, C. J. Fettig, E. M. Hansen, J. L. Hayes, J. A. Hicke, R. G. Kelsey, J. F. Negrón, and S. J. Seybold. 2010. Climate Change and Bark Beetles of the Western United States and Canada: Direct and Indirect Effects. *BioScience* **60**:602-613.
- Blanche, C. A., P. L. Lorio, R. A. Sommers, J. D. Hodges, and T. E. Nebeker. 1992. Seasonal Cambial Growth and Development of Loblolly Pine: Xylem Formation, Inner Bark Chemistry, Resin Ducts, and Resin Flow. *Forest Ecology and Management* **49**:151-165.
- Bockino, N. K., and D. B. Tinker. 2012. Interactions of White Pine Blister Rust and Mountain Pine Beetle in Whitebark Pine Ecosystems in the Southern Greater Yellowstone Area. *Natural Areas Journal* **32**:31-40.
- Boege, K., and R. J. Marquis. 2005. Facing Herbivory as You Grow Up: The Ontogeny of Resistance in Plants. *Trends in Ecology and Evolution* **20**:441-448.
- Bonello, P., T. R. Gordon, D. A. Herms, D. L. Wood, and N. Erbilgin. 2006. Nature and Ecological Implications of Pathogen-Induced Systemic Resistance in Conifers: A Novel Hypothesis. *Physiological and Molecular Plant Pathology* **68**:95-104.
- Boone, C. K., B. H. Aukema, J. Bohlmann, A. L. Carroll, and K. F. Raffa. 2011. Efficacy of Tree Defense Physiology Varies with Bark Beetle Population Density: A Basis for Positive Feedback in Eruptive Species. *Canadian Journal of Forest Research* **41**:1174-1188.
- Borden, J. H., D. S. Pureswaran, and J. P. Lafontaine. 2008. Synergistic Blends of Monoterpenes for Aggregation Pheromones of the Mountain Pine Beetle (Coleoptera: Curculionidae). *Journal of Economic Entomology* **101**:1266-1275.

- Bower, A. D., and S. N. Aitken. 2008. Ecological Genetics and Seed Transfer Guidelines for *Pinus Albicaulis* (Pinaceae). *American Journal of Botany* **95**:66-76.
- Bullington, L. S., Y. Lekberg, R. A. Snieszko, and B. Larkin. 2018. The Influence of Genetics, Defensive Chemistry and the Fungal Microbiome on Disease Outcome in Whitebark Pine Trees. *Molecular Plant Pathology*.
- Buotte, P. C., J. A. Hicke, H. K. Preisler, J. T. Abatzoglou, K. F. Raffa, and J. A. Logan. 2016. Climate Influences on Whitebark Pine Mortality from Mountain Pine Beetle in the Greater Yellowstone Ecosystem. *Ecological Applications* **26**:2507–2524.
- Buotte, P. C., J. A. Hicke, H. K. Preisler, J. T. Abatzoglou, K. F. Raffa, and J. A. Logan. 2017. Recent and Future Climate Suitability for Whitebark Pine Mortality from Mountain Pine Beetles Varies Across the Western U.S. *Forest Ecology and Management* **399**:132-142.
- Campbell, E. M., and J. A. Antos. 2000. Distribution and Severity of White Pine Blister Rust and Mountain Pine Beetle on Whitebark Pine in British Columbia. *Canadian Journal of Forest Research* **30**:1051-1059.
- Campbell, E. S., and J. C. A. Taylor. 2007. Monoterpene Production in Redberry Juniper Foliage following Fire. *Rangeland Ecology & Management* **60**:104-109.
- Cannac, M., T. Barboni, L. Ferrat, A. Bighelli, V. Castola, J. Costa, D. Trecul, F. Morandini, and V. Pasqualini. 2009. Oleoresin Flow and Chemical Composition of Corsican pine (*Pinus nigra* subsp. *laricio*) in Response to Prescribed Burnings. *Forest Ecology and Management* **257**:1247-1254.
- Chang, T., A. J. Hansen, and N. Piekielek. 2014. Patterns and Variability of Projected Bioclimatic Habitat for *Pinus albicaulis* in the Greater Yellowstone Area. *PLoS One* **9**:e111669.
- Cobb, N. S., J. B. Mitton, and T. G. Whitham. 1994. Genetic Variation Associated with Chronic Water and Nutrient Stress in Pinyon Pine. *American Journal of Botany* **81**.
- Cole, D. N. 1990. Recreation in Whitebark Pine Ecosystems: Demand, Problems, and Management Strategies. Pages 305-309 in U. S. F. Service, editor. *Intermountain Research Station*, Ogden, UT.
- Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource Availability and Plant Antiherbivore Defense. *Science* **230**:895-899.
- Conedera, M., W. Tinner, C. Neff, M. Meurer, A. F. Dickens, and P. Krebs. 2009. Reconstructing Past Fire Regimes: Methods, Applications, and Relevance to Fire Management and Conservation. *Quaternary Science Reviews* **28**:555-576.
- Dawson, T. P., S. T. Jackson, J. I. House, I. C. Prentice, and G. M. Mace. 2011. Beyond Predictions: Biodiversity Conservation in a Changing Climate. *Science* **332**:53-58.
- de la Mata, R., S. M. Hood, and A. Sala. 2017. Insect Outbreak Shifts the Direction of Selection from Fast to Slow Growth Rates in the Long-Lived Conifer *Pinus ponderosa*. *Proceedings of the National Academy of Science* **114**:7391-7396.
- DeAngelis, J. D., T. E. Nebeker, and J. D. Hodges. 1986. Influence of Tree Age and Growth Rate on the Radial Resin Duct System in Loblolly Pine (*Pinus taeda*). *Canadian Journal of Botany* **64**:1046-1049.
- Driscoll, D. A., D. B. Lindenmayer, A. F. Bennett, M. Bode, R. A. Bradstock, G. J. Cary, M. F. Clarke, N. Dexter, R. Fensham, G. Friend, M. Gill, S. James, G. Kay, D. A. Keith, C. MacGregor, J. Russell-Smith, D. Salt, J. E. M. Watson, R. J. Williams, and A. York. 2010.

- Fire Management for Biodiversity Conservation: Key Research Questions and Our Capacity to Answer Them. *Biological Conservation* **143**:1928-1939.
- Endara, M.-J., and P. D. Coley. 2011. The Resource Availability Hypothesis Revisited: A Meta-Analysis. *Functional Ecology* **25**:389-398.
- Erbilgin, N., and K. F. Raffa. 2001. Modulation of Predator Attraction to Pheromones of Two Prey Species by Stereochemistry of Plant Volatiles. *Oecologia* **127**:444-453.
- Ferrenberg, S., J. M. Kane, and J. M. Langenhan. 2015. To Grow or Defend? Pine Seedlings Grow Less But Induce More Defences When a Key Resource is Limited. *Tree Physiology* **35**:107-111.
- Ferrenberg, S., J. M. Kane, and J. B. Mitton. 2014. Resin Duct Characteristics Associated with Tree Resistance to Bark Beetles Across Lodgepole and Limber Pines. *Oecologia* **174**:1283-1292.
- Ferrenberg, S., J. M. Langenhan, S. A. Loskot, L. M. Rozal, and J. B. Mitton. 2017. Resin Monoterpene Defenses Decline within Three Widespread Species of Pine (*Pinus*) along a 1530-m Elevational Gradient. *Ecosphere* **8**:e01975.
- Fettig, C. J., K. D. Klepzig, R. F. Billings, A. S. Munson, T. E. Nebeker, J. F. Negrón, and J. T. Nowak. 2007. The Effectiveness of Vegetation Management Practices for Prevention and Control of Bark Beetle Infestations in Coniferous Forests of the Western and Southern United States. *Forest Ecology and Management* **238**:24-53.
- Franceschi, V. R., T. Krekling, A. A. Berryman, and E. Christiansen. 1998. Specialized Phloem Parenchyma Cells in Norway Spruce (*Pinaceae*) Bark are an Important Site of Defense Reactions. *American Journal of Botany* **85**:601-615.
- Franceschi, V. R., P. Krokene, E. Christiansen, and T. Krekling. 2005. Anatomical and Chemical Defenses of Conifer Bark Against Bark Beetles and Other Pests. *New Phytologist* **167**:353-375.
- Franceschi, V. R., P. Krokene, T. Krekling, and E. Christiansen. 2000. Phloem Parenchyma Cells are Involved in Local and Distant Defense Responses to Fungal Inoculation or Bark-Beetle Attack in Norway Spruce (*Pinaceae*). *American Journal of Botany* **87**:314-326.
- Franklin, J. F., and J. K. Agee. 2003. Forging a Science-Based National Forest Fire Policy. *Issues in Science and Technology* **20**.
- Gaylord, M. L., T. E. Kolb, and N. G. McDowell. 2015. Mechanisms of Piñon Pine Mortality after Severe Drought: A Retrospective Study of Mature Trees. *Tree Physiology* **35**:806-816.
- Gaylord, M. L., T. E. Kolb, W. T. Pockman, J. A. Plaut, E. A. Yezzer, A. K. Macalady, R. E. Pangle, and N. G. McDowell. 2013. Drought Predisposes Pinon-Juniper Woodlands to Insect Attacks and Mortality. *New Phytologist* **198**:567-578.
- Gaylord, M. L., T. E. Kolb, K. F. Wallin, and M. R. Wagner. 2007. Seasonal Dynamics of Tree Growth, Physiology, and Resin Defenses in a Northern Arizona Ponderosa Pine Forest. *Canadian Journal of Forest Research* **37**:1173-1183.
- Goeking, S., and D. Izlar. 2018. *Pinus albicaulis* Engelm. (Whitebark Pine) in Mixed-Species Stands throughout Its US Range: Broad-Scale Indicators of Extent and Recent Decline. *Forests* **9**:131.
- Goeking, S. A., D. K. Izlar, and T. C. Edwards. 2018. A Landscape-Level Assessment of Whitebark Pine Regeneration in the Rocky Mountains, USA. *Forest Science* **fx029**:1-13.

- Goodsman, D. W., I. Lusebrink, S. M. Landhausser, N. Erbilgin, and V. J. Lieffers. 2013. Variation in Carbon Availability, Defense Chemistry and Susceptibility to Fungal Invasion along the Stems of Mature Trees. *New Phytologist* **197**:586-594.
- Halofsky, J. E., D. C. Donato, D. E. Hibbs, J. L. Campbell, M. Donaghy Cannon, J. B. Fontaine, J. R. Thompson, R. G. Anthony, B. T. Bormann, L. J. Kayes, B. E. Law, D. L. Peterson, and T. A. Spies. 2011. Mixed-Severity Fire Regimes: Lessons and Hypotheses from the Klamath-Siskiyou Ecoregion. *Ecosphere* **2**:1-19.
- Hammerbacher, A., A. Schmidt, N. Wadke, L. P. Wright, B. Schneider, J. Bohlmann, W. A. Brand, T. M. Fenning, J. Gershenzon, and C. Paetz. 2013. A Common Fungal Associate of the Spruce Bark Beetle Metabolizes the Stilbene Defenses of Norway Spruce. *Plant Physiology* **162**:1324-1336.
- Hannrup, B., C. Cahalan, G. Chantre, M. Grabner, B. Karlsson, I. L. Bayon, G. L. Jones, U. Müller, H. Pereira, J. C. Rodrigues, S. Rosner, P. Rozenberg, L. Wilhelmsson, and R. Wimmer. 2004. Genetic Parameters of Growth and Wood Quality Traits in *Picea abies*. *Scandinavian Journal of Forest Research* **19**:14-29.
- Harrington, T. C. 2005. Ecology and Evolution of Mycophagous Bark Beetles and their Fungal Partners. Pages 257-291 in F. E. Vega and M. Blackwell, editors. *Ecological and Evolutionary Advances in Insect-Fungal Associations*. Oxford University Press.
- Hayes, J. L., and B. L. Strom. 1994. 4-Allylanisole as an Inhibitor of Bark Beetle (Coleoptera: Scolytidae) Aggregation. *Journal of Economic Entomology* **87**:1586-1594.
- Herms, D. A., and W. J. Mattson. 1992. The Dilemma of Plants: To Grow or Defend. *The Quarterly Review of Biology* **67**:283-335.
- Hood, S. M., S. Baker, and A. Sala. 2016. Fortifying the Forest: Thinning and Burning Increase Resistance to a Bark Beetle Outbreak and Promote Forest Resilience. *Ecological Applications* **26**:1984-2000.
- Hood, S. M., and A. Sala. 2015. Ponderosa Pine Resin Defenses and Growth: Metrics Matter. *Tree Physiology* **35**:1223-1235.
- Hood, S. M., A. Sala, E. K. Heyerdahl, and M. Boutin. 2015. Low-Severity Fire Increases Tree Defense Against Bark Beetle Attacks. *Ecology* **96**:1846-1855.
- Huber, D. P. W., R. Gries, J. H. Borden, and H. D. Pierce Jr. 2000. A Survey of Antennal Responses by Five Species of Coniferophagous Bark Beetles (Coleoptera: Scolytidae) to Bark Volatiles of Six Species of Angiosperm Trees. *Chemoecology* **10**:103-113.
- Iglesias, V., T. R. Krause, and C. Whitlock. 2015. Complex Response of White Pines to Past Environmental Variability Increases Understanding of Future Vulnerability. *PLoS One* **10**:e0124439.
- Imaji, A., and K. Seiwa. 2010. Carbon Allocation to Defense, Storage, and Growth in Seedlings of Two Temperate Broad-Leaved Tree Species. *Oecologia* **162**:273-281.
- Jactel, H., J. Petit, M.-L. Desprez-Loustau, S. Delzon, D. Piou, A. Battisti, and J. Koricheva. 2012. Drought Effects on Damage by Forest Insects and Pathogens: A Meta-Analysis. *Global Change Biology* **18**:267-276.
- Jamieson, M. A., L. A. Burkle, J. S. Manson, J. B. Runyon, A. M. Trowbridge, and J. Zientek. 2017. Global Change Effects on Plant-Insect Interactions: The Role of Phytochemistry. *Current Opinion in Insect Science* **23**:70-80.

