



Growth, Chemistry, and Genetic Profiles of Whitebark Pine Forests Affected by Climate-Driven Mountain Pine Beetle Outbreaks

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Climate change-driven *Dendroctonus ponderosae* outbreaks in semi-naïve *Pinus albicaulis* may result in rapid natural selection for trees with genotypes and phenotypes associated with survival. In this study, we investigated whether survivors were genetically and chemically different from a living cohort of trees that escaped predation due to smaller size and estimated genetic diversity. We also examined how growth rate and climate sensitivity varied between beetle-killed and surviving trees. *Dendroctonus ponderosae* predominantly kills large diameter trees; therefore, we predicted that large surviving trees would have distinctive genetic profiles and, due to bottlenecks and drift, survivors would have lower genetic diversity than the abundant smaller mature trees that escaped predation. We found survivors were indeed genetically divergent from the smaller trees but, contrary to expectations, the smaller trees had lower diversity. This suggests that while beetles may select for trees with particular genotypes, other factors are also driving population genetic sub-structuring. Individual tree terpene profiles were diverse and varied by population but showed no clear relationship to survivorship. Two groups of trees with divergent sensitivities to climate were observed in each population, but neither was a clear indicator of survivorship or susceptibility to beetle attack. Growth rate was the best predictor of survivorship with survivors growing significantly slower than beetle-killed trees over their lifetimes although growth rates converged in years just prior to increased beetle activity. Overall, our results suggest that *P. albicaulis* forests show considerable divergence among populations and within-population genetic sub-structuring, and that they may contain complex mosaics of adaptive potentials to a variety of stressors including *D. ponderosae*. To protect the ability of this tree to adapt to increasing pressure from beetles, blister rust, and climate change, a top priority should be the maintenance of standing genetic diversity and adaptive shifts in allele frequencies.

Keywords: *Dendroctonus ponderosae* (mountain pine beetle), genetic diversity, climate change, adaptation, dendrochronology, *Pinus albicaulis*

INTRODUCTION

A recent outbreak of *Dendroctonus ponderosae* (mountain pine beetle) in *Pinus albicaulis* (whitebark pine) has killed trees across large areas. *Pinus albicaulis* is a semi-naïve host for *D. ponderosae* (Raffa et al., 2013). It grows in the subalpine zone where cold typically protects it from predation by the beetle. Exceptions occur when warm droughts allow beetles to move upslope and kill trees until normal colder conditions return (Perkins and Swetnam, 1996). However, chronic warming due to climate change has allowed the beetle to persist in many subalpine areas of western North America supporting outbreaks and widespread mortality of mature *P. albicaulis* (Buotte et al., 2016, 2017; Six et al., 2018). Outbreaks are expected to select for increased resistance in tree hosts over time and selection pressure may be especially strong in a naïve non-coevolved host that lacks a co-evolutionary history with the beetle. In such forests, most trees can be expected to have low levels of defense resulting in high levels of mortality of susceptible genotypes leaving behind rare individuals that exhibit some degree of resistance.

As has been observed in other naïve hosts experiencing outbreaks of *D. ponderosae* (Clark et al., 2010; Cudmore et al., 2010; Raffa et al., 2013; Burke et al., 2017), *P. albicaulis* appears to have more reduced defenses than populations of *Pinus* that have experienced regular pressure from the beetle (Raffa et al., 2017). This has led to fears that the tree may experience severe declines or even local extinction in areas where the beetle is now resident. However, survival of some mature *P. albicaulis* in forests hard-hit in recent *D. ponderosae* outbreaks, and the discovery that these trees may be genetically distinct from those that are chosen for attack, indicates adaptation may be occurring in response to increased selection pressure by the beetle (Six et al., 2018). Little research has investigated the possibility of rapid *in situ* adaptation in conifers due to climate change-driven insect outbreaks. Most bioclimatic models assume that all individuals of a tree species respond similarly and typically incorporate only a few broad ecological parameters (Rice and Emery, 2003; Aitken et al., 2008). These models seldom include phenotypic plasticity, genetic diversity, local adaptation, or the potential for near-term evolution, factors that could lead to substantially different outcomes (Davis and Shaw, 2001; Harte et al., 2004; Savolainen et al., 2007; Aitken et al., 2008). Clearly, there is a need for genetic and phenotypic data to inform our understanding of the potential for evolution to support *in situ* persistence of tree species to improve model outcomes and to inform conservation approaches.

