2016

RESIN DUCT DEFENSES IN PONDEROSA PINE DURING A MOUNTAIN PINE BEETLE OUTBREAK: GENETIC EFFECTS, MORTALITY, AND RELATIONSHIPS WITH GROWTH

Sean Pinnell

RESIN DUCT DEFENSES IN PONDEROSA PINE DURING A MOUNTAIN PINE BEETLE OUTBREAK: GENETIC EFFECTS, MORTALITY, AND RELATIONSHIPS WITH GROWTH

By

SEAN RICHARD PINNELL

B.S. California State University, Fresno, Fresno, CA, 2009
M.S. California State University, Fresno, Fresno, CA, 2013

Thesis
presented in partial fulfillment of the requirements
for the degree of

Master of Science
in Organismal Biology and Ecology

The University of Montana
Missoula, MT

May 2016

Approved by:

Scott Whittenburg, Dean of The Graduate School
Graduate School

Anna Sala, Chair
Division of Biological Sciences

Lila Fishman
Division of Biological Sciences

Cara Nelson
College of Forestry and Conservation

ABSTRACT

Pinnell, Sean R., M.S. May 2016 Organismal Biology and Ecology

Resin duct defenses in ponderosa pine during a mountain pine beetle outbreak: genetic effects, mortality, and relationships with growth

Chairperson: Anna Sala

Despite formidable tree defenses against insect pests, including resin produced in resin ducts, bark beetles have recently caused extensive outbreaks in western North American forests. To date, our understanding of the extent to which resin duct defenses remain effective at the peak of an outbreak, the relative genetic vs. environmental controls on resin duct production, the degree to which drought reduces resin defenses in naturally occurring outbreaks, and whether and when defense production competes for resources with growth remains limited. Resolving these questions is relevant in the face of climate change, and increased risk of outbreaks. I took advantage of a 40-year-old ponderosa pine (*Pinus ponderosa*) genetic trial where a recent mountain pine beetle outbreak (*Dendrochtonus ponderosae*) killed 36% of trees. I examined phenotypic and genetic variation in resin ducts, their consequences on survival during the outbreak, the influence of drought on resin duct defenses, and potential growth-defense tradeoffs. Using dendrochronology, I retrospectively measured growth and resin duct traits in thirty families from low to high mortality from before to during the outbreak. Resin duct defenses significantly decreased mortality during the peak of the outbreak both at the phenotypic and genetic levels. However, differences in resin duct defenses between trees that survived and those that died and between high and low mortality families were small prior to the outbreak but were amplified during the outbreak, which was preceded by a drought. Resin duct production, but not size, was under strong genetic control. Analyses at both the phenotypic and genetic levels indicated that drought significantly predisposed some trees and families to mortality via reductions of resin duct defenses, particularly duct size. I found no evidence of a resin duct defense-growth tradeoff. Rather, faster growing trees and families produced more resin ducts than slower ones. Despite this, however, faster growing families did not suffer lower mortality. These contradictory results suggest breeding for fast growth increases resin duct defenses and survival, but at high beetle densities during an outbreak, other factors possibly involving induced defenses, appear to ultimately offset this effect.

ACKNOWLEDGMENTS

First and foremost I would like to acknowledge my advisor Anna Sala for her support and guidance during my graduate training, her infinite wisdom and patience were paramount to my success at the University of Montana. Also, I would like to acknowledge Raul de la Mata for providing me the necessary data and professional expertise to begin my research, without Raul's work on this project my research would not have been possible. I'd like to acknowledge my fellow lab mates Gerard Sapés, Beth Roskilly, and especially Sharon Hood for all of their support both technically and personally. I also thank Tatiana Kelly for all her hard work and much appreciated assistance in the field, and to Emily Heyerdahl and Elaine Sutherland at the USDA Forest Service for their technical assistance with all matters related to dendrochronology. Finally I would like to thank the McIntire-Stennis grant through the College of Forestry & Conservation for funding this project.

TABLE OF CONTENTS

INTRODUCTION

Recently, the mountain pine beetle (*Dendroctonus ponderosae)* has caused extensive, and in some cases unprecedented, outbreaks leading to large-scale mortality in pine forests from Alaska to Northern Mexico (Allen et al. 2010, Meddens et al. 2012). Mountain pine beetle (MPB) outbreaks are a regularly reoccurring natural disturbance that has maintained and shaped species diversity over large time scales. Not surprisingly, the coevolution of conifers with tree killing insects and pathogens over millions of years, has led to a formidable defense system where resin production is critical (Franceschi et al. 2005). However, our understanding of the variability and effectiveness of defense mechanisms against the bark beetle remains incomplete. First, the role of resin defenses is thought to decrease and even to become inconsequential as beetle densities increase (Boone et al. 2011). However, some studies have shown that beetle-killed trees in natural stands have higher resin ducts (where resin is produced and stored) relative to surviving trees (Kane and Kolb 2010, Gaylord et al. 2013, Ferrenberg et al. 2014, Gaylord et al. 2015, Hood and Sala 2015), pointing to the possibility that resin defenses may remain effective at high beetle densities. Second, defense production is known to be under strong genetic and environmental control (Coley et al. 1985, Herms and Mattson 1992, Hamilton et al. 2001), and there is now consensus that warmer temperatures and drought associated with climate change have intensified current bark beetle epidemics (Raffa et al. 2008, Bentz et al. 2010, Marini et al. 2012, Netherer et al. 2015) in part due to negative effects of drought on defense production (Netherer et al. 2015, Gaylord et al. 2015). Given the strong consequences of defense production on survival and fitness, the relative genetic vs. environmental control of resin duct production becomes particularly relevant, but our knowledge of how they interact remains

limited (Sampedro et al. 2011, Moreira et al. 2012, Sampedro 2014) particularly with respect to drought. Third, while limited experimental evidence suggests that drought reduces resin defenses (Gaylord et al. 2013, Netherer et al. 2015, Gaylord et al. 2015), evidence in naturally occurring outbreaks is very limited. Fourth, whether and when defense production competes for resources with growth (Herms and Mattson 1992) remains controversial, but could have important consequences for forest breeding programs aimed at increasing growth, particularly in the face of climate change and increased risk of outbreaks. Here, I took advantage of a genetic trial in ponderosa pine (*Pinus ponderosa*) that was naturally subjected to a mountain pine beetle outbreak, to examine the phenotypic and genetic variation in defense traits, their consequences on survival, responses to drought, and potential growth-defense tradeoffs.

Pines, the hosts of the MPB, defend themselves against bark beetles by producing resin ducts where oleoresin (hereafter resin), a viscous lipid-soluble mixture of volatile and nonvolatile terpenoid and phenolic compounds is synthesized and stored (Hodges et al. 1979, Strom et al. 2002). Therefore, resin ducts function both as a constitutive defense present prior to attack, and as an inducible defense that produces additional resin, often with altered chemical composition, in response to wounding or infection (Franceschi et al. 2005, Kolosova and Bohlmann 2012, Hood and Sala 2015). When beetles bore into a tree, resin ducts are severed, causing resin to flow to the attack site and to physically push out or trap entering beetles, thus impairing successful attack. Resin defenses against the beetle can be extremely dynamic depending on beetle population density, and are thought to be most important at endemic stages (Raffa et al. 2008, Boone et al. 2011) when low beetle population density limit pheromone-mediated mass attacks necessary to successfully overwhelm most trees defenses. At this stage, beetles tend to attack small, less vigorous and poorly defended trees, which in turn limit beetle

reproduction and population increases, thus perpetuating the endemic stage. Certain environmental triggers such as increased temperatures during larval development can cause beetle populations to occasionally increase to epidemic levels (Aukema et al. 2008, Raffa et al. 2008). Increases in beetle populations increase the probability that larger trees will be mass attacked regardless of tree defenses (Wallin and Raffa 2004, Boone et al. 2011). Moreover, a density-dependent switch in beetle behavior occurs as beetle population densities increase, such that at epidemic stages beetles increasingly prefer larger, more resource-rich trees, despite having higher defenses, likely because larger trees provide a better habitat for beetle reproduction (Boone et al. 2011). Consistently, (Boone et al. 2011) showed that host defenses were effective at deterring beetle attack when beetle populations were low, but became inconsequential after stand-level beetle densities surpassed a critical threshold. Recent studies, however, have shown that beetle-killed trees in natural stands have lower resin ducts than surviving trees (Kane and Kolb 2010, Gaylord et al. 2013, Ferrenberg et al. 2014, Gaylord et al. 2015, Hood and Sala 2015), suggesting that resin defenses may remain effective during outbreaks. However, whether mortality in these studies occurred at high beetle densities remains unclear and additional research on the effectiveness of resin duct defenses during an outbreak is warranted.

Plant defense traits are highly influenced by environmental and genetic factors (Coley et al. 1985, Herms and Mattson 1992, Hamilton et al. 2001). Experimental evidence from pines and other conifers indicate that resin flow and secondary chemicals are heritable genetic traits and thus subject to selection from insects, pathogens, and environmental factors (Rosner and Hannrup 2004, Sampedro et al. 2011, Westbrook et al. 2013). Although resin flow and chemistry are critical upon attack, resin flow is extremely variable and often difficult to measure (Blanche et al. 1992, Lombardero et al. 2000, Knebel et al. 2008, Cannac et al. 2009, Moreira et al. 2015,

Hood et al. 2015). Recent studies showing higher resin ducts in surviving trees (see above) indicate that resin ducts may be a more convenient, stable and reliable measure of defense potential because they are recorded over time in the wood. These results are further corroborated with the fact that both, resin duct size and the total area of resin ducts correlates with resin flow, the critical metric that influences the beetle's ability to escape entrapment and successfully bore into the tree (Hood and Sala 2015). Consistent with genetic variation of resin flow, studies so far have also found genetic variation in resin ducts (Moreira et al. 2012, Westbrook et al. 2015). However, our knowledge of genetic and environmental effects on specific resin duct traits (e.g. number and size of individual ducts and their total area) that are specifically correlated to resin flow, and their subsequent effects on plant fitness and survival at high beetle densities is still limited. Furthermore, if resin duct defenses reduce mortality as indicated by recent studies (see above), and if they exhibit a significant genetic signal, then we would expect phenotypes and genotypes with greater resin duct defenses to exhibit lower mortality during an outbreak. To our knowledge no study has evaluated phenotypic and genetic variation of resin duct defenses and their consequences on mortality during a naturally occurring beetle outbreak.