- Kane, J. M., and T. E. Kolb. 2010. Importance of Resin Ducts in Reducing Ponderosa Pine Mortality from Bark Beetle Attack. *Oecologia* **164**:601-609.
- Kane, J. M., J. M. Varner, M. R. Metz, and P. J. van Mantgem. 2017. Characterizing Interactions Between Fire and Other Disturbances and Their Impacts on Tree Mortality in Western U.S. Forests. *Forest Ecology and Management* **405**:188-199.
- Keane, R. E. 2000. The Importance of Wilderness to Whitebark Pine Research and Management. Pages 84-92 in U. S. F. Service, editor. Rocky Mountain Research Station, Ogden, UT.
- Keane, R. E., and S. F. Arno. 1993. Rapid Decline of Whitebark Pine in Western Montana: Evidence from 20-Year Remeasurements. *Western Journal of Applied Forestry* **8**:44-47.
- Keane, R. E., D. F. Tomback, C. A. Aubry, A. D. Bower, E. M. Campbell, C. L. Cripps, M. B. Jenkins, M. F. Mahalovich, M. Manning, S. T. McKinney, M. P. Murray, D. L. Perkins, D. P. Reinhart, C. Ryan, A. W. Schoettle, and C. M. Smith. 2012. A Range-Wide Restoration Strategy for Whitebark Pine (*Pinus albicaulis*). in U. S. D. o. Agriculture, editor. Forest Service, Rocky Mountain Research Station.
- Keefover-Ring, K., A. Trowbridge, C. J. Mason, and K. F. Raffa. 2016. Rapid Induction of Multiple Terpenoid Groups by Ponderosa Pine in Response to Bark Beetle-Associated Fungi. *Journal of Chemical Ecology* **42**:1-12.
- Keeling, C. I. 2016. Bark Beetle Research in the Postgenomic Era. Pages 265–294 in C. Tittiger and G. Blomquist, editors. *Pine Bark Beetles - Advances in Insect Physiology*. Simon Fraser University, Burnaby, BC, Canada.
- Kendall, K. C., and R. E. Keane. 2000. The Decline of Whitebark Pine. Pages 123-145 in D. F. Tomback, S. F. Arno, and R. E. Keane, editors. *Whitebark Pine Communities: Ecology and Restoration*. Island Press, Washington, DC.
- Kichas, N. E., S. M. Hood, G. T. Pederson, R. G. Everett, and D. B. McWethy. 2020. Whitebark Pine (*Pinus albicaulis*) Growth and Defense in Response to Mountain Pine Beetle Outbreaks. *Forest Ecology and Management* **457**.
- Kichas, N. E., A. M. Trowbridge, K. F. Raffa, S. C. Malone, S. M. Hood, R. G. Everett, D. B. McWethy, and G. T. Pederson. 2021. Growth and Defense Characteristics of Whitebark Pine (*Pinus albicaulis*) and Lodgepole Pine (*Pinus contorta* var *latifolia*) in a High-Elevation, Disturbance-Prone Mixed-Conifer Forest in northwestern Montana, USA. *Forest Ecology and Management* **493**.
- Kim, M.-S., S. J. Brunsfeld, G. I. McDonald, and N. B. Klopfenstein. 2003. Effect of White Pine Blister Rust (*Cronartium ribicola*) and Rust-Resistance Breeding on Genetic Variation in Western White Pine (*Pinus monticola*). *Theoretical and Applied Genetics* **106**:1004-1010.
- Klepzig, K. D., E. L. Kruger, E. B. Smalley, and K. F. Raffa. 1995. Effects of Biotic and Abiotic Stress on Induced Accumulation of Terpenes and Phenolics in Red Pines Inoculated with Bark Beetle-Vectored Fungus. *Journal of Chemical Ecology* **21**:601-626.
- Kolb, T. E., J. K. Agee, P. Z. Fulé, N. G. McDowell, K. Pearson, A. Sala, and R. H. Waring. 2007. Perpetuating Old Ponderosa Pine. *Forest Ecology and Management* **249**:141-157.
- Lewinsohn, E., M. Gijzen, and R. Croteau. 1991. Defense Mechanisms of Conifers. *Plant Physiology* **96**:44-49.
- Littell, J. S., D. McKenzie, D. L. Peterson, and A. L. Westerling. 2009. Climate and Wildfire Area Burned in Western US Ecoprovinces, 1916-2003. *Ecological Applications* **19**:1003-1021.

- Liu, J.-J., R. A. Snieszko, M. Murray, N. Wang, H. Chen, A. Zamany, R. N. Sturrock, D. P. Savin, and A. Kegley. 2016. Genetic Diversity and Population Structure of Whitebark Pine (*Pinus albicaulis* Engelm.) in Western North America. *PLoS One* **11**:e0167986.
- Liu, J.-J., R. A. Snieszko, A. Zamany, H. Williams, N. Wang, A. Kegley, D. P. Savin, H. Chen, and R. N. Sturrock. 2017a. Saturated Genic SNP Mapping Identified Functional Candidates and Selection Tools for the *Pinus monticola* Cr2 Locus Controlling Resistance to White Pine Blister Rust. *Plant Biotechnology Journal*.
- Liu, J.-J., H. Williams, X. R. Li, A. W. Schoettle, R. A. Snieszko, M. Murray, A. Zamany, G. Roke, and H. Chen. 2017b. Profiling Methyl Jasmonate-Responsive Transcriptome for Understanding Induced Systemic Resistance in Whitebark Pine (*Pinus albicaulis*). *Plant Molecular Biology* **95**:359-374.
- Loehman, R. A., R. E. Keane, L. M. Holsinger, and Z. Wu. 2017. Interactions of landscape disturbances and climate change dictate ecological pattern and process: spatial modeling of wildfire, insect, and disease dynamics under future climates. *Landscape Ecology* **32**:1447-1459.
- Logan, J. A., W. W. Macfarlane, and L. Willcox. 2010. Whitebark Pine Vulnerability to Climate-Driven Mountain Pine Beetle Disturbance in the Greater Yellowstone Ecosystem. *Ecological Applications* **20**:895-902.
- Lombardero, M. J., M. P. Ayres, P. L. Lorio Jr, and J. J. Ruel. 2000. Environmental Effects on Constitutive and Inducible Resin Defences of *Pinus taeda*. *Ecology Letters* **3**:329-339.
- Loomis, W. F. 1932. Growth-Differentiation Balance vs Carbohydrate-Nitrogen Ratio. Pages 240-245 in *Proceedings of the American Society for Horticultural Science*.
- Macfarlane, W. W., J. A. Logan, and W. R. Kern. 2013. An Innovative Aerial Assessment of Greater Yellowstone Ecosystem Mountain Pine Beetle-Caused Whitebark Pine Mortality. *Ecological Applications* **23**:421-437.
- Maloney, P. E., T. F. Smith, C. E. Jensen, J. Innes, D. M. Rizzo, and M. P. North. 2008. Initial Tree Mortality and Insect and Pathogen Response to Fire and Thinning Restoration Treatments in an Old-Growth Mixed-Conifer Forest of the Sierra Nevada, California. *Canadian Journal of Forest Research* **38**:3011-3020.
- Mason, C. J., K. Keefover-Ring, C. Villari, J. G. Klutsch, S. Cook, P. Bonello, N. Erbilgin, K. F. Raffa, and P. A. Townsend. 2019. Anatomical Defences against Bark Beetles Relate to Degree of Historical Exposure between Species and are Allocated Independently of Chemical Defences within Trees. *Plant Cell & Environment* **42**:633-646.
- Mattson, W. J., and R. A. Haack. 1987. The Role of Drought in Outbreaks of Plant-Eating Insects. *BioScience* **37**:110-118.
- McWethy, D. B., T. Schoennagel, P. E. Higuera, M. Krawchuk, B. J. Harvey, E. C. Metcalf, C. Schultz, C. Miller, A. L. Metcalf, B. Buma, A. Virapongse, J. C. Kulig, R. C. Stedman, Z. Ratajczak, C. R. Nelson, and C. Kolden. 2019. Rethinking resilience to wildfire. *Nature Sustainability* **2**:797-804.
- Millar, C. I., and D. L. Delany. 2019. Interaction between Mountain Pine Beetle-Caused Tree Mortality and Fire Behavior in Subalpine Whitebark Pine Forests, Eastern Sierra Nevada, CA; Retrospective Observations. *Forest Ecology and Management* **447**:195-202.
- Millar, C. I., R. D. Westfall, D. L. Delany, M. J. Bokach, A. L. Flint, and L. E. Flint. 2012. Forest Mortality in High-Elevation Whitebark Pine (*Pinus albicaulis*) Forests of Eastern

- California, USA; Influence of Environmental Context, Bark Beetles, Climatic Water Deficit, and Warming. *Canadian Journal of Forest Research* **42**:749-765.
- Miller, D. R., and J. H. Borden. 2000. Dose-Dependent and Species-Specific Responses of Pine Bark Beetles (Coleoptera: Scolytidae) to Monoterpenes in Association with Pheromones. *The Canadian Entomologist* **132**:183-195.
- Mills, L. S., and D. F. Doak. 1993. The Keystone-Species Concept in Ecology and Conservation. *BioScience* **43**:219-224.
- Mitton, J. B., M. C. Grant, and A. M. Yoshino. 1998. Variation in Allozymes and Stomatal Size in Pinyon (*Pinus edulis*, Pinaceae), Associated with Soil Moisture. *American Journal of Botany* **85**:1262-1265.
- Mizell, R. F., 3rd, J. L. Frazier, and T. E. Nebeker. 1984. Response of the Clerid Predator *Thanasimus dubius* (F.) to Bark Beetle Pheromones and Tree Volatiles in a Wind Tunnel. *Journal of Chemical Ecology* **10**:177-187.
- Moreira, X., K. A. Mooney, S. Rasmann, W. K. Petry, A. Carrillo-Gavilán, R. Zas, L. Sampedro, and V. Novotny. 2014. Trade-Offs Between Constitutive and Induced Defences Drive Geographical and Climatic Clines in Pine Chemical Defences. *Ecology Letters* **17**:537-546.
- Moreira, X., L. Sampedro, R. Zas, and I. S. Pearse. 2016. Defensive Traits in Young Pine Trees Cluster into Two Divergent Syndromes Related to Early Growth Rate. *PLoS One* **11**:e0152537.
- Moreira, X., R. Zas, A. Solla, and L. Sampedro. 2015. Differentiation of Persistent Anatomical Defensive Structures is Costly and Determined by Nutrient Availability and Genetic Growth-Defence Constraints. *Tree Physiology* **35**:112-123.
- Mumm, R., and M. Hilker. 2006. Direct and Indirect Chemical Defence of Pine against Folivorous Insects. *Trends in Plant Science* **11**:351-358.
- Muola, A., P. Mutikainen, L. Laukkanen, M. Lilley, and R. Leimu. 2010. Genetic Variation in Herbivore Resistance and Tolerance: The Role of Plant Life-History Stage and Type of Damage. *Journal of Evolutionary Biology* **23**:2185-2196.
- Nagy, N. E., V. R. Franceschi, H. Solheim, T. Krekling, and E. Christiansen. 2000. Wound-Induced Traumatic Resin Duct Development in Stems of Norway Spruce (Pinaceae): Anatomy and Cytochemical Traits. *American Journal of Botany* **87**:302-313.
- Neale, D. B., and O. Savolainen. 2004. Association Genetics of Complex Traits in Conifers. *Trends in Plant Science* **9**:325-330.
- Paine, T. D., K. F. Raffa, and T. C. Harrington. 1997. Interactions Among Scolytid Bark Beetles, their Associated Fungi, and Live Host Conifers. *Annual Review of Entomology* **42**:179-206.
- Pederson, G. T., J. L. Betancourt, and G. J. McCabe. 2013. Regional Patterns and Proximal Causes of the Recent Snowpack Decline in the Rocky Mountains, U.S. *Geophysical Research Letters* **40**:1811-1816.
- Pederson, G. T., L. J. Graumlich, D. B. Fagre, T. Kipfer, and C. C. Muhlfeld. 2009. A Century of Climate and Ecosystem Change in Western Montana: What do Temperature Trends Portend? *Climatic Change* **98**:133-154.