The ability of *P. albicaulis* to adapt (here defined as an increase in allele frequencies associated with favorable changes in phenotypes) to increased bark beetle pressure, white pine blister rust (a disease caused by an invasive pathogen, *Cronartium ribicola*), and changes in climate, will rely to a great degree, on the genetic diversity already existing within affected populations or accessible through gene flow (Kremer et al., 2012, 2014). Many forest trees, including conifers, have high levels of genetic diversity that result in a variety of tree responses to environmental conditions both within and among populations (Savolainen et al., 2007; Kremer et al., 2012; McDowell et al., 2020). This variability

forms the basis for subsequent evolution at local scales and may support considerable *in situ* adaptation in response to climate change (Petit and Hampe, 2006). For *P. albicaulis* to persist in the face of increasing pressure by *D. ponderosae* and changing environmental conditions, shifts not only in allele frequencies supporting resistant phenotypes, but also the maintenance of standing genetic diversity, will be crucial.

Conifers are often viewed as slow to evolve because they are long-lived and wind-pollinated. However, many have shown a considerable capacity for rapid local adaptation even with extensive gene flow (Petit and Hampe, 2006). In fact, populations of trees that experience high mortality rates via highly selective agents such as insects and drought may be the fastest to evolve in response to new climatic conditions (Kuparinen et al., 2010). Climate change can lead to increased rates of adaptation of trees both directly and indirectly. Direct effects include decreased or enhanced growth or increased mortality rates in response to changes in temperature and precipitation (Kremer et al., 2014). These, in turn, can initiate a cascade of indirect effects including altered levels of biotic disturbance. This can currently be seen globally where warming temperatures are leading to greater survival and reproductive rates of many forest insects, and tree hosts stressed by drought or heat are becoming more vulnerable to insect herbivory (Jactel et al., 2019; Kolb et al., 2019).

Aggressive tree-killing bark beetle population dynamics are intimately linked to climatic conditions affecting insect demographics and tree physical and chemical defenses (Raffa et al., 2016). Warming can lead to higher beetle population growth rates (Creeden et al., 2014) and highly selective host tree choice by bark beetles can result in differential mortality among trees of the same species due to selection for specific size classes and a suite of other traits, independent of stress (Stephenson et al., 2019). Aggressive bark beetles choose individual trees for colonization using a combination of visual and chemical cues that results in either the initiation of a pheromone-mediated mass attack or rejection (Raffa et al., 2016). The type and quantity of secondary defense chemicals produced by a tree, especially terpenoids, help beetles locate trees, both at a distance, and upon contact (Raffa and Smalley, 1995; Keeling and Bohlmann, 2006; Tittiger and Blomquist, 2017). Additionally, bark beetles exploit several monoterpenes to produce the aggregation pheromones they use to initiate and sustain the mass attacks that allow them to overcome tree defenses (Seybold et al., 2000). The production of secondary defensive chemicals is strongly influenced by tree genotype (Keeling and Bohlmann, 2006; Bullington et al., 2018), which leads to variation in the chemical profiles expressed by individual trees, influencing their relative attractiveness to beetles. Furthermore, trees experiencing drought stress can exhibit decreases in constitutive and induced defenses (Arango-Velez et al., 2011) suggesting that shifts in tree chemistry in response to water deficit may influence the ability of beetles to successfully attack trees. However, how this may translate to effects on beetle choice of individual trees is unclear. In piñon pine, Trowbridge et al. (2019) found that increased emission rates of terpenoids during warm periods were offset by decreases in production resulting in no net change in emission rates overall. This may generally be the case, but more work is

produce different types, amounts, or ratios of secondary chemical compounds that result in the rejection of these trees by beetles but that the chemicals involved are in groups other than the terpenoids we included.