Both direct and indirect effects of warming and drought have been identified as one of the main triggers of recent beetle outbreaks in conifer stands (Rouault et al 2006; Dobbertin(Rouault et al. 2006, Dobbertin et al. 2007, Raffa et al. 2008, Seidl et al. 2011, Williams et al. 2013, Kaiser et al. 2013). On the one hand, warming increases insect developmental rates and survival which in turn drive population success (Regniere and Nealis 2007, Powell and Bentz 2009, Weed et al. 2013). On the other, drought and warming have negative physiological effects on trees, which may lead to increased rates of attack and mortality. Drought compromises hydraulic function, carbohydrate production, and carbohydrate supply to

sinks (McDowell et al. 2008, McDowell and Sevanto 2010, Sala et al. 2010, Ryan 2011), all of which could compromise defense production and delivery to attack sites. Despite substantial research on carbon allocation in trees (Litton et al. 2007), our understanding of the mechanisms by which drought may reduce defense production remains limited and so far negative effects of drought on resin (or resin duct) production have only been shown under experimental settings (Netherer et al. 2015, Gaylord et al. 2015). To date, information on negative drought effects on resin duct production in naturally occurring outbreaks is lacking.

The production of both, carbon-based secondary compounds such as resins and resin ducts entails resources and it is costly (Gershenzon 1994, Haukioja et al. 1998). Plant defense theory, therefore, predicts that growth-defense tradeoffs are likely to occur (Herms and Mattson 1992, Heil and Baldwin 2002). However, empirical evidence of growth defense tradeoffs is extremely difficult to obtain (Agrawal et al. 2010). The growth-differentiation-balance hypothesis (GDBH) allows predictions of costs of defense at the intraspecific level based on the degree to which stress affects growth vs carbon assimilation (Herms and Mattson 1992). While several studies in pines have found support for the GDBH (Lorio and Sommers 1986, Wilkens et al. 1998, Lombardero et al. 2000, Gaylord et al. 2007, Novick et al. 2012) the timing of resin and resin duct production may not coincide with growth demands and no trade-off may occur. Further, higher resource acquisition by vigorous, faster growing trees may allow for a greater allocation to both growth and defense (Hood and Sala 2015) in which case tradeoffs are very difficult to demonstrate. Potential growth-defense tradeoffs have important consequences for tree-breeding programs developed to increase growth rates, particularly under climate change and increased incidence of insect outbreaks (Allen et al. 2010, Anderegg et al. 2015).

Here we took advantage of a 40-year-old ponderosa pine genetic trial, which was naturally subjected to a mountain pine beetle outbreak to examine phenotypic and genetic variation in resin ducts, their consequences on survival during the outbreak, and potential growth-defense tradeoffs. Specifically I addressed the following questions: (i) Is survival during an outbreak when beetle densities are high related to greater resin duct defenses at both the individual tree level (phenotypic effect) and at the family level (genetic effect)? (ii) Is there genetic differentiation in resin duct traits? If so, which specific traits are under strongest genetic control? (iii) Does drought affect resin duct defenses, and is this effect constant across families? (i.e. does drought predispose some families more than other to mortality?) And (iv) is there evidence of a growth defense trade-off? This later question was motivated by results from our prior work at this site (R. de la Mata and A. Sala, unpublished) showing a significant negative genetic correlation between growth and survival. Although defenses were not measured and a growth-defense tradeoff could not be demonstrated, results showed that the growth-survival tradeoff was not solely due to beetle preference for larger trees (and hence faster growing families), thus suggesting that differences in mortality between fast and slow growing families may be related to defense allocation.

MATERIALS & METHODS

Study site and plant material

The site is a *P. ponderosa* common garden genetic trial originally established at the Lubrecht experimental forest in Montana (40°32'N, 113°42'W; 1,128 m) in 1974 by the Inland Empire Tree Improvement Cooperative (IETIC). Mean annual air temperature is 7°C and a mean annual precipitation 511 mm, with 44% falling as snow (Nimlos, 1986). Soils are dominantly silty clay loam, with Greenough soils, fine silty, frigid Typic Cryoboralf (Madsen and Hudson, 1997). Seeds were collected from 204 open-pollinated, wild mother trees selected from 44 natural stands in western Montana and Northern Idaho (northern USA rocky Mountains) exhibiting above average growth and form characteristics at stand level. Mother trees were selected based on phenotypically superior growth and form. Cones were collected from 1 to 6 trees per stand spaced between 20 and 400 m apart. Seeds from each individual mother tree were considered a half sibling family and were reared at the USDA Forest Service tree nursery in Coeur d'Alene (Idaho, USA). The site was level and cleared prior to planting one-year-old bare-root seedlings in 1974 on a 3 × 3 m spacing using a randomized complete block design with 4-tree family-row plots in each of 5 blocks for a total of 20 trees per family (4,025 seedlings total). Additional planting of containerized 2-year-old seedlings occurred in 1975 to replace mortality during the first year.

Survival and diameter at breast height (DBH; 1.4 m above ground) were surveyed periodically until 2001 by the IETIC. Mortality was high shortly after planting and decreased thereafter with ca. 62.7% survival by 2001. No beetle-mortality was noted during the last measurement year in 2001 (27 ± 1 year after planting). Beetle activity was initially observed in

2005 and started to intensify at the Lubrecht site thereafter with peak mortality reaching ca. 36% in 2011 (De la Mata et al. in review).

Mortality survey

We used data from De la Mata et al. (in prep) on beetle-induced mortality. Briefly, in June 2013, each tree in the stand (dead or alive) was mapped with a GPS device with submetric accuracy (Trimble® Geo HX). For each tree, live or dead status as well as whether death occurred before or after 2001 (based on the 2001 survey) was noted. Beetle-killed trees after 2001 were identified based on abundant resin pitch tubes. The approximate year of successful attack (<2008, 2009, 2010, 2011, 2012) was retrospectively determined based on needle presence, needle color, and twig and bark characteristics. Year of death was assumed to be one year following attack.

During bark beetle outbreaks, live trees adjacent to attacked trees are more likely to be attacked and killed than live trees away from attacked trees (contagion effect). Further, live hosts in heavily attacked areas of the stand may die regardless of defenses because high beetle densities allow mass attacks to overwhelm well-defended trees. Conversely, trees in areas with low mortality may survive not necessarily because they are well defended but because beetle pressure is low. Therefore, mortality probability needs to be adjusted to account for the contagion effect (spatial susceptibility or autocorrelation). Spatial susceptibility was calculated using geostatistical techniques (De la Mata et al. in prep), and beetle-mortality was then adjusted.

Precipitation data to test about drought effects was obtained from the Lubrecht Flume SNOTEL weather station (46°53’N, 113°19’W; 1,427 m) United States Department of

Agriculture Natural Resources Conservation Service. We used annual accumulated precipitation (mm yr^{-1}) over hydrological year from 1995 to 2010.

Resin duct defenses, growth, and mortality

To explore if resin duct traits relate to MPB induced mortality, we selected fifteen families of highest and fifteen of lowest spatially adjusted mortality. Although twenty trees were initially planted per family, mortality from planting to prior to the outbreak reduced the sample size. We therefore randomly selected 6-10 trees per family (dead or alive) depending on the number of trees that were alive prior to the outbreak within each family.

Tree sampling for annual tree growth and axial resin duct measurements was conducted in July and August of 2014. We extracted two 5-mm diameter increment cores from bark to pith at DBH (1.4 m) from each tree at north and south cardinal directions using a manual increment borer. DBH and bark depth were also measured during this time. Cores were mounted on wood blocks and progressively sanded until cellular structures were visible through a stereoscopic microscope. We created high-resolution images (2,400 d.p.i.) of each core using an Epson platform scanner and measured ring widths to the nearest 0.001 mm using CooRecorder v7.8 (Cybis Elekronik & Data AB, Saltsjöbaden, Sweden). For each tree we averaged ring width measurements between both cores using standard dendrochronogical methods. We used both the initial estimate of tree death from the 2013 survey (De la Matta, in prep) and dendrochronological cross-dating techniques to determine the exact year a tree died. For dead trees we assumed the last growth ring formed to be the year of death (beetle flight occurs in July). We used COFECHA (Grissino-Mayer 2001) to assess the strength of our cross-dating techniques. All dead trees died between the years of 2008-2012 from beetle attack.