- Pederson, G. T., S. T. Gray, C. A. Woodhouse, J. L. Betancourt, D. B. Fagre, J. S. Littell, E. Watson, B. H. Luckman, and L. J. Graumlich. 2011. The Unusual Nature of Recent Snowpack Declines in the North American Cordillera. *Science* **333**:332-335.
- Perrakis, D. D. B. 2008. Fire-Bark Beetle Interactions: Exploring Links Between Fire Injury, Resin Defenses, and Beetle-Induced Mortality in Ponderosa Pine Forests. University of Washington.
- Perrakis, D. D. B., and J. K. Agee. 2006. Seasonal Fire Effects on Mixed-Conifer Forest Structure and Ponderosa Pine Resin Properties. *Canadian Journal of Forest Research* **36**:238-254.
- Perrakis, D. D. B., J. K. Agee, and A. Eglitis. 2011. Effects of Prescribed Burning on Mortality and Resin Defenses in Old Growth Ponderosa Pine (Crater Lake, Oregon): Four Years of Post-Fire Monitoring. *Natural Areas Journal* **31**:14-25.
- Phillips, M. A., and R. B. Croteau. 1999. Resin-Based Defenses in Conifers. *Trends in Plant Science - Reviews* **4**:1360-1385.
- Powell, E. N., and K. F. Raffa. 2011. Fire Injury Reduces Inducible Defenses of Lodgepole Pine against Mountain Pine Beetle. *Journal of Chemical Ecology* **37**:1184-1192.
- Quiring, D. T. 1992. Rapid Change in Suitability of White Spruce for a Specialist Herbivore, *Zeiraphera canadensis*, as a Function of Leaf Age. *Canadian Journal of Zoology* **70**:2132-2138.
- Raffa, K. F. 2014. Terpenes Tell Different Tales at Different Scales: Glimpses into the Chemical Ecology of Conifer - Bark Beetle - Microbial Interactions. *Journal of Chemical Ecology* **40**:1-20.
- Raffa, K. F., and A. A. Berryman. 1983. The Role of Host Plant Resistance in the Colonization Behavior and Ecology of Bark Beetles (Coleoptera: Scolytidae). *Ecological Monographs* **53**:27-49.
- Raffa, K. F., C. J. Mason, P. Bonello, S. Cook, N. Erbilgin, K. Keefover-Ring, J. G. Klutsch, C. Villari, and P. A. Townsend. 2017. Defence Syndromes In Lodgepole - Whitebark Pine Ecosystems Relate To Degree Of Historical Exposure To Mountain Pine Beetles. *Plant Cell & Environment* **40**:1791-1806.
- Raffa, K. F., E. N. Powell, and P. A. Townsend. 2013. Temperature-Driven Range Expansion of an Irruptive Insect Heightened by Weakly Coevolved Plant Defenses. *Proceedings of the National Academy of Science* **110**:2193-2198.
- Ranty, B., D. Aldon, V. Cotellet, J.-P. Galaud, P. Thuleau, and C. Mazars. 2016. Calcium Sensors as Key Hubs in Plant Responses to Biotic and Abiotic Stresses. *Frontiers in Plant Science* **7**:327.
- Retzlaff, M. L., S. B. Leirfallom, and R. E. Keane. 2016. A 20-Year Reassessment of the Health and Status of Whitebark Pine Forests in the Bob Marshall Wilderness Complex, Montana, USA. in U. S. D. o. Agriculture, editor. U.S. Forest Service, Rocky Mountain Research Station.
- Rigling, A., H. Brühlhart, O. U. Bräker, F. Theodor, and F. H. Schweingruber. 2003. Effects of Irrigation on Diameter Growth and Vertical Resin Duct Production in *Pinus sylvestris* L. on Dry Sites in the Central Alps, Switzerland. *Forest Ecology and Management* **175**:285-296.

- Rodríguez-García, A., R. López, J. A. Martín, F. Pinillos, and L. Gil. 2014. Resin Yield in *Pinus pinaster* is Related to Tree Dendrometry, Stand Density and Tapping-Induced Systemic Changes in Xylem Anatomy. *Forest Ecology and Management* **313**:47-54.
- Rosner, S., and B. Hannrup. 2004. Resin Canal Traits Relevant for Constitutive Resistance of Norway Spruce Against Bark Beetles: Environmental and Genetic Variability. *Forest Ecology and Management* **200**:77-87.
- Roy, B. A., H. M. Alexander, J. Davidson, F. T. Campbell, J. J. Burdon, R. Snieszko, and C. Brasier. 2014. Increasing Forest Loss Worldwide from Invasive Pests Requires New Trade Regulations. *Frontiers in Ecology and the Environment* **12**:457-465.
- Sala, A., F. Piper, and G. Hoch. 2010. Physiological Mechanisms of Drought-Induced Tree Mortality are Far from Being Resolved. *New Phytologist* **186**:274-281.
- Salminen, J.-P., and M. Karonen. 2011. Chemical Ecology of Tannins and other Phenolics: We Need a Change in Approach. *Functional Ecology* **25**:325-338.
- Schaller, A. 2008. *Induced Plant Resistance to Herbivory*. 1 edition. Springer, Dordrecht, Netherlands.
- Schwandt, J. W., I. B. Lockman, J. T. Kliejunas, and J. A. Muir. 2010. Current Health Issues and Management Strategies for White Pines in the Western United States and Canada. *Forest Pathology* **40**:226-250.
- Seybold, S. J., D. P. W. Huber, J. C. Lee, A. D. Graves, and J. Bohlmann. 2006. Pine Monoterpenes and Pine Bark Beetles: A Marriage of Convenience for Defense and Chemical Communication. *Phytochemistry Reviews* **5**:143-178.
- Siemens, D. H., R. Haugen, S. Matzner, and N. Vanasma. 2009. Plant Chemical Defence Allocation Constrains Evolution of Local Range. *Molecular Ecology* **18**:4974-4983.
- Six, D. L. 2003. Bark Beetle-Fungus Symbioses. Page 368 in K. Bourtzis and T. A. Miller, editors. *Insect Symbiosis*. CRC Press.
- Six, D. L. 2012. Ecological and Evolutionary Determinants of Bark Beetle-Fungus Symbioses. *Insects* **3**:339-366.
- Six, D. L., C. Vergobbi, and M. Cutter. 2018. Are Survivors Different? Genetic-Based Selection of Trees by Mountain Pine Beetle During a Climate Change-Driven Outbreak in a High-Elevation Pine Forest. *Frontiers in Plant Science* **9**:993.
- Smith, R. H. 2000. Xylem Monoterpenes of Pines: Distribution, Variation, Genetics, Function. Pages 1-454 in U. S. D. A. F. Service, editor. *Pacific Southwest Research Station*, Albany, California.
- Smith, T. F., D. M. Rizzo, and M. P. North. 2005. Patterns of Mortality in an Old-Growth Mixed-Conifer Forest of the Southern Sierra Nevada, California. *Forest Science* **51**:266-275.
- Stamp, N. 2003. Out Of The Quagmire Of Plant Defense Hypotheses. *The Quarterly Review of Biology* **78**:23-55.
- Stephens, S. L., and L. W. Ruth. 2005. Federal Forest-Fire Policy in the United States. *Ecological Applications* **15**:532-542.
- Strom, B. L., R. A. Goyer, L. L. Ingram, G. D. L. Boyd, and L. H. Lott. 2002. Oleoresin Characteristics of Progeny of Loblolly Pines that Escaped Attack by the Southern Pine Beetle. *Forest Ecology and Management* **158**:169-178.
- Tomback, D. F., and P. Achuff. 2010. Blister Rust and Western Forest Biodiversity: Ecology, Values and Outlook for White Pines. *Forest Pathology* **40**:186-225.

- Tomback, D. F., S. F. Arno, and R. E. Keane. 2001. Whitebark Pine Communities: Ecology and Restoration. Island Press, Washington, D.C.
- Tomback, D. F., and K. C. Kendall. 2001. Biodiversity Losses: The Downward Spiral. Pages 243-262 in D. F. Tomback, S. F. Arno, and R. E. Keane, editors. Whitebark Pine Communities: Ecology and Restoration. Island Press, Washington, DC.
- Tomback, D. F., L. M. Resler, R. E. Keane, E. R. Pansing, A. J. Andrade, and A. C. Wagner. 2016. Community Structure, Biodiversity, and Ecosystem Services in Treeline Whitebark Pine Communities: Potential Impacts from a Non-Native Pathogen. *Forests* **7**:21.
- Tomback, D. F., S. K. Sund, and L. A. Hoffmann. 1993. Post-Fire Regeneration of *Pinus albicaulis*: Height–Age Relationships, Age Structure, and Microsite Characteristics. *Canadian Journal of Forest Research* **23**:113-119.
- Trapp, S., and R. Croteau. 2001. Defensive Resin Biosynthesis in Conifers. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**:689-724.
- Trowbridge, A. M. 2014. Evolutionary Ecology of Chemically Mediated Plant-Insect Interactions. Pages 143-176 *Ecology and the Environment*.
- van Mantgem, P. J., and N. L. Stephenson. 2007. Apparent Climatically Induced Increase of Tree Mortality Rates in a Temperate Forest. *Ecology Letters* **10**:909-916.
- van Mantgem, P. J., N. L. Stephenson, J. C. Byrne, L. D. Daniels, J. F. Franklin, P. Z. Fulé, M. E. Harmon, A. J. Larson, J. M. Smith, A. H. Taylor, and T. T. Veblen. 2009. Widespread Increase of Tree Mortality Rates in the Western United States. *Science* **323**:521-524.
- Vázquez-González, C., L. Sampedro, V. Rozas, J. Voltas, and R. Zas. 2021. Population Differentiation in Climate Sensitivity of Resin Duct Formation during Growth Resumption in *Pinus pinaster*. *Dendrochronologia* **67**.
- Veblen, T. T., K. S. Hadley, E. M. Nel, T. Kitzberger, M. Reid, and R. Villalba. 1994. Disturbance Regime and Disturbance Interactions in a Rocky Mountain Subalpine Forest. *The Journal of Ecology* **82**.
- Wainhouse, D., J. T. Staley, R. Jinks, and G. Morgan. 2009. Growth and Defence in Young Pine and Spruce and the Expression of Resistance to a Stem-Feeding Weevil. *Oecologia* **158**:641-650.
- Warren, J. M., H. L. Allen, and F. L. Booker. 1999. Mineral Nutrition, Resin Flow and Phloem Phytochemistry in Loblolly Pine. *Tree Physiology* **19**:655-663.
- Warwell, M. V., and R. G. Shaw. 2017. Climate-Related Genetic Variation in a Threatened Tree Species, *Pinus albicaulis*. *American Journal of Botany* **104**:1205-1218.
- Westbrook, J. W., A. R. Walker, L. G. Neves, P. Munoz, M. F. Resende, Jr., D. B. Neale, J. L. Wegrzyn, D. A. Huber, M. Kirst, J. M. Davis, and G. F. Peter. 2015. Discovering Candidate Genes that Regulate Resin Canal Number in *Pinus taeda* Stems by Integrating Genetic Analysis across Environments, Ages, and Populations. *New Phytologist* **205**:627-641.
- Westerling, A. L., H. G. Hidalgo, D. R. Cayan, and T. W. Swetnam. 2006. Warming and Earlier Spring Increase Western US Forest Wildfire Activity. *Science* **313**:940-943.
- Williams, D. W., and A. M. Liebhold. 2002. Climate Change and the Outbreak Ranges of Two North American Bark Beetles. *Agricultural and Forest Entomology* **4**:87-99.
- Wimmer, R., and M. Grabner. 1997. Effects of Climate on Vertical Resin Duct Density and Radial Growth of Norway Spruce (*Picea abies*). *Trees* **11**:271-276.
- Wu, H., and Z.-h. Hu. 1997. Comparative Anatomy of Resin Ducts of the Pinaceae. *Trees* **11**.

- Zhao, T., P. Krokene, N. Björklund, B. Långström, H. Solheim, E. Christiansen, and A.-K. Borg-Karlson. 2010. The Influence of *Ceratocystis polonica* Inoculation and Methyl Jasmonate Application on Terpene Chemistry of Norway spruce, *Picea abies*. *Phytochemistry* **71**:1332-1341.