It is not known whether larger resin duct area results in greater constitutive defense. If it does then there should be greater resin flow in slow growing *P. albicaulis*. However, resin flow in *P. albicaulis* is usually very low and sometimes non-existent. During this study, we measured resin flow over a 24-h period in July 2017 for 40 survivors and 40 generals at Vipond Park and only four trees produced measurable, but low, amounts of resin with two falling into each category (data not shown). Additionally, larger resin ducts in other conifers have not been found to result in greater resin flow (Hodges et al., 1981; Hood and Sala, 2015). Slow-growing survivors may invest more in producing greater resin duct area and resin within their xylem, but the effect on beetles may be more qualitative than quantitative, especially if they also invest differently in the secondary chemicals they produce. An investigation into the secondary chemistry of *P. albicaulis* with differing resin duct areas may help identify the means by which beetles can exert 'choice at a distance' given survivors are not attacked and beetles do not encounter resin physically.

Interestingly, climate sensitivity was not predictive of tree survivorship. At Poverty Flat and Vipond Park, beetle-killed trees and survivors both contained large proportions of sensitive and non-sensitive trees (Supplementary Appendices 2, 3). Our results agree with those of Cooper et al. (2018) who found no relationship of climate variables to survivorship in *P. contorta* and of Kichas et al. (2020) who found no relationship of these variables to survivorship or resin duct area in *P. albicaulis*.

In correlations comparing BAI/BAs for individual trees and climate variables, again we found no clear difference in climate sensitivity between survivors, beetle-killed trees, and generals (Table 8). However, we did find differences between sensitive and non-sensitive trees, particularly a positive correlation to PDSI indicating a negative growth response of sensitive trees to drier conditions (Supplementary Appendices 2–4). In contrast, non-sensitive trees did not respond positively to PDSI, but rather, were more likely to positively respond to March temperatures or negatively to December temperatures (CO). Warmer March temperatures may allow for earlier growth in these trees while the negative relationship to December temperature may relate to negative effects of warmer temperatures on snowpack retention. Kichas et al. (2020) observed reduced growth during wetter winters for *P. albicaulis* at a site in northern Montana and Perkins and Swetnam (1996) noted positive responses to winter and spring precipitation on growth of *P. albicaulis* and suggested that feedbacks among climate variables likely produce nonlinear effects influencing snowpack and consequently growth (Perkins and Swetnam, 1996). The sensitive-insensitive dichotomy that we observed in tree responses to climate variables at all three sites further suggests that genetic sub-structuring within *P. albicaulis* populations increases the diversity of tree responses to climatic conditions, potentially increasing resilience of the forest as a whole to fluctuating conditions over time.

There appeared to be a relationship of drought to outbreak development at Poverty Flat and Vipond Park (Supplementary Figures 2, 3) but no relationship at Lacy Creek (Supplementary Figure 3). In fact, Lacy Creek trees did not reduce growth rates during the most recent drought and actually exhibited dips in growth during wet periods in the 60s and 90s. This highlights the importance of tree responses, and likely genetics, along with climate in outbreak development.

Importance to Conservation

Whitebark pine is in decline across much of its range due to the effects of the non-native disease white pine blister rust, *D. ponderosae* outbreaks, and climate change (Keane et al., 2012). This is a 'wicked problem' in that successful conservation of this tree will require approaches that deal with all three threats. However, as different as these threats are from one another, they share one commonality, and that is that the ability of the species to persist will depend on its potential for adaption to these stressors that, in turn, depends on genetic diversity.