Resin duct measurements were made in ImageJ (version 1.46r, National Institute of Health, Bethesda, MD, USA) to the nearest 1 x 10^{-7} mm^2 using the ellipse tool. Each resin duct was assigned the calendar year in which that duct formed validated by our cross-dated ring width measurements. We measured resin ducts formed from 1995 until 2010 excluding the years of 2011-2014 due to low sample sizes for dead trees. We calculated five axial resin duct variables as described in (Hood and Sala 2015): resin duct production (no. $year^{-1}$), duct size (mm^2), total duct area (mm^2 $year^{-1}$), duct density (no. mm^2 $year^{-1}$), and relative duct area (% of total resin duct area relative to xylem area –excluding ducts- in each annual ring). We used basal area increment (BAI) as an annual growth metric (cm^2 $year^{-1}$) by using both ring width and tree-specific cross-sectional data (inside bark diameter) under the assumption that tree rings represent concentric circles. BAI (cm^2 $year^{-1}$) was calculated using the dplR package v 1.6.0 in R v 3.0.1 (Bunn 2010).

All statistical analyses described below were performed using the R statistical software (Team 2013). We tested differences in resin duct number (no. ducts yr^{-1}), duct size (mm^2), total duct area (mm^2 ring $area^{-1}$), and relative duct area (% annual ring) between live and dead trees. These variables were selected on the basis of their effect on resin flow (duct number, duct size and total duct area) and as a metric of potential growth defense tradeoff (relative duct area) (Hood and Sala 2015). We used one-tailed *t*-test on log-or square root-transformed data or by Wilcoxon tests when assumptions of normality were not met by transformations. Analyses were performed separately for the periods prior to the beetle outbreak (1995-2003) and the period when the beetle outbreak likely started (2004-2010). These two periods were distinguished based on the assessment of beetle-mortality in the stand. Mortality in 2008 was already at 4%, which is substantially higher than background mortality rates of 1-1.5% (Franklin et al. 1987, van

Mantgem et al. 2003, Allen et al. 2010) and is considered to reflect an outbreak (Raffa and Berryman 1983, Raffa et al. 2008). We thus assumed that beetle activity started a few years earlier, in 2004, when beetle activity in the area became noticed. Simple *t*-tests do not account for differences in growth and spatial susceptibility (areas of high and low attack density). We therefore did an analysis of co-variance (ANCOVA) using type-II sums of squares for each response variable, with tree status (live and dead) as a factor and DBH and spatial susceptibility (see above) as co-variates.

To determine which resin duct traits were best predictors of mortality in individual trees, we ran multiple logistic regression analyses to model the probability of beetle-induced mortality across all years of the study (1995-2010) as a function of spatial susceptibility and resin duct traits as predictor variables. Each model was run separately using each resin duct trait as an independent variable. Because beetle preference for larger hosts is strongly dependent on beetle population densities during and outbreak (Boone et al. 2011), we also included DBH as a predictor variable.

Resin duct traits generally decreased over time, from before to during the outbreak (see *Results*). To evaluate whether resin duct defenses differed between high and low mortality families, we first calculated the percent change of each resin duct variable for each individual tree from before (using the average from 1995-2003) to during the outbreak (average from 2004-2010). We then pooled the % change of each resin duct variable per family and tested whether the change in resin duct defenses predicted mortality at the family level. We used simple linear regression between family and % change in resin duct defenses as the dependent variable and spatially adjusted mortality rate as the predictor variable.

Genetic influence of resin duct traits

Genetic differentiation in resin duct variables were tested using a linear mixed effects model with tree nested within family as a random effect. In order to account for the autocorrelation between successive years, year was also treated as a random effect. Family was considered a random factor and the associated variance components were estimated by restricted maximum likelihood (Faraway 2005). Two assess whether family (random effect) had a significant effect over each resin duct variable, a comparative model approach was used, where two times the log-likelihood when including and excluding the random family effect are compared. This statistic follows a chi-squared distribution and chi-squared *p*-values are reported. We also used a parametric bootstrap approach to more accurately estimate the *p*-values for the likelihood ratio test for each resin defense trait (Faraway 2005).

Drought effects on resin duct production

We used a linear mixed effects model approach (see above) to test whether drought caused a divergence in resin duct production from before to during the outbreak in individual trees that died vs. those that survived, and also in high vs. low mortality families. At the individual tree level, year and tree were treated as random effects, while precipitation and status (dead or alive) were fixed. A similar approach was used at the family level with the following modifications: year and family were treated as random effects, with tree nested within family also as a random effect and rank (high or low mortality families) and precipitation as fixed effects.

Trade-offs between growth and defense

Correlation analyses between resin duct variables and growth (DBH) were used to evaluate potential growth-defense trade-offs (note that DBH is a surrogate of growth rate as all trees were the same age). Analyses were done at the individual (phenotypic) and family (genotypic) level. When statistical model assumptions were not met with simple linear regression, we used a generalized linear model (GLM) and specified a logarithmic link function to stabilize residuals.

RESULTS

Role of defenses during the outbreak

Within our random subsample of trees per family (n=232), a total of 84 trees were dead. Several dead tree core samples, however, were in poor condition and had to be removed from the analyses, leaving a total of 68 dead trees available for cross dating. Based on cross dating, dead trees died between 2008 and 2012, although the majority (94%of our sampled trees) died in 2009 and 2010, the two years of peak mortality during the outbreak (13% relative to 4-7% in other years; de la Mata et al. in prep).

Duct size and total duct area decreased from before to during the outbreak (Figs. 1-2). (Note that reductions in total duct area are expected because trees become larger over time, thus reducing ring width over which total duct area is measured). Relative duct area, in contrast, increased slightly from before to during the outbreak but only in trees that survived the attack relative to trees that were killed (Fig. 1). Results were similar when low and high mortality families were compared (Fig. 2). At the individual level (dead vs. alive) during, but not before, the outbreak duct size, total duct area, and relative duct area were all significantly higher in trees that survived relative than those that were killed, suggesting that trees with greater resin duct defenses during the outbreak are more likely to survive (Fig. 3). Of these variables, only total duct area was significantly higher in live trees prior to the outbreak (Fig. 3e). After adjusting for spatial susceptibility and growth, differences between live and dead trees were reduced, but relative duct area during the outbreak (but not before) remained significantly higher in live relative to dead trees ($P > 0.05$; Fig. 4f). At the family level (comparisons between high and low mortality families) results were similar to those at the individual level (not shown).

Results from logistic regression analyses across all years of the study (1995-2010) showed that only relative duct area was a significant predictor of tree mortality (odds ratio of 0.009; $P = 0.041$; Table 1). In contrast, DBH did not have any significant effect on mortality (Table 1).

At the family level, decreases in duct size and relative duct area from before to during the outbreak were negatively correlated with mortality ($P = 0.004$ and 0.013, respectively; Fig. 5). That is, families that suffered greater mortality had stronger reductions of duct size and relative duct area from before to during the outbreak. In contrast, there was no relationship for total duct area ($P > 0.05$; Fig 5b).

Genetic influence of resin duct traits

We found no significant genetic effects for duct size and total duct area, but a significant genetic effect for relative duct area, duct density and number (Table 2).

Effect of drought on resin duct defenses

Average rainfall during 1999-2002, a period shortly before the outbreak, was below the long-term average. These dry years occurred after a period of above average precipitation (Fig. 6a). Reduced precipitation prior to the outbreak coincided with a decrease in radial growth from prior years of above average precipitation (Fig. 6b). BAI decreased from before to during the outbreak but was similar for both dead and live trees in both periods, indicating that the drought prior to the outbreak did not have a stronger effect on BAI in trees that died (Fig. 3).

As expected, at the phenotypic level, there was a significant effect of mortality on all resin duct variables (Table 3, Fig. 1). Drought (annual precipitation) had a significant effect on total duct area but not on duct size or relative duct area (Table 3). The interaction precipitation x

mortality status (live and dead trees) was highly significant for duct size ($P < 0.0001$), marginally significant for relative duct area ($P = 0.055$), but not significant for duct area ($P > 0.05$; Table 3, Fig. 1). At the family level (genotypic effect) mortality (high vs. low mortality families) was significant for duct size and total duct area but was marginally insignificant for relative duct area (Table 3, Fig. 2). Annual precipitation had a significant effect on total duct area but not on duct size or relative duct area (Table 3, Fig. 2). The interaction drought x family mortality rank (low and high mortality families) was statistically significant for duct size and total duct area ($P < 0.05$; Table. 3, Fig. 2), but not for relative duct area ($P > 0.05$; Fig. 2).

Resin duct defenses, growth, and mortality

Resin duct size and total duct area were positively correlated to DBH both at the individual tree (phenotypic) and at the family level (genotypic; $P < 0.05$; Fig. 7). While there was a similar trend for relative duct area, it was not statistically significant ($P > 0.05$; Fig. 7). Trees that died tended to have higher BAI prior to and during the outbreak although these differences were not statistically significant (Fig. 6b, analyses not shown). Because our selection of families to sample was based on adjusted mortality rather than growth, the DBH of individual trees and families did not correlate to spatially corrected mortality (Fig. 8).

DISCUSSION

Although conifers exhibit a well-developed defense system against beetles, the role of defenses is thought to decrease during epidemic stages of an outbreak when beetle population densities are very high (Raffa and Smalley 1995, Boone et al. 2011). Here, we show that resin duct defenses in ponderosa pine decreased mortality at the peak of an outbreak, both at the phenotypic and genetic level. As expected, we found genetic differentiation in resin duct traits, (although only for traits related to cambial activity) therefore providing a source of variation for selection to act upon. Interestingly, resin duct defenses between dead and live trees and between high and low mortality families were generally smaller and often not statistically significant before the outbreak but were amplified during the outbreak. Therefore, predisposition of trees or families to mortality prior to the outbreak is small when based on resin duct defenses alone. In contrast, our results indicate that the drought immediately prior to the outbreak predisposed some trees and families to mortality during the outbreak. Although defenses are assumed to be costly, we found no evidence of a growth-resin duct defense tradeoff. Rather, some resin duct traits strongly increased with growth both at the individual and family level. However, growth rate (DBH) was similar between the high and low mortality families (i.e. faster growing families did not exhibit greater mortality despite greater resin duct defenses). In fact, our previous results at this site with all 204 families showed that faster growing families (greater DBH) suffered greater, rather than lower, mortality (de la Mata et al. in prep). These seemingly contradictory results suggest that while resin duct defenses decrease mortality at the peak of an outbreak, other factors (likely induced defenses) ultimately offset this effect.