APPENDICES

APPENDIX A

SUPPLEMENTARY DATA FOR CHAPTER 3 “WHITEBARK PINE (*PINUS ALBICAULIS*)
GROWTH AND DEFENSE IN RESPONSE TO MOUNTAIN PINE BEETLE OUTBREAKS”

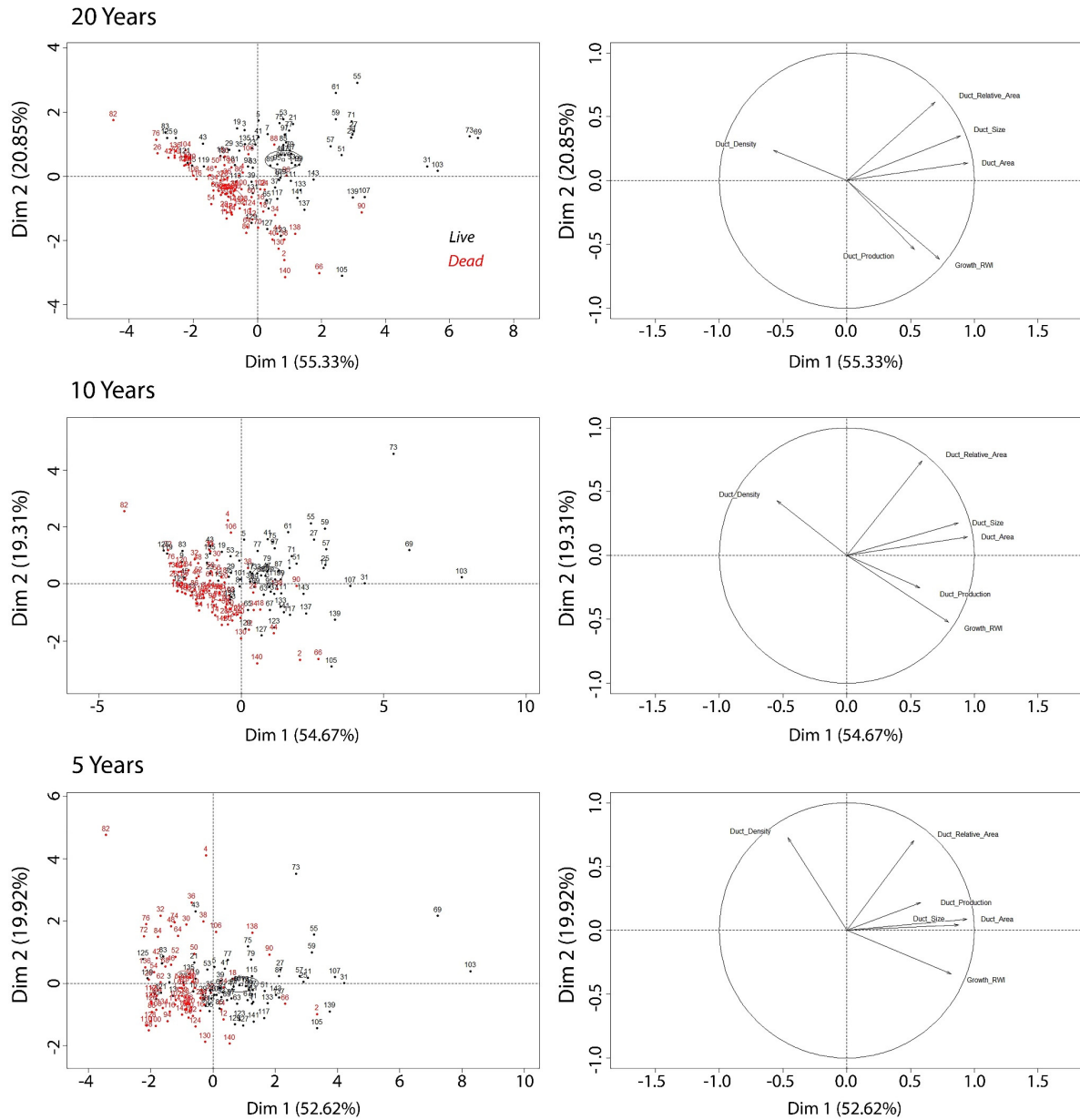


Figure S1. Principal components analysis of live and dead whitebark pine (144 samples, 72 live, 72 dead) for the 20–, 10– and 5–year windows prior to mortality of the dead pair. Ellipses are 95th percentile confidence intervals for each grouping (live - black; dead - red). Variables factor maps (right panels) illustrate the relative contribution of growth and defense metrics to the ordinations.

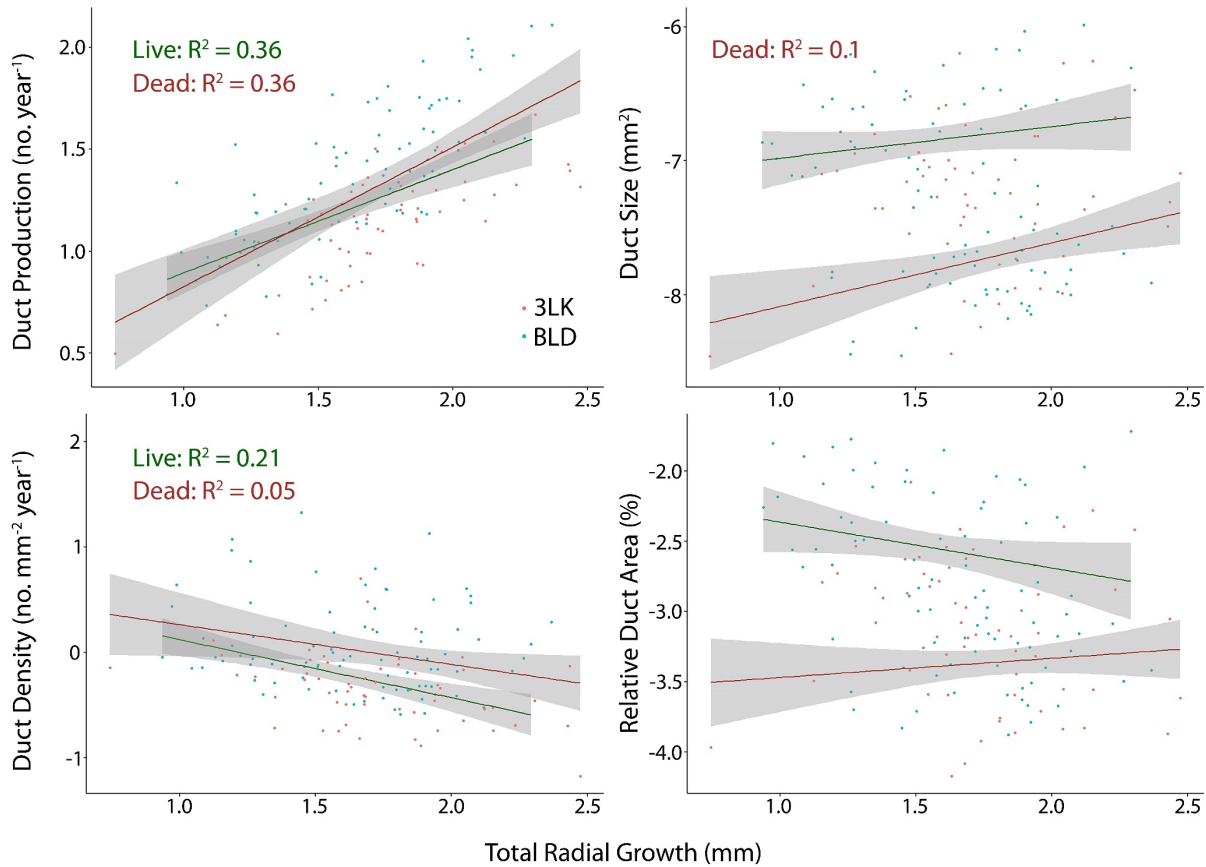


Figure S2. Relationship of average vertical resin ducts metrics and growth for live and dead whitebark pine across the full time period. Pairs of live and dead whitebark pine are color-coded by site: 3LK (magenta) and BLD (cyan).

Note: variables were log-transformed to meet the assumptions of linear regression.

Table S1. Generalized linear models predicting survival of whitebark pine during disturbance outbreaks. Log likelihood reflects the probability of the data given the model, while K represents the relative number of sample parameters for each model. Top five models are shown out of ten models developed.

Model	AIC	Δ AIC	LogL	K
5year_Size + Total_Relative_Area	88.2	0	-41.1	2
5year_Size + Total_Relative_Area + 5year_Area	89.7	1.4	-40.8	3
10year_Size + Total_Relative_Area + 5year_Area + 5year_Series	95.5	7.3	-42.8	4
10year_Size + Total_Relative_Area	97.1	8.8	-42.5	2
10year_Size + Total_Relative_Area + 5year_Area	98.1	9.9	-45.1	3

Abbreviations: Area (resin duct area); Relative_Area (relative resin duct area); Size (resin duct size); Series (ring width index); 5year (5-year window prior to mortality); 10year (10-year window); Total (all overlapping years).

Table S2. Pearson's product moment correlation coefficients for growth and defense metrics and climate variables across both live and dead whitebark pine. Correlations were assessed across the full chronology (1911–2004; 93 years) as well as an early 20th century period (1911–1960; 49 years) and late 20th century period (1961–2004; 43 years). Climate data was obtained from PRISM at a spatial resolution of 4 km using the AN version of the dataset. Winter: previous December, January, February, March / Spring: April, May / Summer: June, July, August

	Time Period		
	Full (1911–2004)	1911–1960	1961–2004
Growth			
<u>Winter Precip</u>			
Live	<i>–0.41</i> (<i>p</i> < 0.0001)	–0.18 (<i>p</i> = 0.213)	<i>–0.57</i> (<i>p</i> < 0.0001)
Dead	–0.01 (<i>p</i> = 0.949)	–0.01 (<i>p</i> = 0.962)	0.08 (<i>p</i> = 0.605)
<u>Winter z–PDSI</u>			
Live	<i>–0.38</i> (<i>p</i> = 0.0002)	–0.28 (<i>p</i> = 0.05)	<i>–0.48</i> (<i>p</i> = 0.0009)
Dead	–0.09 (<i>p</i> = 0.379)	–0.11 (<i>p</i> = 0.469)	0.04 (<i>p</i> = 0.795)
<u>Spring Precip</u>			
Live	0.01 (<i>p</i> = 0.944)	–0.19 (<i>p</i> = 0.169)	0.12 (<i>p</i> = 0.446)
Dead	<i>–0.22</i> (<i>p</i> = 0.03)	–0.19 (<i>p</i> = 0.182)	–0.06 (<i>p</i> = 0.683)
<u>Spring Temp</u>			
Live	<i>0.36</i> (<i>p</i> = 0.0003)	<i>0.41</i> (<i>p</i> = 0.003)	<i>0.39</i> (<i>p</i> = 0.008)
Dead	<i>0.19</i> (<i>p</i> = 0.05)	<i>0.33</i> (<i>p</i> = 0.019)	0.1 (<i>p</i> = 0.517)
<u>Spring z–PDSI</u>			
Live	–0.11 (<i>p</i> = 0.312)	<i>–0.31</i> (<i>p</i> = 0.029)	–0.01 (<i>p</i> = 0.95)
Dead	<i>–0.26</i> (<i>p</i> = 0.013)	–0.27 (<i>p</i> = 0.061)	–0.1 (<i>p</i> = 0.517)
<u>Summer Precip</u>			
Live	0.09 (<i>p</i> = 0.353)	0.11 (<i>p</i> = 0.465)	0.07 (<i>p</i> = 0.655)
Dead	–0.06 (<i>p</i> = 0.557)	0.07 (<i>p</i> = 0.626)	0.05 (<i>p</i> = 0.761)
<u>Summer z–PDSI</u>			
Live	0.07 (<i>p</i> = 0.516)	–0.03 (<i>p</i> = 0.843)	0.1 (<i>p</i> = 0.509)
Dead	–0.1 (<i>p</i> = 0.33)	–0.01 (<i>p</i> = 0.95)	0.02 (<i>p</i> = 0.903)
Duct Production			
<u>Winter Precip</u>			
Live	<i>–0.27</i> (<i>p</i> = 0.009)	–0.22 (<i>p</i> = 0.12)	<i>–0.47</i> (<i>p</i> = 0.001)
Dead	–0.14 (<i>p</i> = 0.183)	–0.22 (<i>p</i> = 0.12)	–0.05 (<i>p</i> = 0.762)
<u>Winter z–PDSI</u>			
Live	<i>–0.33</i> (<i>p</i> = 0.001)	<i>–0.3</i> (<i>p</i> = 0.036)	<i>–0.44</i> (<i>p</i> = 0.003)
Dead	<i>–0.23</i> (<i>p</i> = 0.028)	<i>–0.3</i> (<i>p</i> = 0.034)	–0.15 (<i>p</i> = 0.33)
<u>Spring Precip</u>			
Live	<i>–0.21</i> (<i>p</i> = 0.04)	–0.18 (<i>p</i> = 0.204)	0.02 (<i>p</i> = 0.899)
Dead	<i>–0.27</i> (<i>p</i> = 0.008)	–0.23 (<i>p</i> = 0.104)	–0.14 (<i>p</i> = 0.371)
<u>Spring Temp</u>			
Live	0.11 (<i>p</i> = 0.29)	0.04 (<i>p</i> = 0.802)	0.26 (<i>p</i> = 0.087)
Dead	0.09 (<i>p</i> = 0.386)	0.09 (<i>p</i> = 0.514)	0.02 (<i>p</i> = 0.88)
<u>Spring z–PDSI</u>			
Live	<i>–0.22</i> (<i>p</i> = 0.035)	–0.17 (<i>p</i> = 0.229)	–0.06 (<i>p</i> = 0.678)
Dead	<i>–0.27</i> (<i>p</i> = 0.008)	–0.25 (<i>p</i> = 0.086)	–0.15 (<i>p</i> = 0.34)
<u>Summer Precip</u>			
Live	<i>–0.26</i> (<i>p</i> = 0.012)	–0.22 (<i>p</i> = 0.134)	–0.23 (<i>p</i> = 0.137)
Dead	–0.16 (<i>p</i> = 0.112)	–0.09 (<i>p</i> = 0.516)	–0.01 (<i>p</i> = 0.943)