Standing genetic diversity is in large part how long-lived organisms such as trees persist in the face of shifting climatic and other stressors over time (Petit and Hampe, 2006). We found evidence of substantial population sub-structuring in *P. albicaulis* that may indicate a strong adaptive potential. Different cohorts and categories of trees within each population exhibited divergent genetic (Figure 1) and phenotypic profiles (Figures 2, 3) as well as differential responses to climate (Table 8). Older trees possessed greater genetic diversity (Table 2) but younger (general) trees grew better under more recent conditions (Figure 4) suggesting they may be better adapted to warmer conditions. Although generals had lower genetic diversity, gene flow among other cohorts including large survivors should act to maintain genetic diversity and to produce new genetic combinations on which selection can act. However, the rapid growth rates exhibited by generals may also be a cause for concern given that *D. ponderosae* appears to prefer faster growing trees. This may influence overall stand survival and genetic diversity as these trees reach sizes susceptible to beetle attack. Overall, our results suggest *P. albicaulis* forests contain a complex mosaic of adaptive potentials to a variety of stressors but that the ability of populations to persist will likely vary across the geographic range of the tree depending in the genetic diversity within and among populations (Jorgenson and Hamrick, 1997; Liu et al., 2016).

To protect the ability of this tree to adapt to current and future conditions, the maintenance of genetic diversity should be a top priority, and practices that can reduce diversity or that may introduce maladaptive genes or swamp local adaptation should be avoided. Reliance on natural regeneration is best because it involves locally adapted seed sources drawn from the full array of diversity present in the stand and seedlings that establish will have done so under a local climatic selection filter. Where silvicultural practices are applied, they should be implemented with caution. In cases where planting is required, care should be used in sourcing seed, as even locations close to one another may not be appropriate for collections. For example, trees at Lacy Creek and Vipond Park, located only 27 km apart, showed differences in genetic and terpenoid profiles, growth rates, and sensitivity to

climate. Even when using seed from the site where seedlings will be planted, care must be taken to collect from multiple cohorts, as one cohort may not reflect the full genetic diversity of the site as shown by differences between survivors and generals at our sites. To maximize effectiveness and minimize inadvertent harm, it will be crucial in all conservation efforts to consider the strength of local adaptation and genetic structuring within and among populations as well as the sources and degree of gene flow and the multitude of stressors with which the tree must contend (McKay et al., 2005).

Our results also indicate that thinning prescriptions aimed at increasing tree growth in whitebark pine should be applied with considerable caution. Greater tree ring width/faster growth has been widely used as a proxy for tree vigor and as an indicator of greater resistance to bark beetles (Bhuyan et al., 2017). This view has led to thinning projects in whitebark pine aimed at increasing growth and vigor (Retzlaff et al., 2018) and resistance to *D. ponderosae*. However, while the relationship between growth and resistance to beetles has been shown to be positive in some pines and variable in others (Millar et al., 2012; Knapp et al., 2013; Keen et al., 2020), the opposite appears to be true for some populations of *P. contorta* (Cooper et al., 2018) and for *P. albicaulis*. In our study, as well as in that of Kichas et al. (2020), faster overall growth was the strongest predictor of mortality due to *D. ponderosae* indicating that such treatments will not have their intended effect in *P. albicaulis* and may even be detrimental. Furthermore, naturally low resin production, even by survivor and open-grown *P. albicaulis*, indicates thinning will have little-to-no effect on enhancing constitutive defenses against the insect.

Anthropogenic change is creating or enhancing a number of stressors on forests. To aid forests in adapting to these stressors, we need to move beyond traditional spacing and age-class prescriptions and take into account the genetic variability within and among populations and the impact our actions may have on adaptive potential and forest trajectories. Because so little is known about the genetic diversity in most forest trees, and because it is key to effective conservation, studies of genetic diversity and structuring in forest trees should be a top priority in forest adaptation and conservation efforts.

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

DS conceived of the study, participated in all aspects of collections and analysis, and wrote the manuscript. DP did most sample collections in Idaho and participated in project conception and writing. EB did much of the sample processing for genetic and tree ring analysis, and oversaw data management. DS conducted genetic analyses and along with PB conducted the statistical analyses of tree growth and climate with help from JH. GB conducted the chemical analyses while DS, AT, and MH conducted the statistical analysis and interpretations of the data. All the authors contributed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2021.671510/full#supplementary-material>

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