Resin duct defenses: phenotypic and genotypic effects on mortality

Constitutive resin flow in pines is the first line of defense against bark beetle attack, acting both as a physical barrier that pushes out entering beetles as well as a potent chemical deterrent (Erbilgin et al. 2003, 2006). Previous studies have shown that resin ducts, where resin is produced and stored, are a good surrogate of defense. For example, (Hood and Sala 2015) showed that resin flow in ponderosa pine (the metric relevant for pitching out beetles) is significantly and positively correlated with total resin duct area and resin duct size of the five most recent years. Consistently, studies have also shown that dead or successfully attacked trees have lower resin duct defenses relative to live or unsuccessfully attacked trees (Kane and Kolb 2010, Ferrenberg et al. 2014, Hood et al. 2015). While these results suggest that resin duct defenses reduce the risk of mortality, it is not clear whether trees sampled in these studies died when beetle densities were highest during the peak of the outbreak. This is important because the role of constitutive and induced defenses has been shown to decrease with beetle densities and even to become inconsequential at epidemic stages (Boone et al. 2011), thus suggesting that resin duct defenses do not affect mortality at the peak of an outbreak. Indeed, constitutive resin defenses are most important at endemic stages when mass attacks are limited by low beetle densities and beetles can only successfully attack weaker and less defended trees (Boone et al. 2011). However, at epidemic stages when beetle densities are highest, a density-dependent coordinated shift in beetle behavior allows beetles to preferentially attack larger, higher quality hosts for brood production despite their higher defenses (Wood 1982, Wallin and Raffa 2000, 2004), a response possible via a mass attack strategy that overwhelm host defense capacity.

Consistent with previous results showing that resin ducts are a good surrogate for overall defense capacity, but in contrast to studies showing little or no effect of defenses at the peak of an outbreak, we found that trees that survived the outbreak had greater resin duct defenses (duct size, total duct area and relative duct area) during the outbreak (Figs. 1, 3). Likewise, families that incurred lower mortality had lower decreases of resin duct traits (duct size and relative duct area) from before to during the outbreak (Figs. 2,5). Although differences in resin duct defenses between dead and live trees during the outbreak were reduced when accounting for tree size and spatial susceptibility, they remained significant for relative duct area (Fig. 4f). Our tree ring analyses confirmed that the majority of dead trees sampled in this study (94%) were attacked the years when mortality during the outbreak was highest (de la Mata et al. in prep). Therefore, our results provide evidence that resin duct defenses were effective at decreasing mortality even at the peak of the outbreak that overall killed 36% of the trees (de la Mata et al. in prep). Accounting for tree size and spatial susceptibility likely reduced the differences between live and dead trees during the outbreak (Fig. 4) because of contrasting effects of tree size and the role of defenses in areas of high and low spatial susceptibility (and, presumably, beetle densities). Thus, while at the stand level resin duct defenses decreased mortality at the peak of the outbreak, at smaller spatial scales (i.e. when accounting for spatial susceptibility and size) the role of defenses likely varied with beetle pressure. That is, greater defenses in larger trees may be effective at lower beetle pressure (lower spatial susceptibility) but less so at higher beetle pressures (high spatial susceptibility). The overall effectiveness of resin duct defenses in reducing mortality was also corroborated by our analyses of drought effects (Table 3) and by the results from logistic regression analyses showing that relative duct area was a significant predictor of mortality (Table 1).

Genetic influence of resin duct traits

Resin defenses in conifers have strong fitness consequences and are significantly influenced by genetic and environmental factors. For instance, (Moreira et al. 2015) found that duct density and relative duct area were influenced by genotype in *Pinus pinaster*, and (Westbrook et al. 2015) found that duct production (duct no. yr^{-1}) was genetically correlated to xylem growth in *Pinus taeda*. However, less is known about genetic effects on resin duct size and total area (the metrics related to resin flow; Hood and Sala 2015). The formation of resin duct canals occurs within the cambial zone and outermost layers of developing xylem due to a developmental shift in cambial cells that initiates resin duct epithelial cells in lieu of wood-forming tracheids (Schaller 2008). The production of these resin ducts is associated with increased biosynthesis and accumulation of terpenoid oleoresins. The developmental and metabolic processes associated with the production of resin ducts and synthesis of resins require gene upregulation of multiple enzymatic pathways (Martin et al. 2002, Faldt et al. 2003, McKay et al. 2003, Miller et al. 2005, Huber et al. 2005, Byun-McKay et al. 2006). Therefore, it is reasonable to predict that resin duct traits directly related to cambial activity are strongly influenced by such gene regulation and therefore under stronger genetic control. Consistent with this, we found that genetic effects were only significant for resin duct variables that are influenced by cambial activity (duct production, density, and relative area) but not size of ducts or total duct area. The lack of genetic signature on duct size and total duct area suggests that these variables are under stronger environmental control (Table 2). Indeed, both of these variables relate to cell size, a trait strongly influenced by water-driven cell expansion. Because genetic effects on resin duct traits translated to differential mortality, outbreaks have the potential to drive selection for better-defended phenotypes (de la Mata et al. in prep).

Drought effects on resin duct defenses

Trees that later survived and low mortality families had generally greater resin duct defenses than trees that died and high mortality families, respectively. However, these differences were mostly significant during the outbreak, but generally not before the outbreak (Figs. 1-4). There are two possible non-mutually exclusive explanations for these results. One is that high beetle pressure somehow caused an induction of resin duct production in some trees and families to a greater extent than others. Although resin duct size and total duct area decreased over time in all trees (Figs. 1-4), a potential induction in some trees or families could have minimized this decrease, thus generating the differences between trees that subsequently died and those that survived and families with high and low mortality. Although induction of resin duct production in pines has been shown in response to mechanical wounding (Fahn and Zamski 1970), hormone treatment (Moreira et al. 2015), or fire (Hood et al. 2015), differences in resin duct traits between live and dead trees and between high and low mortality families (higher in surviving trees and in low mortality families) consistently appeared at the onset of the drought prior to the outbreak (Figs. 1-2). These patterns, therefore, are not consistent with a beetle induction because the outbreak occurred later.

Several studies have shown that drought and warming have enhanced the recent bark beetle outbreaks in western North America (Raffa et al. 2008, Bentz et al. 2010, Marini et al. 2012, Mitton and Ferrenberg 2012, Netherer et al. 2015), an effect thought to be due to drought-related reductions in carbohydrate availability and, therefore, on defense production (Seager et al. 2007, Bentz et al. 2010, Raffa et al. 2013, Netherer et al. 2015). Both resin duct structures and resin itself (made of carbon-rich terpenes) are costly to produce (Gershenzon 1994, Franceschi et al. 2005, Moreira et al. 2015). By limiting photosynthesis, extended or severe drought can deplete carbon reserves (Dunn and Peter 1993, Gaylord et al. 2013) which could limit allocation

to defense (Herms and Mattson 1992, Lombardero et al. 2000, Desprez-Loustau et al. 2006, Netherer et al. 2015). Consistently, both experimental drought (Gaylord et al. 2013, Netherer et al. 2015, Gaylord et al. 2015) and reduced precipitation (Rigling 2003; Hood et al. 2015) have been shown to decrease resin duct-related defense production. The significant negative effect of drought on total duct area we report here (Table 3) is consistent with these studies. Importantly, our results show that the drought preceding the outbreak had a differential effect on resin duct traits in trees that later died relative to those that survived and in high relative to low mortality families (Table 3). Although the specific traits for which drought caused this divergence varied depending on whether the analysis was at the phenotypic or genotypic level, duct size, a variable apparently under strong environmental control (Table 2) and significantly related to resin flow (Hood and Sala 2015) was significant in both cases. These results clearly show that drought predisposed some trees and families to higher mortality relative to others. That is, before the drought, most resin duct traits were similar in trees that survived the outbreak relative to those that died, and in low and high mortality families, but values for some resin duct traits within both groups significantly diverged at the onset of the drought with differences persisting during the outbreak (Figs. 1-4).

Our results, however, do not provide clear evidence of the mechanisms by which some trees and families are more susceptible to the negative effects of drought on defenses relative to others. Before the outbreak and just prior to the drought event, BAI tended to be higher in trees that subsequently died (Fig. 6b), suggesting that fast growth may compromise resin duct defense production under drought. However, these differences, while consistent, were not statistically significant, precluding inference on whether fast growth compromises defenses under drought and subsequent survival (see below). Prior to the drought and the outbreak, total duct area and

relative duct area tended to be smaller in trees that subsequently died relative to those that survived (Fig. 3), however, only differences in total duct area, a variable correlated with resin flow (Hood and Sala 2015), were statistically significant. Combined, these results suggest that slight differences in relative allocation to growth and defense prior to the outbreak may have been exacerbated by the drought. The significant effect of relative duct area as a predictor of mortality (Table 1) is also consistent with this. In summary, while the mechanisms involved are not completely resolved, our results provide clear evidence that a drought preceding a naturally occurring outbreak predisposed some trees and families to greater mortality relative to others via a decrease of resin duct defenses.