Table S2 Continued

<u>Summer z-PDSI</u>			
<i>Live</i>	-0.28 (p = 0.006)	-0.25 (p = 0.08)	-0.23 (p = 0.13)
<i>Dead</i>	-0.19 (p = 0.067)	-0.14 (p = 0.344)	-0.05 (p = 0.759)
Duct Size			
<u>Winter Precip</u>			
<i>Live</i>	-0.26 (p = 0.01)	-0.06 (p = 0.674)	-0.46 (p = 0.002)
<i>Dead</i>	0.13 (p = 0.207)	0.19 (p = 0.182)	0.22 (p = 0.146)
<u>Winter z-PDSI</u>			
<i>Live</i>	-0.26 (p = 0.011)	-0.17 (p = 0.241)	-0.42 (p = 0.005)
<i>Dead</i>	-0.002 (p = 0.988)	0.04 (p = 0.78)	0.12 (p = 0.429)
<u>Spring Precip</u>			
<i>Live</i>	0.06 (p = 0.597)	-0.21 (p = 0.14)	0.15 (p = 0.324)
<i>Dead</i>	-0.24 (p = 0.019)	-0.23 (p = 0.105)	-0.08 (p = 0.628)
<u>Spring Temp</u>			
<i>Live</i>	0.14 (p = 0.187)	0.24 (p = 0.089)	0.12 (p = 0.447)
<i>Dead</i>	0.11 (p = 0.294)	0.21 (p = 0.136)	-0.05 (p = 0.73)
<u>Spring z-PDSI</u>			
<i>Live</i>	0.01 (p = 0.928)	-0.24 (p = 0.089)	0.09 (p = 0.54)
<i>Dead</i>	-0.25 (p = 0.017)	-0.26 (p = 0.072)	-0.07 (p = 0.631)
<u>Summer Precip</u>			
<i>Live</i>	0.04 (p = 0.717)	-0.02 (p = 0.88)	-0.01 (p = 0.938)
<i>Dead</i>	-0.19 (p = 0.063)	-0.2 (p = 0.159)	-0.05 (p = 0.754)
<u>Summer z-PDSI</u>			
<i>Live</i>	0.01 (p = 0.894)	-0.08 (p = 0.586)	-0.02 (p = 0.902)
<i>Dead</i>	-0.24 (p = 0.023)	-0.27 (p = 0.06)	-0.08 (p = 0.59)
Duct Area			
<u>Winter Precip</u>			
<i>Live</i>	-0.4 (p < 0.0001)	-0.23 (p = 0.103)	-0.52 (p = 0.0003)
<i>Dead</i>	-0.06 (p = 0.546)	-0.17 (p = 0.251)	0.14 (p = 0.368)
<u>Winter z-PDSI</u>			
<i>Live</i>	-0.45 (p < 0.0001)	-0.39 (p = 0.005)	-0.49 (p = 0.0008)
<i>Dead</i>	-0.19 (p = 0.068)	-0.38 (p = 0.007)	0.05 (p = 0.771)
<u>Spring Precip</u>			
<i>Live</i>	-0.17 (p = 0.11)	-0.37 (p = 0.009)	0.07 (p = 0.661)
<i>Dead</i>	-0.34 (p = 0.0008)	-0.51 (p = 0.0002)	-0.09 (p = 0.542)
<u>Spring Temp</u>			
<i>Live</i>	0.24 (p = 0.019)	0.23 (p = 0.115)	0.27 (p = 0.08)
<i>Dead</i>	0.14 (p = 0.175)	0.31 (p = 0.027)	-0.04 (p = 0.781)
<u>Spring z-PDSI</u>			
<i>Live</i>	-0.22 (p = 0.037)	-0.37 (p = 0.008)	-0.02 (p = 0.88)
<i>Dead</i>	-0.34 (p = 0.0007)	-0.54 (p < 0.0001)	-0.09 (p = 0.541)
<u>Summer Precip</u>			
<i>Live</i>	-0.16 (p = 0.123)	-0.19 (p = 0.166)	-0.09 (p = 0.575)
<i>Dead</i>	-0.23 (p = 0.025)	-0.34 (p = 0.017)	-0.002 (p = 0.985)
<u>Summer z-PDSI</u>			
<i>Live</i>	-0.2 (p = 0.052)	-0.29 (p = 0.036)	-0.08 (p = 0.584)
<i>Dead</i>	-0.27 (p = 0.008)	-0.44 (p = 0.001)	-0.04 (p = 0.817)

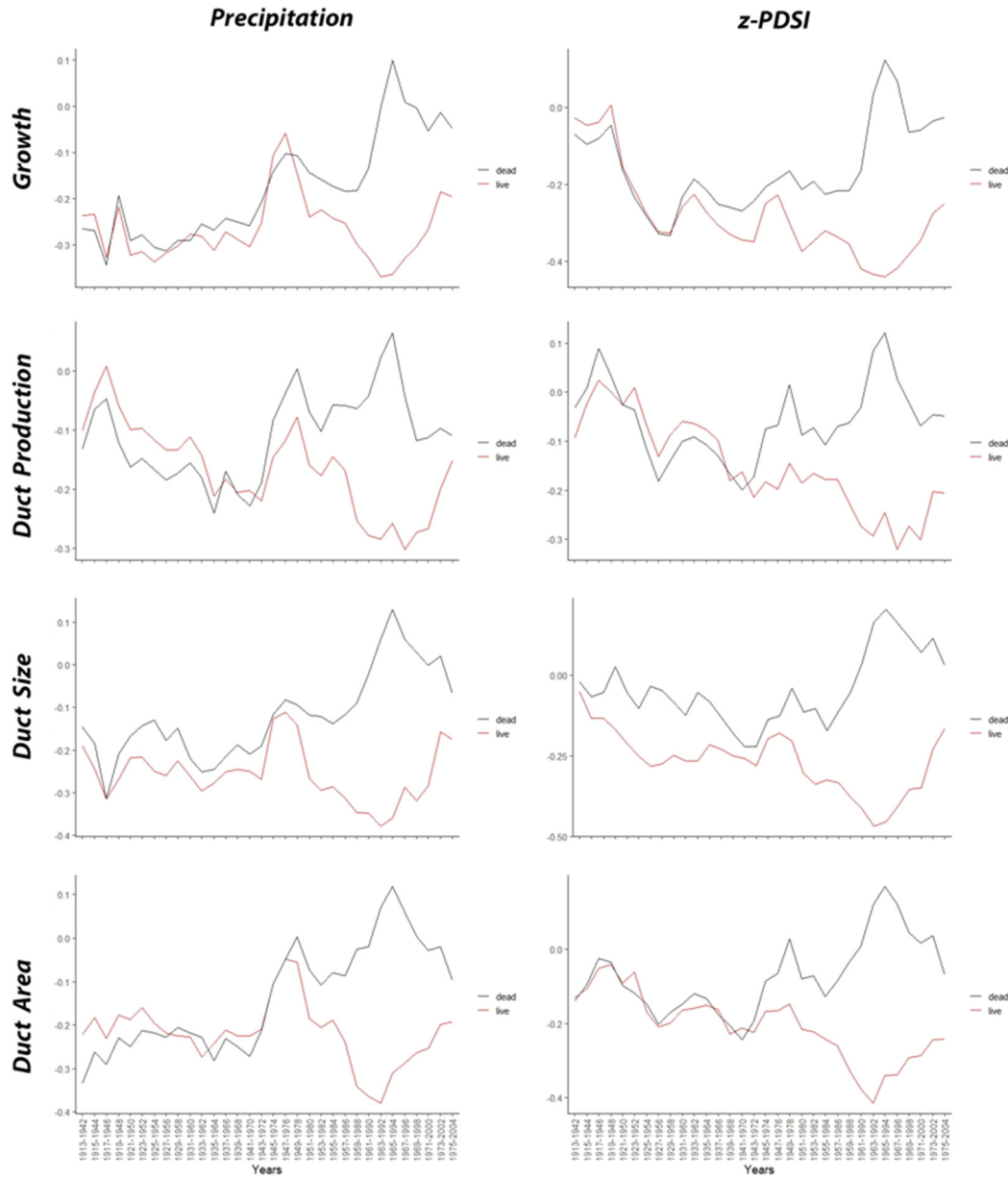
Winter (DJFM)

Figure S3. 30-year moving correlations with a two-year time-step for growth and defense metrics, winter precipitation (left), and winter z-PDSI (right) for live (red) and dead (black) whitebark pine.

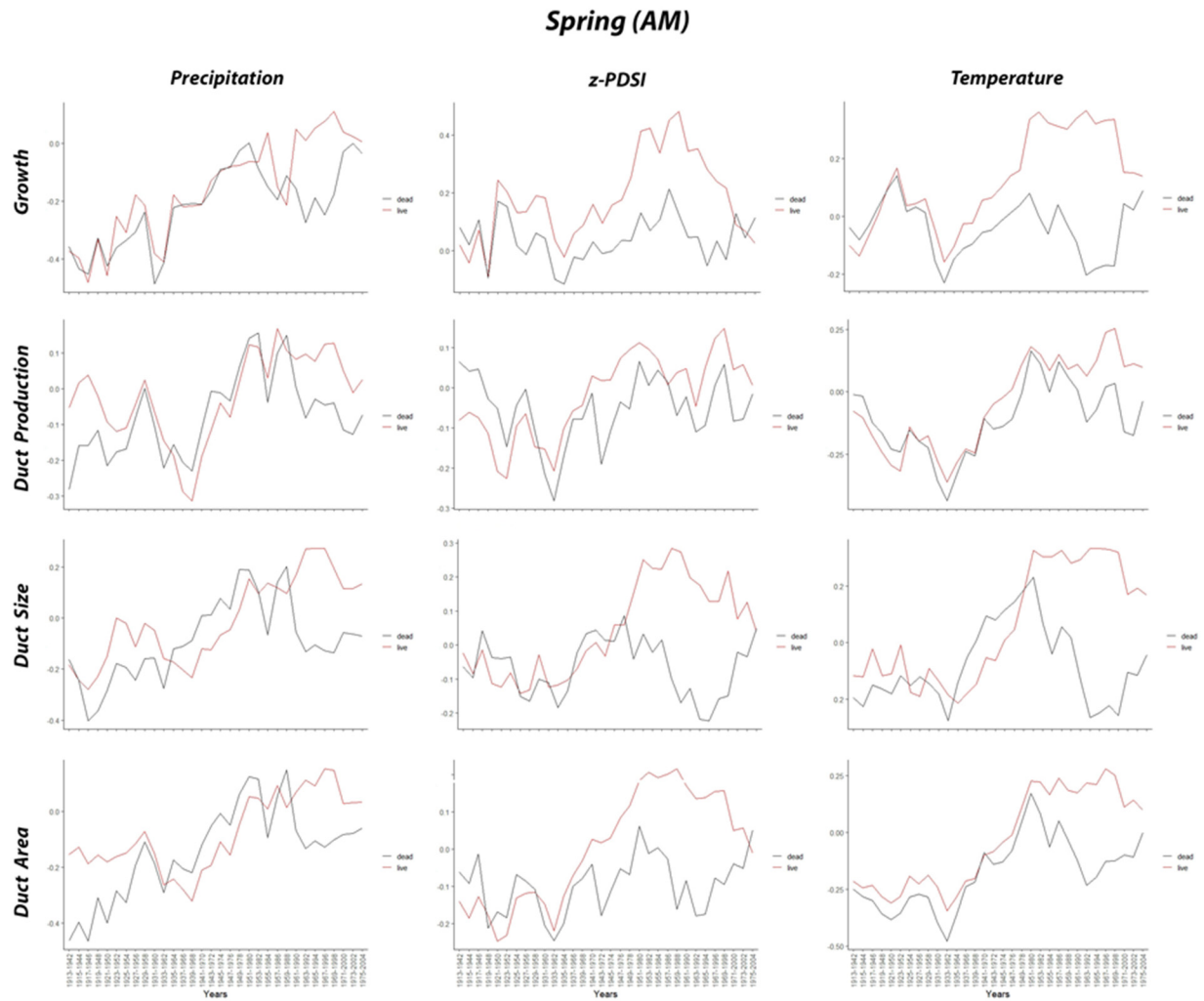


Figure S4. 30-year moving correlations with a two-year time-step for growth and defense metrics, spring precipitation (left), spring z-PDSI (right), and spring temperature (right) for live (red) and dead (black) whitebark pine.