Trade-offs between growth and defense

Tradeoffs between growth and defense have long been hypothesized because defenses are costly to produce and limited resources cannot be simultaneously allocated to all fitness-enhancing functions (Rose and Charlesworth 1981, Schluter et al. 1991, Stearns 1992, Messina 1993, Messina and Fox 2001, Roff et al. 2002). These tradeoffs, however, are surprisingly difficult to demonstrate (Lorio and Sommers 1986, Mattson and Haack 1987, Conner 2003, Agrawal et al. 2010, Sampedro 2014) and results in the literature are often inconsistent. For instance, genetic correlations between growth and resin duct defenses have been shown to be negative (Reid and Watson 1966, Blanche et al. 1992), positive (Fahn and Zamski 1970, Rosner and Hannrup 2004), or not significant (Wimmer and Grabner 1997). Here, we found no evidence of a growth-resin duct defense tradeoff. Instead, faster growing individuals and genotypes (families) had more and larger ducts and larger total duct area (Fig. 7). Similar results at the phenotypic level have been reported for ponderosa pine (Kane and Kolb 2010, Hood and Sala 2015). The positive effect of tree size on resin duct defenses highlights the well-known vigor

effect whereby larger plants acquire more resources for investment in all functions, including defense (Agrawal 2011). Because of this vigor effect, a better metric for examining tradeoffs may be relative resin duct area, which reflects investment in defense relative to growth. However, relative duct area did not vary significantly with growth at the individual and family levels (Fig. 7). Therefore, our results do not support a tradeoff between growth and resin duct defenses. If anything, faster growing families expressed greater resin duct defenses relative to slower growing families.

Despite the fact that faster growing families had greater resin duct defenses (Fig. 7) and that resin duct defenses decreased beetle mortality (Figs. 3-4), faster growing families did not experience lower mortality in our sampled families. Although trees that subsequently died tended to have higher growth rates (BAI) across all years of the study (Fig. 6b), these differences and those in DBH (tree diameter) were not statistically significant (Fig. 8). Moreover, our results at the same site based on the full set of half-sib families planted at the site (204 instead of the 30 we sampled for this study) show a significant negative genetic correlation between growth and survival (i.e. faster growing families exhibited greater, rather than lower, mortality; De la Mata et al. in prep). This effect, however, was driven by a single year during the epidemic stages of the outbreak: the second year (2010) of the two years of highest mortality. Further, a detailed analysis showed that this effect was not driven by the beetle preference for larger, better defended trees (and therefore faster growing families) at high beetle densities, suggesting an intrinsic growth survival trade-off of the host trees (De la Mata et al. in prep). If faster growing trees and families produce more resin duct defenses (Fig. 7) and if these defenses increase survival during an outbreak (Figs. 1-4), why was growth not related (Fig. 8) or negatively genetically correlated with survival (De la Mata et al. in prep)? A possible explanation is that

slower growing families exhibited an induction of resin flow and/or resin chemistry in response to the high beetle pressure in 2009, and that during the subsequent year this effect was strong enough to offset the increase of constitutive resin duct defenses with growth rates (De la Mata et al. in prep). Further research is needed to elucidate whether differential induced responses in slow vs. fast growing trees and families are responsible for the patterns we observed.

Conclusion

Insect-related pests, including bark beetles, are predicted to intensify under climate change due to direct positive effects of higher temperatures on insects as well as indirect effects of drought and warming on beetle-associated organisms and host trees (Bentz et al. 2010, Anderegg et al. 2012). In contrast to previous studies indicating a minor role (if any) of tree defenses in preventing mortality at high beetle densities during an outbreak, we show, both at the phenotypic and genetic levels, that resin duct defenses decreased mortality during the peak of an outbreak when beetle densities were highest. We also show that a drought preceding the outbreak predisposed some trees and families to higher mortality due to differential production of resin duct defenses. Importantly, while resin duct defenses decreased mortality and faster growing trees and families produced more resin duct defenses, faster growing families did not exhibit lower mortality (based on the subsample in this study) or exhibited greater mortality (based on all 204 families at the site; de la Mata et al. in prep). We speculate that these counterintuitive results are due to a greater induced response in slow growing families that offsets the positive effects on constitutive defenses in fast growing families.

Understanding the positive effects of tree growth on constitutive and induced defenses and genetic and environmental effects on growth and defense allocation strategies should be taken under consideration by forest management decisions. Based on our results, breeding

programs that aim to propagate fast, vigorously growing trees will also increase constitutive resin duct defenses (Westbrook et al. 2015). However, our results also show that this does not necessarily decrease mortality during an outbreak. On the contrary, it may increase mortality (De la Mata et al. in prep). This suggests that breeding programs aimed at increasing growth rates may reduce the likelihood of beetle-induced mortality during endemic stages and the transition from endemic to epidemic stages (when constitutive defenses matter most), but once at epidemic stages faster growth may decrease survival. How growth rates affect the relative investment to constitutive vs. inducible responses, their potential tradeoffs, and how these relative investments are affected by genetics and drought deserves further research.

TABLES & FIGURES

Variable	β (SE)	*P*-value	Odds ratio
Relative duct area (% annual ring)	-2.37 (1.16)	0.041	0.009
Tree diameter (DBH) (cm)	0.043 (0.04)	0.330	1.043
Spatial susceptibility	0.5	< 0.0001	1.690

Table 1. Logistic regression results for effects on spatial susceptibility, DBH and relative resin duct area on the probability of mortality across all years of the study (1995-2010); model $\chi^2(3)=188.4$, $R^2= 0.67$ (Hosmer-Lemeshow), 0.55 (cox-Snell), 0.79 (Nagelkerke). Of several resin duct traits tested independently, only relative duct area was statistically significant.

Resin duct variable	Family effect χ^2	*p*-value
Duct size (mm^2)	1.54	0.213
Total duct area ($mm^2\ yr^{-1}$)	2.37	0.123
Relative duct area (% annual ring)	8.80	**0.003**
Resin duct density ($ducts \cdot mm^2 \cdot yr^{-1}$)	13.40	**<0.001**
Resin duct production (no. ducts yr^{-1})	5.15	**0.023**

Table 2. Summary of linear mixed effects models on the genetic influence over resin duct defense parameters across all years of the study (1995-2010). Significant *p*-values are shown in bold.

	Drought		Mortality		Drought x Mortality	
Phenotypic	*F*	*P*	*F*	*P*	*F*	*P*
Duct size (mm^2)	2.72	0.118	14.9	**<0.001**	15.64	**<0.0001**
Total duct area ($mm^2\ yr^{-1}$)	5.19	**0.037**	5.11	**0.023**	2.49	0.115
Relative duct area (% annual ring)	0.12	0.735	6.35	**0.011**	3.67	0.055
Genotypic						
Duct size (mm^2)	1.49	0.240	8.19	**0.004**	6.24	**0.012**
Total duct area ($mm^2\ yr^{-1}$)	4.93	**0.041**	7.07	**0.007**	7.61	**0.005**
Relative duct area (% annual ring)	0.01	0.943	3.48	0.061	6.24	0.113

Table 3. Tests of the effects of drought (annual precipitation), mortality and their interaction in the mixed models used to test drought effects at the individual (upper; mortality is dead vs. live) and family levels (lower; mortality is high vs. low mortality families) across all years of the study (1995-2010). The significance of the main fixed effects and the interaction term were tested via restricted maximum likelihood (see text). Significant *p*-values are shown in bold.

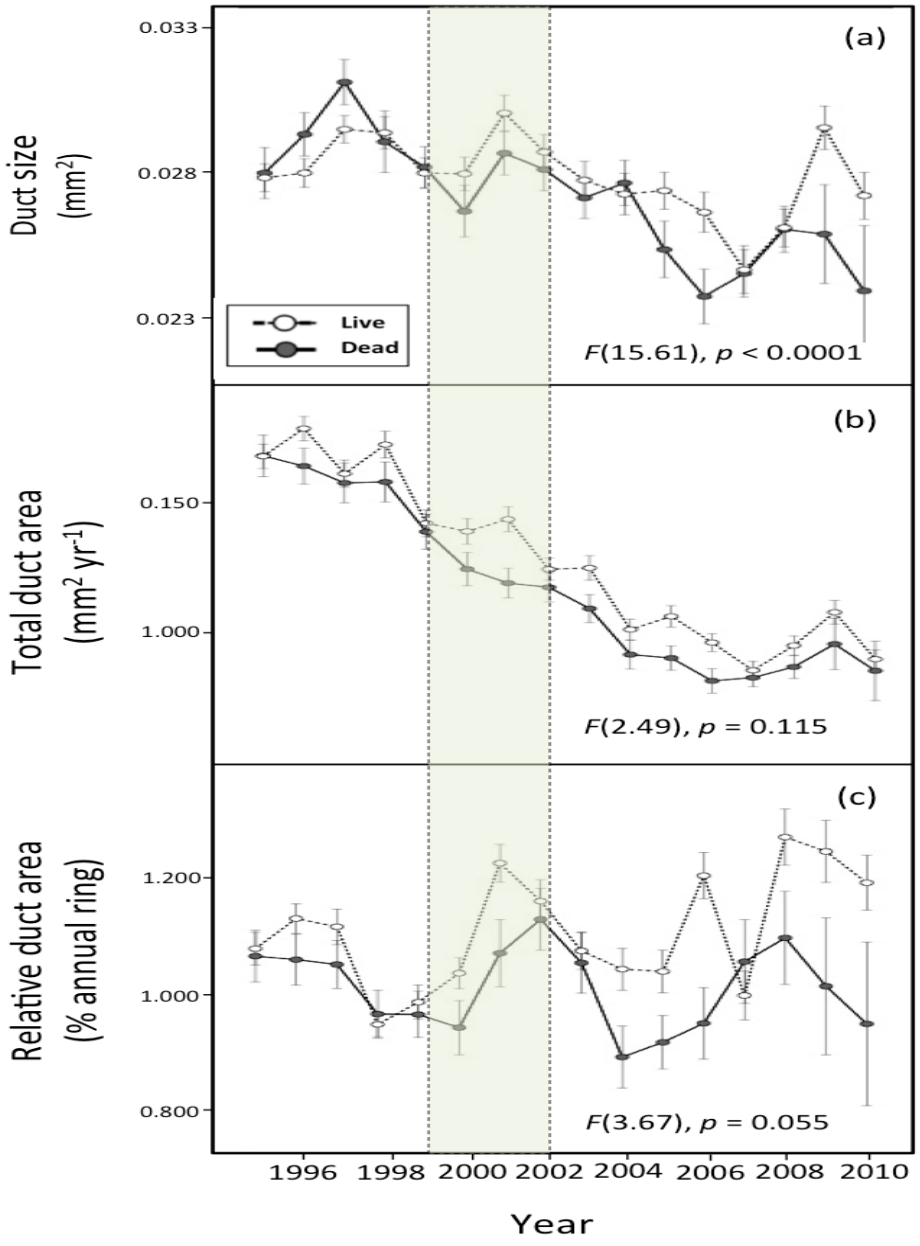

Figure 1. Temporal variation in resin duct traits between live and dead trees across all years of the study (1995-2010). Shaded area shows the drought prior to the outbreak (1999-2002). *p*-values show differences in resin duct traits between live and dead trees due to drought. Error bars are ±SE.

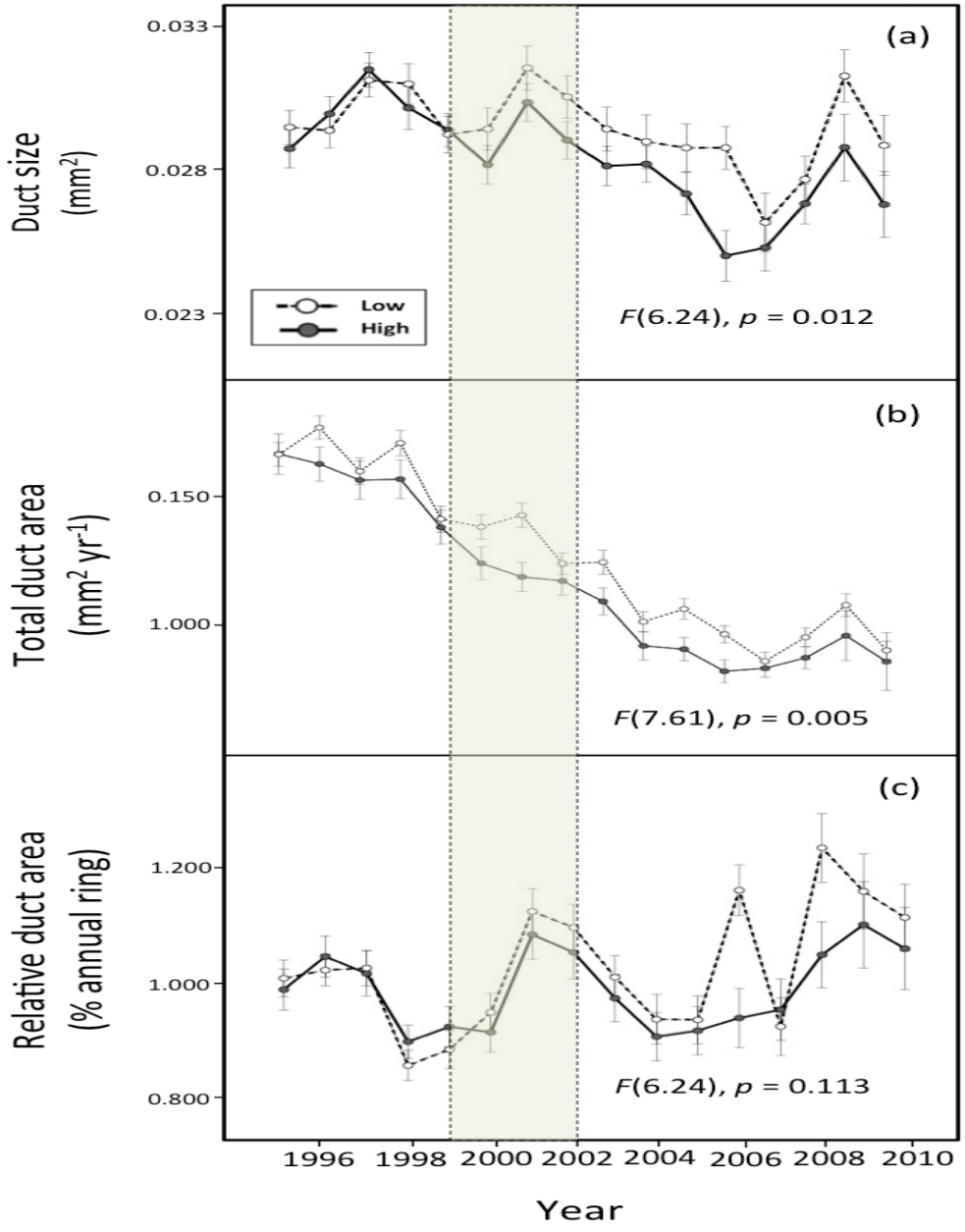

Figure 2. Temporal variation in resin duct traits between low and high mortality families across all years of the study (1995-2010). Shaded area shows the drought period prior to the outbreak (1999-2002). *p*-values show differences in resin duct traits between low and high mortality families due to drought. Error bars are ±SE.

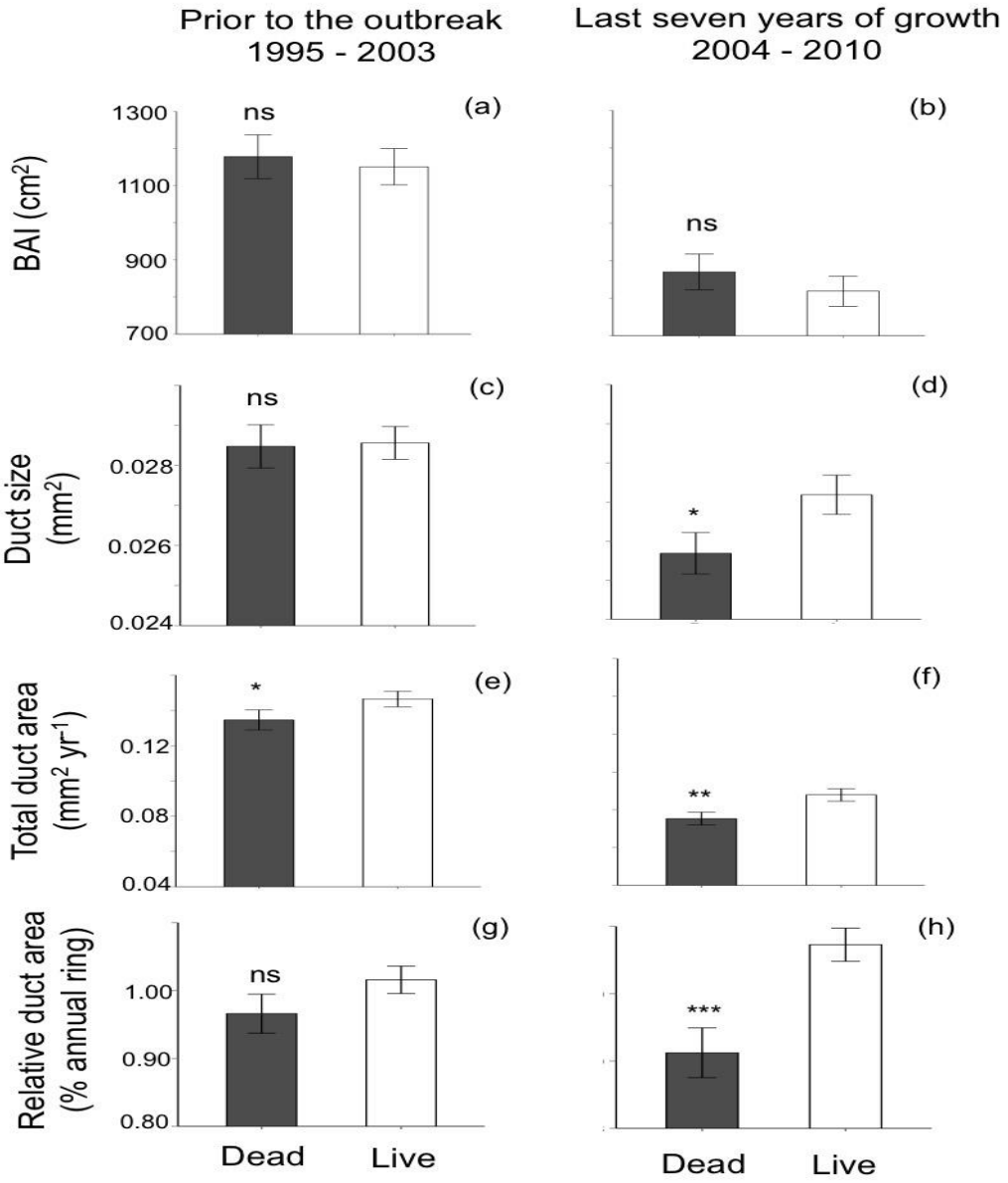

Figure 3. BAI and resin duct traits of individual live and dead trees prior to the outbreak (bar-plots left), and during the last seven years when the outbreak developed (bar-plots right). Error bars are ±SE. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