APPENDIX B

SUPPLEMENTARY DATA FOR CHAPTER 4 “GROWTH AND DEFENSE
CHARACTERISTICS OF WHITEBARK PINE (*PINUS ALBICAULIS*) AND LODGEPOLE
PINE (*PINUS CONTORTA* VAR *LATIFOLIA*) IN A HIGH-ELEVATION, DISTURBANCE-
PRONE MIXED-CONIFER FOREST IN NORTHWESTERN MONTANA, USA”

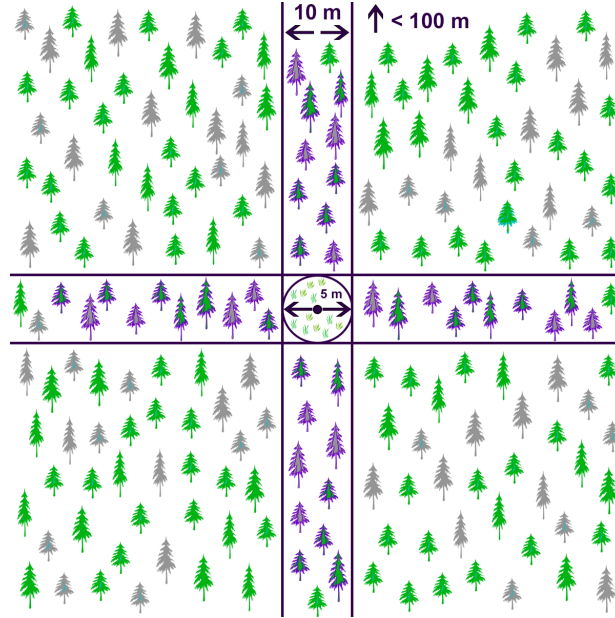


Figure S1. Conceptual diagram of the plot sampling protocol. From plot center, four 10m wide belt transects were extended in each cardinal direction and the closest 10 trees (> 15 cm DBH) in each transect were sampled (to a maximum distance of 100 m). A 5.6 m diameter plot was established around plot center to measure understory vegetation. Coloring: Live trees (green); dead trees (grey); sampled trees for fire history study (purple highlighting). From Kichas et al. (2020).

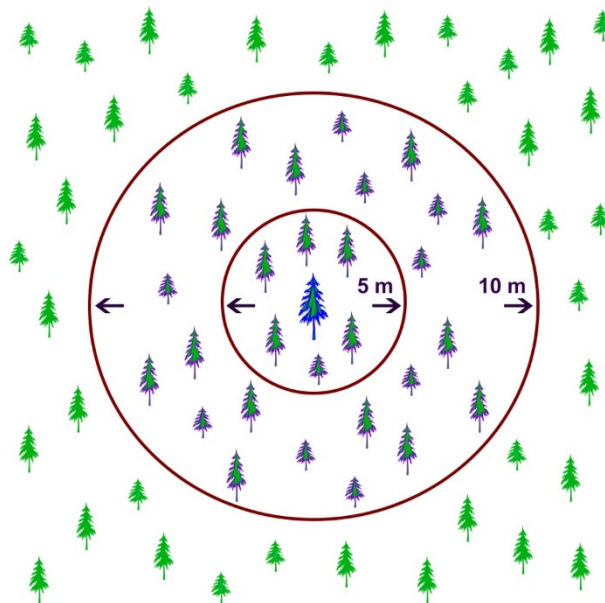


Figure S2. Conceptual diagram of sampling design. Individual whitebark (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*) trees (blue outline) were located and increment cores and phloem tissues obtained for growth and defense analyses. Overstory competition around

each subject tree was also recorded (species and DBH information) for all live / dead trees (≥ 1 cm DBH) within 5 m and all trees (≥ 5 cm DBH) to a distance of 10 m. Understory species and percent cover estimates were also made within a smaller 5.6 m diameter plot around each subject tree.

Table S1. Spearman correlation coefficients of mono- and sesqui- terpene concentrations ($\mu\text{g} / \text{g}$ fresh weight) with tree ring growth and resin duct anatomical measurements for whitebark (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*) during the 20-year period prior to sampling (1998–2018). Correlations were conducted on metrics averaged over the 20-year period.

	Monoterpenes		Sesquiterpenes	
	<i>Pinus albicaulis</i>	<i>Pinus contorta</i>	<i>Pinus albicaulis</i>	<i>Pinus contorta</i>
Growth (BAI)	0.16	0.22	0.27	0.22
Duct Production	0.13	−0.08	0.22	−0.12
Duct Size	0.21	0.17	0.45	0.14
Duct Area	0.27	0.11	0.48	0.05
Duct Density	0.32	−0.09	−0.08	−0.18
Relative Duct Area	0.32	−0.08	−0.003	−0.25

Note: Bold values indicate significant correlations ($p < 0.025$). DBH = diameter at breast height; BAI = basal area increment.

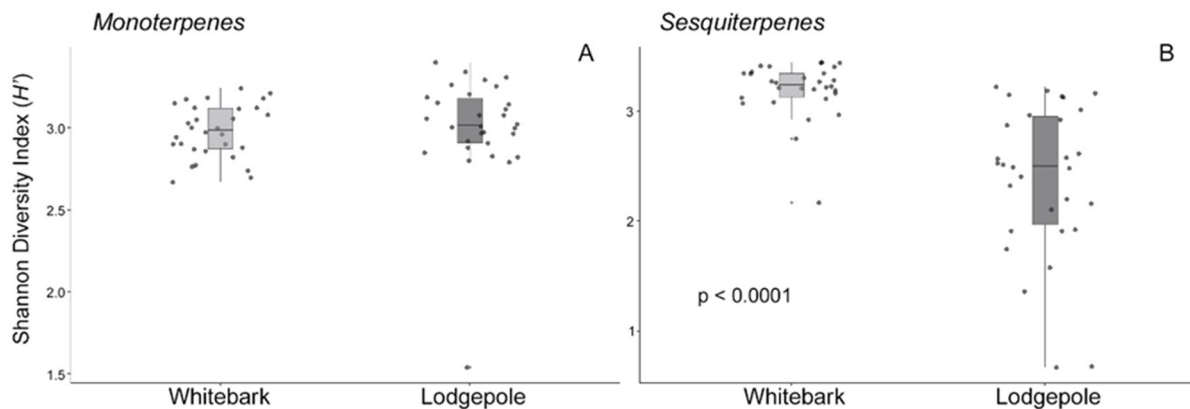


Figure S3. Constitutive monoterpene diversity (calculated as Shannon's H' , a measure of within-sample diversity; A) and constitutive sesquiterpene diversity (B) for whitebark (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*).

Table S2. Spearman correlation coefficients of mono- and sesqui- terpene diversity (calculated as Shannon's H' , a measure of within-sample diversity) with tree ring growth and resin duct anatomical measurements for whitebark (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*). Correlations were conducted on metrics averaged over the full record (1914–2018).

	Monoterpene Diversity		Sesquiterpene Diversity	
	<i>Pinus albicaulis</i>	<i>Pinus contorta</i>	<i>Pinus albicaulis</i>	<i>Pinus contorta</i>
Growth (BAI)	0.33	0.06	0.16	0.22
Duct Production	0.43	-0.18	0.12	0.08
Duct Size	0.17	-0.03	0.17	0.09
Duct Area	0.37	-0.12	0.22	0.11
Duct Density	0.12	0.02	0.07	< 0.01
Relative Duct Area	0.08	-0.06	0.22	-0.14

Note: Bold values indicate significant correlations ($p < 0.05$). DBH = diameter at breast height; BAI = basal area increment.

Table S3. Major monoterpenes and sesquiterpenes ($\mu\text{g} / \text{g}$ fresh weight) collected from phloem tissues of whitebark (*P. albicaulis*) and lodgepole pine (*P. contorta* var. *latifolia*) sampled from a high elevation mixed-conifer forest stand on the Flathead Indian Reservation. Shading of individual compounds reflects significant difference ($\alpha = 0.05$) between the two species (light grey = greater concentration in whitebark pine / dark grey = greater concentration in lodgepole pine). Contribution refers to the relative percent contribution of each compound to the total monoterpene or sesquiterpene profile for each species.

No.	Compound	ID*	<i>Pinus albicaulis</i>		Contri bution	<i>Pinus contorta</i>		Contri bution	Kruskal- Wallis Test
			Mean	S.E.		Mean	S.E.		p-value
<i>Monoterpenes (Hydrocarbons)</i>									
1	α -thujene	NIST _{>80}	21.32	4.85	0.25	9.43	1.09	0.16	36.183
2	(-)- α -pinene	SS	676.31	141.41	7.99	113.46	12.49	1.93	< 0.001
3	(+)- α -pinene	SS	399.72	86.57	4.72	48.51	4.81	0.83	0.017
4	MTP 1 [†]	NIST	n.d.	n.d.	n.d.	1.97	0.59	0.03	0.026
5	MTP 2 [†]	NIST	29.69	10.92	0.35	10.65	1.00	0.18	18.848
6	MTP 3 [†]	NIST	3.73	0.88	0.04	6.18	0.63	0.11	< 0.001
7	MTP 4 [†]	NIST	3.46	1.62	0.04	4.95	1.63	0.08	22.284
8	(-)-sabinene	NIST _{>80}	2.25	0.47	0.03	10.87	1.51	0.18	< 0.001
9	(+)-sabinene	NIST _{>80}	332.22	111.56	3.93	21.15	2.79	0.36	< 0.001
10	β -myrcene	SS	407.72	69.53	4.82	90.28	13.44	1.54	0.004
11	(+)- β -pinene	SS	10.01	2.05	0.12	3.99	0.54	0.07	0.979
12	(-)- β -pinene	SS	656.45	97.16	7.76	412.79	76.55	7.02	14.104
13	3-carene	SS	724.58	158.63	8.56	243.68	73.62	4.15	27.835
14	α -phellandrene	NIST _{>80}	7.62	1.85	0.09	51.53	5.20	0.88	< 0.001
15	α -terpinene	NIST _{>80}	12.89	2.87	0.15	6.35	0.97	0.11	9.589
16	(-)- δ -limonene	SS	1413.29	235.07	16.70	293.93	101.08	5.00	< 0.001
17	(+)- δ -limonene	SS	3.08	1.40	0.04	17.44	1.98	0.30	< 0.001
18	β -phellandrene	NIST _{>80}	3059.84	355.55	36.16	4411.69	398.14	75.05	< 0.001
19	β -ocimene	SS	423.81	49.17	5.01	4.57	1.86	0.08	< 0.001
20	γ -terpinene	SS	16.01	3.59	0.19	4.69	0.69	0.08	0.038
21	MTP 5 [†]	NIST	0.42	0.19	< 0.00	0.32	0.26	0.01	10.422
22	terpinolene	SS	176.65	41.16	2.09	72.67	10.74	1.24	2.562
23	ρ -cymenene	NIST _{>80}	1.81	0.91	0.02	0.05	0.05	0.001	0.232