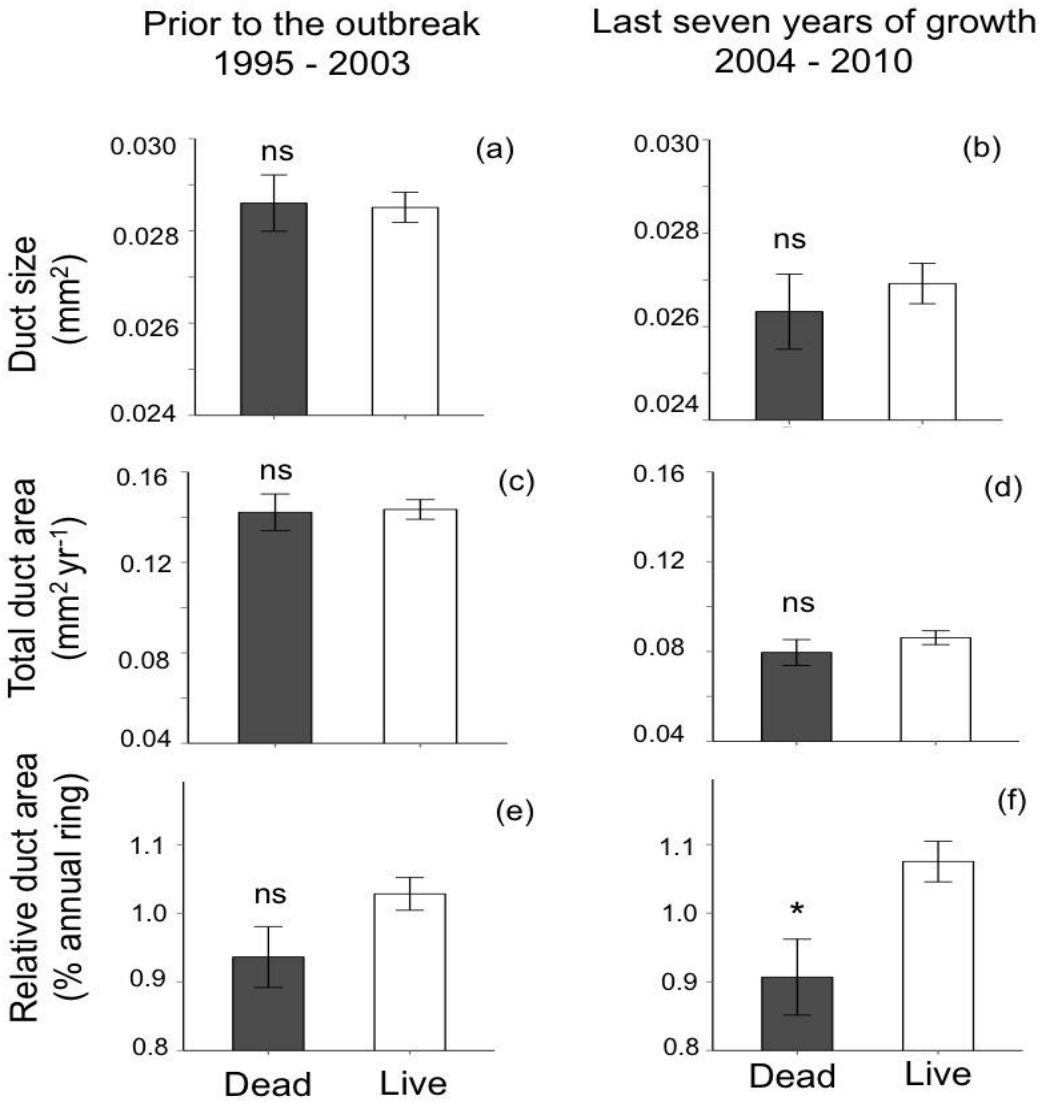

Figure 4. Resin duct traits of individual live and dead trees prior to the outbreak (bar-plots left), and during the last seven years when the outbreak developed (bar plots right) adjusted after accounting for tree growth and spatial susceptibility. Error bars are ±SE, significant values indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

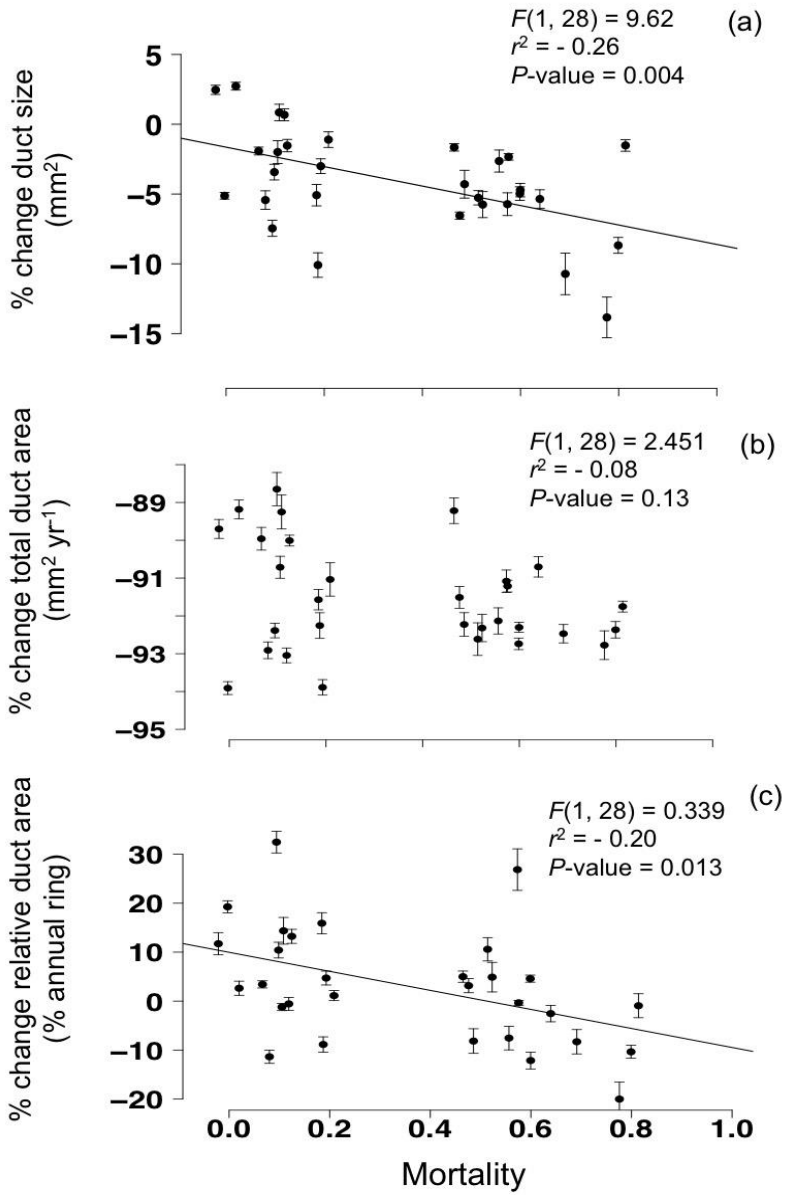

Figure 5. Relationship between percent change in resin duct traits at the family level prior to the outbreak (1995-2003) and during the last seven years when the outbreak developed (2004-2010) across spatially corrected mortality. Each data point represents a family mean consisting of 6-10 individual trees. Error bars are ±SE.