Table S3 Continued

<i>Monoterpenes (Oxygenated Monoterpenes)</i>									
24	MTP 6 [†]	NIST	0.006	0.002	< 0.00	0.001	0.001	< 0.00	1.248
25	cis- β -terpineol	NIST _{>80}	0.01	0.003	< 0.00	0.002	0.001	< 0.00	1.303
26	(-)-linalool	SS	5.76	2.09	0.07	6.29	2.91	0.11	39.413
27	(+)-linalool	NIST _{>80}	1.84	0.51	0.02	3.26	0.69	0.06	1.735
28	citronellal	NIST _{>80}	0.83	0.36	0.01	8.67	3.92	0.15	2.58
29	trans-2-menthenol	NIST _{>80}	1.12	0.46	0.01	1.12	0.34	0.02	10.619
30	dill ether	NIST _{>80}	0.09	0.09	< 0.00	2.89	0.53	0.05	< 0.001
31	MTP 7 [†]	NIST	0.69	0.34	0.01	0.41	0.18	0.01	38.397
32	MTP 8 [†]	NIST	1.00	0.47	0.01	0.17	0.12	< 0.00	9.123
33	Anisole	NIST _{>80}	22.65	3.62	0.27	2.29	1.01	0.04	< 0.001
34	(-)-terpinene-4-ol	NIST _{>80}	0.87	0.32	0.01	0.45	0.19	0.01	21.455
35	(+)-terpinene-4-ol	NIST _{>80}	2.93	1.27	0.03	1.13	0.43	0.02	38.073
36	MTP 9 [†]	NIST	4.71	1.04	0.06	1.10	0.36	0.02	0.059
37	MTP 10 [†]	NIST	0.29	0.17	< 0.00	0.94	0.53	0.02	16.478
38	(-)- α -terpineol	NIST _{>80}	0.93	0.47	0.01	0.75	0.45	0.01	33.194
39	(+)- α -terpineol	NIST _{>80}	0.45	0.25	0.01	0.26	0.17	< 0.00	27.523
40	α -longipinene	NIST _{>80}	30.58	5.36	0.36	3.96	0.99	0.07	0.003
41	bornyl acetate	SS	5.18	1.15	0.06	3.15	0.88	0.05	6.302
<i>Phenolics</i>									
42	4-allylanisole	NIST _{>80}	4.52	1.01	0.05	45.74	8.27	0.78	< 0.001
<i>Sesquiterpenes</i>									
43	α -cubebene	NIST _{>80}	58.54	7.69	2.81	5.69	1.27	0.7	< 0.001
44	α -copaene	NIST _{>80}	152.85	22.89	6.71	7.11	1.59	0.82	< 0.001
45	SQT 1 [†]	NIST	10.27	7.11	0.38	0.28	0.28	0.04	0.004
46	SQT 2 [†]	NIST	4.8	0.95	0.18	2.93	0.93	0.19	19.909
47	longifolene	NIST _{>80}	46.53	11.66	2.64	0	0	0	< 0.001
48	SQT 3 [†]	NIST	66.49	10.23	2.85	0.48	0.23	0.02	< 0.001
49	caryophellene	SS	50.27	6.534	2.3	2.56	0.68	0.58	< 0.001
50	α -guaiene	NIST _{>80}	4.06	2.286	0.16	0	0	0	0.012
51	SQT 4 [†]	NIST	0.15	0.147	0.01	3.34	1.05	0.41	0.084
52	cis- β -farnesene	NIST _{>80}	27.94	3.667	1.39	0.08	0.08	< 0.01	< 0.001
53	humulene	NIST _{>80}	18.88	2.491	0.84	1.16	0.53	0.09	< 0.001
54	(-)- γ -muroloene	NIST _{>80}	16.18	2.666	0.81	2.88	0.91	0.18	< 0.001
55	(+)- γ -muroloene	NIST _{>80}	66.15	9.5	2.86	10.98	2.47	1.06	< 0.001
56	SQT 5 [†]	NIST	5.12	0.889	0.2	0	0	0	< 0.001
57	α -muuroloene	NIST _{>80}	890.25	118.417	40.18	51.03	10.42	6.56	< 0.001
58	γ -elemene	NIST _{>80}	51.61	7.971	2.47	6.94	2.16	0.63	< 0.001
59	SQT 6 [†]	NIST	0	0	0	7.07	1.93	7.44	0.001
60	SQT 7 [†]	NIST	8.47	1.613	0.46	0	0	0	< 0.001
61	SQT 8 [†]	NIST	33.41	5.185	1.43	81.06	17.81	9.66	< 0.001
62	SQT 9 [†]	NIST	53.17	8.489	2.33	103.51	22.95	14.89	< 0.001
63	SQT 10 [†]	NIST	6.78	3.069	0.28	6.67	2.08	1.46	0.926
64	SQT 11 [†]	NIST	2.95	2.951	0.13	5.84	3.01	0.68	0.04
65	SQT 12 [†]	NIST	9.57	3.461	0.37	5.02	1.47	0.37	17.23
66	cubedol	NIST _{>80}	134.93	18.563	6.46	25.08	5.36	2.76	< 0.001
67	SQT 13 [†]	NIST	2.95	2.951	0.13	1.79	0.57	0.11	0.332
68	SQT 14 [†]	NIST	0.18	0.176	0.01	1.30	0.47	0.07	0.805
69	nerolidol	NIST _{>80}	12.55	1.659	0.53	0	0	0	< 0.001
70	cubebol	NIST _{>80}	12.82	1.923	0.55	7.11	1.76	0.6	31.383

Table S3 Continued

71	SQT 15 [†]	NIST	217.89	37.567	9.73	294.31	64.39	37.97	< 0.001
72	SQT 16 [†]	NIST	3.25	0.892	0.11	0.09	0.09	0.01	0.022
73	SQT 17 [†]	NIST	9.56	1.696	0.42	0	0	0	< 0.001
74	SQT 18 [†]	NIST	4.02	1.452	0.15	1.45	0.52	0.09	12.488
75	SQT 19 [†]	NIST	14.83	1.707	0.71	0	0	0	< 0.001
76	SQT 20 [†]	NIST	6.76	1.834	0.32	0.36	0.36	0.02	0.026
77	(-)-copaene	NIST _{>80}	88.65	12.188	3.87	17.04	3.61	2.19	< 0.001
78	(+)-copaene	NIST _{>80}	34.15	4.345	1.83	19.47	3.64	3.58	1.136
79	α -cadinol	NIST _{>80}	14.83	2.425	0.68	21.69	4.80	2.88	0.348
80	α -bisabolol	NIST _{>80}	46.05	9.861	2.3	5.37	1.23	3.95	15.417
81	SQT 21 [†]	NIST	7.31	1.379	0.44	0	0	0	< 0.001

* Identification (ID) of compounds based upon comparison of retention time and mass spectra with synthetic standards (SS) or comparison of mass spectra using NIST Mass Spectral Search Program (NIST); NIST_{>80} = Denotes NIST match quality greater than 80

[†]MTP = unidentified monoterpene; SQT = unidentified sesquiterpene

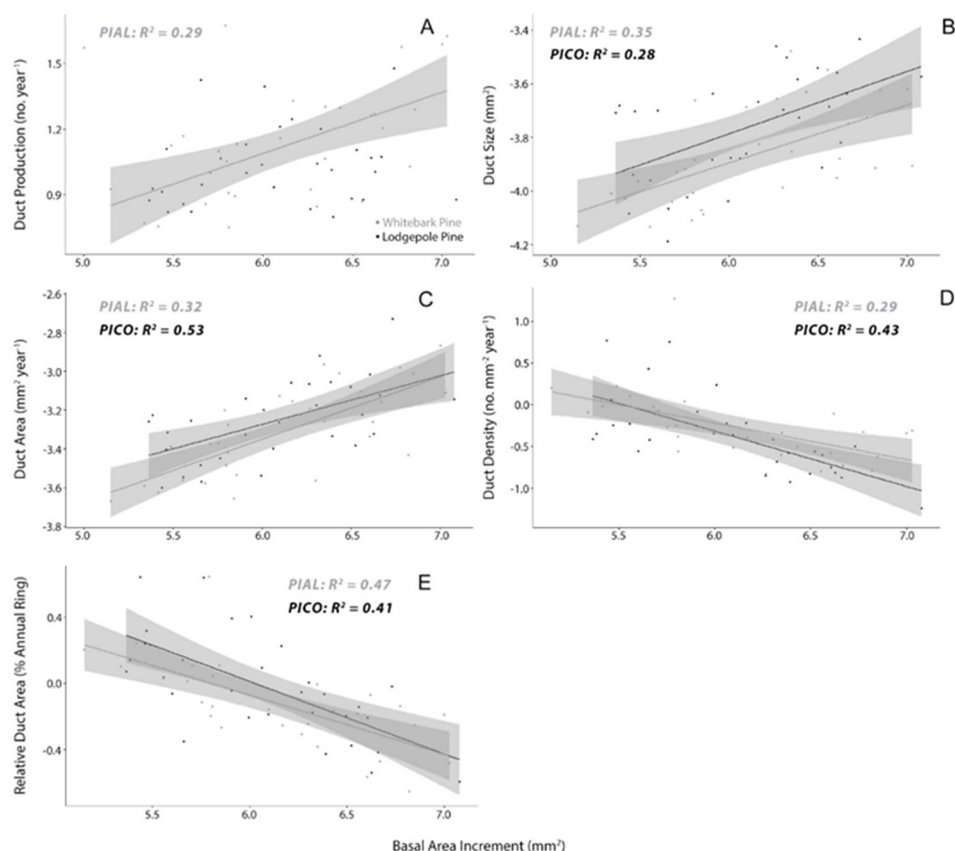


Figure S4. Relationship of radial growth (mm) to resin duct metrics for whitebark (light grey) and lodgepole (black) pines. All metrics were log-transformed to meet the assumptions of linear regression. Only statistically significant models ($\alpha = 0.05$) are depicted.

Table S4. Analysis of variance comparing constitutive mono- and sesqui- terpenes and near-tree competition (by species as well as total competition) at varying densities (high and low basal area estimates; “High Comp” and “Low Comp”) for all whitebark and lodgepole pine.

Competition (Species)	Competitive Load (BA estimates)	Monoterpenes	Sesquiterpenes
Engelmann Spruce	PIAL & PICO	Interaction: $F = 5.14$ $p = 0.0273$	Interaction: $F = 6.31$ $p = 0.0149$
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	Interaction: $F = 4.5$ $p = 0.0499$
	PIAL	$F = 5.818$ $p = 0.0227$	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	$F = 6.219$ $p = 0.0373$	n.s.
	PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PIAL & PICO	n.s.	$F = 6.57$ $p = 0.0131$
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
Lodgepole Pine	PIAL	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	$F = 9.27$ $p = 0.0159$	n.s.
	PIAL & PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PIAL	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
Whitebark Pine	PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PIAL & PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PIAL	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
Douglas-fir	PIAL & PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PIAL	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PIAL & PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
Subalpine Fir	PIAL	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PICO	$F = 4.02$ $p = 0.0546$	n.s.
	High Comp.	n.s.	n.s.
	PIAL & PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PIAL	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PICO	n.s.	n.s.

Table S4 Continued

	Low Comp.	n.s.	n.s.
All Live Comp.	PIAL & PICO	Interaction: $F = 4.78$ $p = \mathbf{0.033}$	$F = 9.69$ $p = \mathbf{0.00292}$
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PIAL	$F = 4.61$ $p = \mathbf{0.0406}$	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PIAL & PICO	n.s.	n.s.
All Live & Dead Comp.	High Comp.	n.s.	$F = 7.07$ $p = \mathbf{0.0171}$
	Low Comp.	n.s.	n.s.
	PIAL	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	n.s.	n.s.
	PICO	n.s.	n.s.
	High Comp.	n.s.	n.s.
	Low Comp.	$F = 5.55$ $p = \mathbf{0.0462}$	n.s.

Note: High Comp = subset of 10 lodgepole and whitebark pines with highest basal area estimates for neighboring competition. Low Comp = subset of 10 lodgepole and whitebark pines with lowest basal area estimates for neighboring competition. BA = basal area; PIAL = whitebark pine; PICO = lodgepole pine.

APPENDIX C

SUPPLEMENTARY DATA FOR CHAPTER 5 “INCREASED WHITEBARK PINE (*PINUS
ALBICAULIS*) GROWTH AND DEFENSE UNDER A WARMER AND REGIONALLY
DRIER CLIMATE”



Geophysical Research Letters

Supporting Information for

Increased Whitebark Pine (*Pinus albicaulis*) Growth and Defense under a Warmer and Regionally Drier Climate

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Introduction

This supplementary information provides additional details on the study area and methods that are presented in the paper. We include a map and description of the study sites on the Flathead Indian Reservation where data were collected, as well as details of sample preparation and processing (Text S1, Figure S1). We also provide raw and detrended time series plots for our composite whitebark pine chronologies (Figure S2) as well as rotated Principal Components Analysis plots for detrended tree growth, resin duct production, duct size, and duct area data (Figure S3). We present an example distribution of climate variables utilized for climate-growth-resin duct analyses (Figure S4) and provide time series datasets containing raw (Data Set S1) and detrended (Data Set S2) tree growth and resin duct data for whitebark pine trees sampled at our study sites.

Text S1.Site Description

The Three Lakes (3LK) site (2,120 m a.s.l.) is located in the Reservation Divide Mountains between the Flathead Indian Reservation and Missoula, Montana (Figure S1). Mean annual temperatures at this location are generally cool, ranging from 2° to 5° C with a 30- to 60-day frost-free period. Precipitation averages 1,143 mm per year, which occurs primarily as snowfall from November through March. The Boulder (BLD) site (1,905 m a.s.l.) is located approximately 74 kilometers away in the western Mission Range on the eastern side of Flathead Lake (Figure S1). Mean annual temperatures for this site are similar to 3LK, with averages ranging from 1° to 6° C with a 20- to 70-day frost-free period. This area receives more precipitation, with an average of 1,397 mm annually occurring primarily as snowfall from November through March. Canopy species at both sites consisted of lodgepole pine (*P. contorta* var *latifolia*) and whitebark pine, with smaller components of subalpine fir (*Abies lasiocarpa*) and Engelmann spruce (*Picea engelmannii*).

Sample Preparation – Increment Cores

Increment cores were collected from live whitebark pine trees near the base of each tree (at or below 15 cm from the root collar) using Hagl6f 4.3 mm diameter increment borers. We required cores that were as close to the pith as possible to accurately determine tree establishment dates. Increment cores were air-dried and processed using standard dendrochronological techniques (Stokes & Smiley, 1996). We measured ring widths to the nearest 0.001 mm using a Velmex measuring stage interfaced with the recording software Measure J2X (Voortech Consulting, 2005). We crossdated tree-ring series visually and graphically using ring-width patterns and frost rings (LaMarche & Hirschboeck, 1984). Crossdating was facilitated using site-specific chronologies from ring-width measurements, which were compiled as part of a previous study (Kichas, Hood, Pederson, Everett, & McWethy, 2020) and statistically checked using COFECHA (Holmes, Adams, & Fritts, 1986).

Individual tree cores were scanned at high resolution (1200 d.p.i.) on an Epson V550 flatbed scanner and annual resin duct features were measured across all years of growth using the program ImageJ (version 1.46r, National Institutes of Health, Bethesda, MD, USA). All resin ducts were measured to the nearest $1 \times 10^{-7} \text{ mm}^2$ using the ellipse tool and the calendar year in which they formed was documented. For defense metrics we followed the methods outlined by Hood and Sala (2015) and collected measurements across five categories, including non-standardized defense measures of resin duct size (mean size of all resin ducts per annual ring; mm^2), duct production (number of ducts year^{-1}), and total duct area (sum of resin duct size: $\text{mm}^2 \text{ year}^{-1}$), as well as resin duct measures that have been standardized to annual tree growth, including duct density (number of resin ducts $\text{mm}^{-2} \text{ year}^{-1}$) and relative duct area (% annual ring).

References

- Ferrenberg, S., Kane, J. M., & Mitton, J. B. (2014). Resin Duct Characteristics Associated with Tree Resistance to Bark Beetles Across Lodgepole and Limber Pines. *Oecologia*, 174(4), 1283-1292. doi:10.1007/s00442-013-2841-2
- Holmes, R. L., Adams, R. K., & Fritts, H. C. (1986). *Tree-ring chronologies of Western North America: California, Eastern Oregon and Northern Great Basin ; with : Procedures used in the chronology development work including users manuals for computer programs COFECHA and ARSTAN*. Tucson, Arizona: Laboratory of Tree-Ring Research, University of Arizona.
- Hood, S. M., & Sala, A. (2015). Ponderosa Pine Resin Defenses and Growth: Metrics Matter. *Tree Physiology*, 35(11), 1223-1235. doi:10.1093/treephys/tpv098
- Kane, J. M., & Kolb, T. E. (2010). Importance of Resin Ducts in Reducing Ponderosa Pine Mortality from Bark Beetle Attack. *Oecologia*, 164(3), 601-609. doi:10.1007/s00442-010-1683-4
- Kichas, N. E., Hood, S. M., Pederson, G. T., Everett, R. G., & McWethy, D. B. (2020). Whitebark Pine (*Pinus albicaulis*) Growth and Defense in Response to Mountain Pine Beetle Outbreaks. *Forest Ecology and Management*, 457. doi:10.1016/j.foreco.2019.117736
- LaMarche, V. C. J., & Hirschboeck, K. K. (1984). Frost Rings in Trees as Records of Major Volcanic Eruptions. *Nature*, 307, 121-126.
- Mason, C. J., Keefover-Ring, K., Villari, C., Klutsch, J. G., Cook, S., Bonello, P., . . . Townsend, P. A. (2019). Anatomical Defences against Bark Beetles Relate to Degree of Historical Exposure between Species and are Allocated Independently of Chemical Defences within Trees. *Plant Cell & Environment*, 42(2), 633-646. doi:10.1111/pce.13449
- Moreira, X., Zas, R., Solla, A., & Sampedro, L. (2015). Differentiation of Persistent Anatomical Defensive Structures is Costly and Determined by Nutrient Availability and Genetic Growth-Defence Constraints. *Tree Physiology*, 35(2), 112-123. doi:10.1093/treephys/tpu106
- Stokes, M. A., & Smiley, T. L. (1996). *An Introduction to Tree-Ring Dating*. Tucson, AZ: The University of Arizona Press.
- Voortech Consulting. (2005). The Tree Ring Measuring Program Project J2X: Voortech Consulting.
- Zhao, S., & Erbilgin, N. (2019). Larger Resin Ducts Are Linked to the Survival of Lodgepole Pine Trees During Mountain Pine Beetle Outbreak. *Frontiers in Plant Science*, 10, 1459. doi:10.3389/fpls.2019.01459

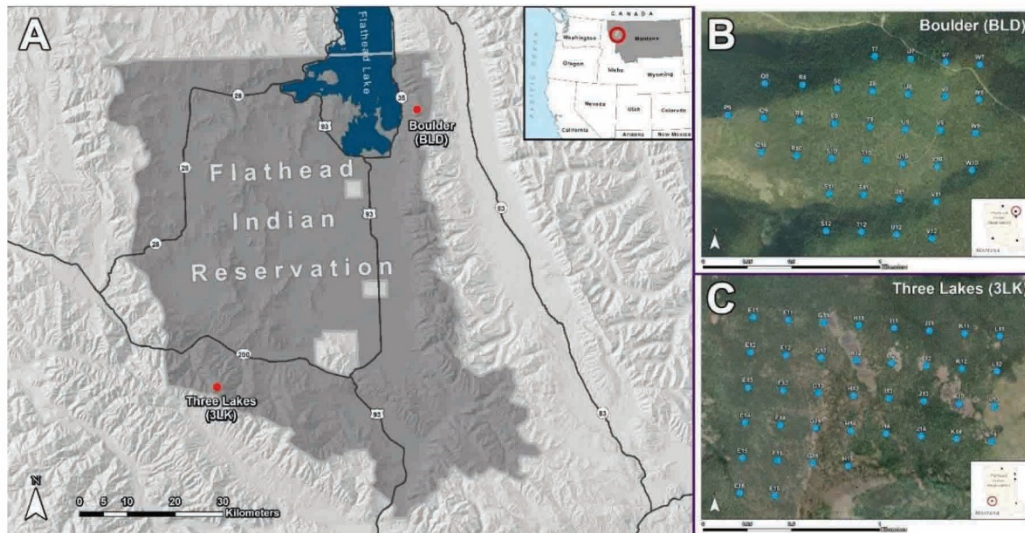


Figure S1. Flathead Indian Reservation in northwestern Montana with the two sites (A) and a detailed map of the Boulder (BLD) study site (B) and Three Lakes (3LK) study site (C). Permanent plots (blue circles) were located on a 200 m grid as part of a larger fire history reconstruction project for the Confederated Salish and Kootenai Tribes. Plots cover approximately 100 ha at 3LK and 88 ha at BLD.

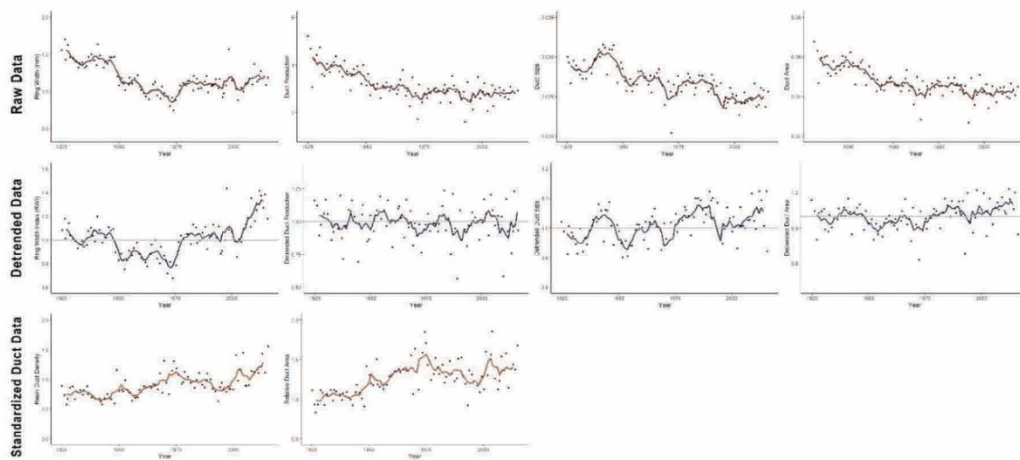


Figure S2. Time series plots of raw tree-ring data (top row), detrended data (middle), and duct metrics that have been standardized to tree growth (bottom row). Tree growth (ring widths: mm), resin duct production (no. ducts year⁻¹), duct size (mm²), duct area (mm² year⁻¹), and resin duct metrics that have been standardized to tree growth: duct density (no. ducts mm⁻² year⁻¹) and relative duct area (% annual ring).

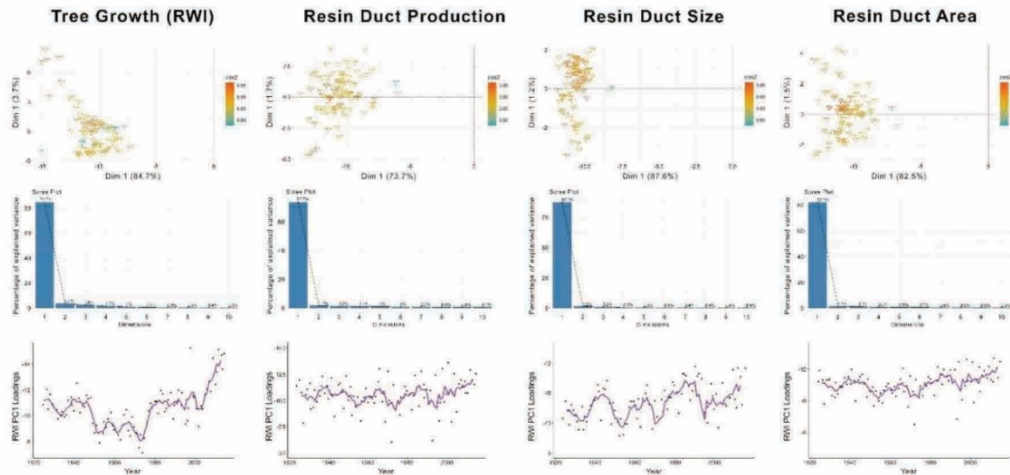


Figure S3. *Top:* Rotated principal component analysis on detrended tree growth (ring-width index) and detrended resin duct production / size / area. *Middle:* Scree plots showing percentage of variance explained by individual principal components (dimensions). *Bottom:* Time series plots of first principal component (PC1) loadings (note: y-axis reversed to help illustrate similarity to time series plots for detrended data).

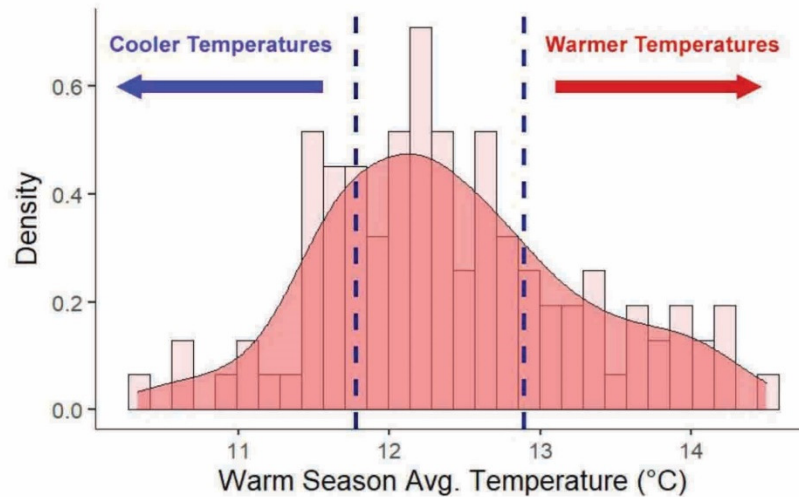


Figure S4. Example distribution of climate variable (Warm Season Average Temperature) used for analysis. Years in the upper and lower quantiles were pooled together and detrended whitebark pine growth and defense metrics were compared across the tail-ends of the respective variable's distribution (e.g., years with the coolest temperatures on record and years with the highest temperatures on record).

Table S1. Spearman correlation coefficients for raw, non-detrended whitebark pine tree size (diameter at breast height; DBH), tree growth (ring width: mm), unstandardized resin duct metrics [non-italicized; resin duct production (no. ducts year⁻¹), duct size (mm²), duct area (mm² year⁻¹)], and resin duct metrics standardized to tree growth [italicized; duct density (no. ducts mm⁻² year⁻¹) and relative duct area (% annual ring)]. Bold text indicates significance at the $p < 0.05$ level with a sample size $n = 112$. Standardized duct metrics reflect a dilution effect, as they geometrically decline with increased tree size even if there is no actual decrease in duct production and/or total resin duct area, as has been demonstrated in other studies (Ferrenberg, Kane, & Mitton, 2014; Hood & Sala, 2015; Kane & Kolb, 2010; Mason et al., 2019; Moreira, Zas, Solla, & Sampedro, 2015; Zhao & Erbilgin, 2019).

Whitebark pine	DBH	Ring Widths	Duct Production	Duct Size	Duct Area	Duct Density	Relative Duct Area
Growth (mm)	0.61						
Duct Production	0.33	0.59					
Duct Size	0.17	0.11	0.01				
Duct Area	0.3	0.39	0.46	0.86			
<i>Duct Density</i>	-0.36	-0.55	0.15	-0.18	-0.08		
<i>Relative Duct Area</i>	-0.3	-0.67	-0.17	0.37	0.24	0.71	

Data Set S1. Time series data for raw whitebark pine tree growth and defense metrics. *RW* = ring widths (mm), *Production* = duct production (no. ducts year⁻¹), *Size* = duct size (mm²), *Area* = duct area (mm² year⁻¹), *Density* = duct density (no. ducts mm⁻² year⁻¹), *RelArea* = relative duct area (% annual ring), n = sample size.

Data Set S2. Time series data for detrended whitebark pine growth and defense metrics. The standardization is done by dividing each series by the geometric trend to produce units in a dimensionless index. *RWI* = ring width index, *Production_Detrend* = detrended duct production, *Size_Detrend* = detrended duct size, *Area_Detrend* = detrended duct area.