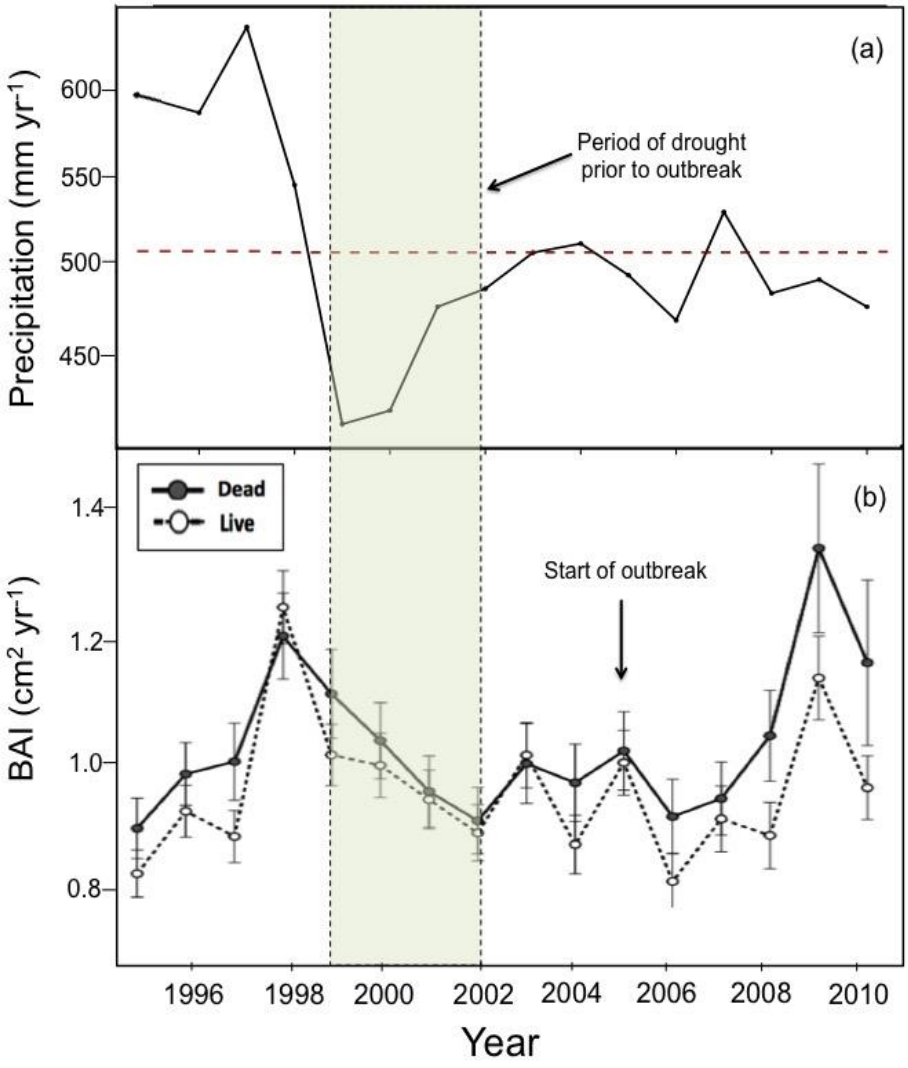

Figure 6. Comparison of (a) total annual accumulated precipitation and (b) mean BAI (±SE) of dead and live trees across all years of the study (1995-2010). Red horizontal dashed line in panel (a) shows average long-term precipitation (1979-2015) at the Lubrecht SNOTEL site. Shaded area shows the drought period prior to the outbreak. Each data point (b) represents the pooled mean of individual trees within a given year that either died or survived beetle attack.

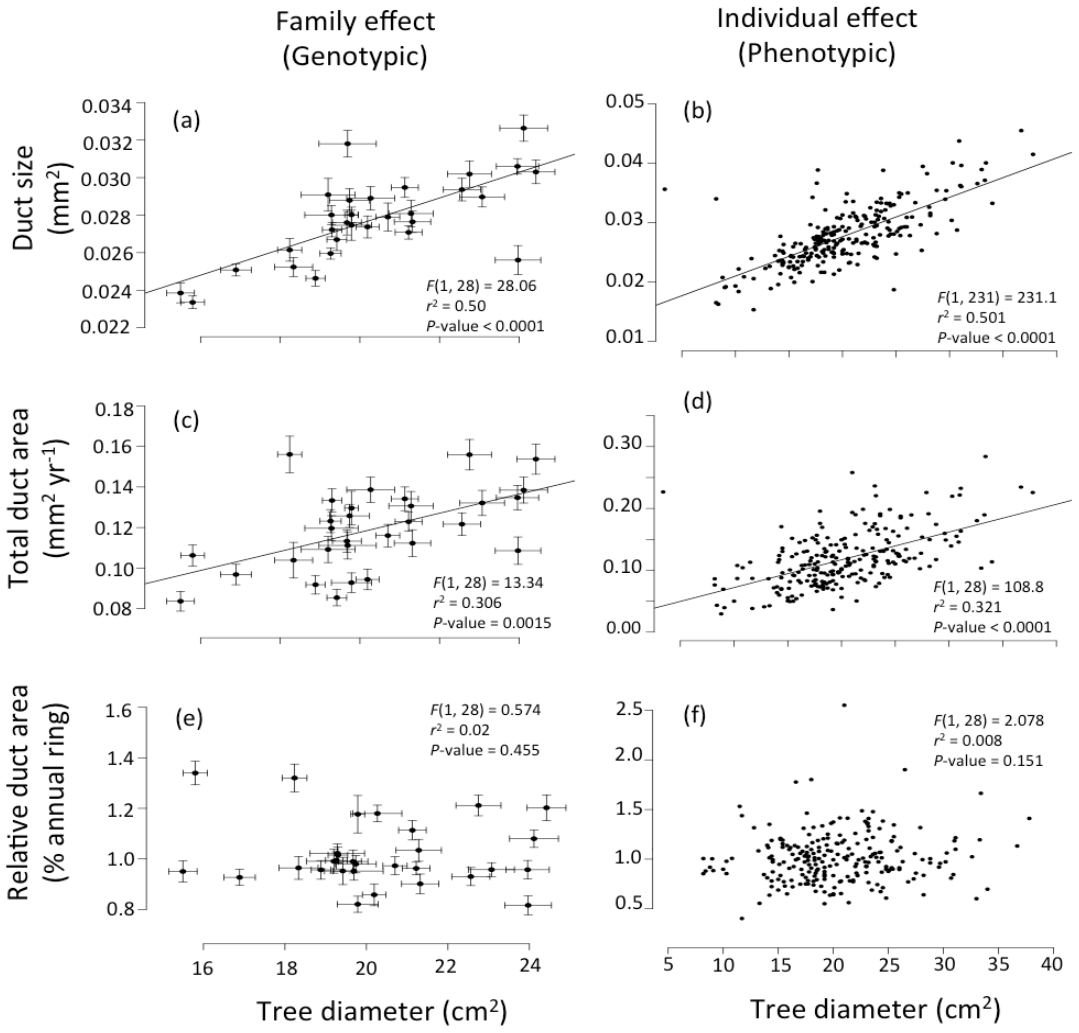

Figure 7. Relationship between resin duct traits and growth (DBH) at the family level (left) and individual tree level (right) for all years of the study (1995-2010). Each data point on the left panels represents a family mean consisting of 6-10 individual trees. Error bars are ±SE. Error bars were omitted from individual plots for clarity.

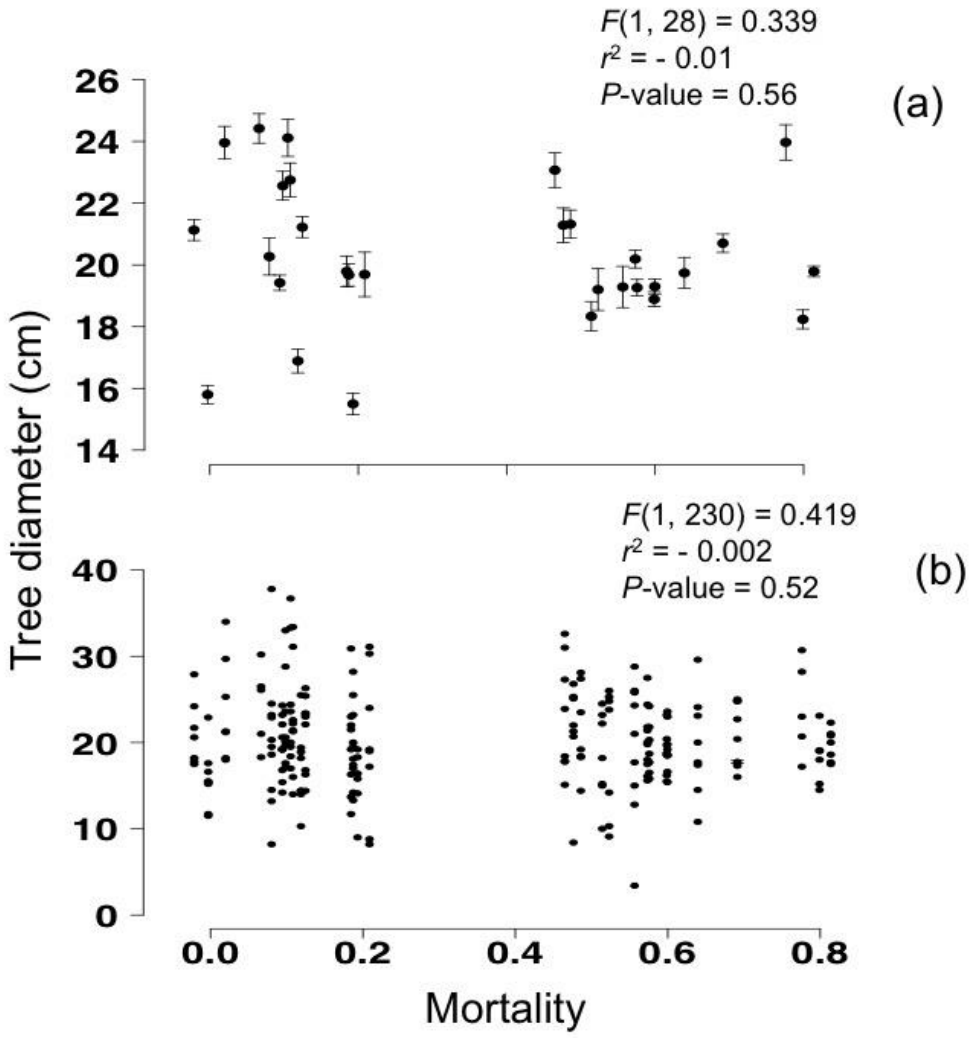

Figure 8. Relationship between growth and spatially corrected mortality at the family level (a) and at the individual tree level (b) across all years of the study (1995-2010). Each data point (a) represents a family mean consisting of 6-10 individual trees. Error bars are ±SE. Error bars were omitted from (b) for clarity

REFERENCES

Agrawal, A. A. 2011. Current trends in the evolutionary ecology of plant defence. Functional Ecology 25:420–432.

Agrawal, A. A., J. K. Conner, and S. Rasmann. 2010. Tradeoffs and negative correlations in evolutionary ecology. Evolution since Darwin: the first 150:243–268.

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.

Anderegg, W. R. L., J. A. Berry, and C. B. Field. 2012. Linking definitions, mechanisms, and modeling of drought-induced tree death. Trends in Plant Science 17:693–700.

Anderegg, W. R. L., J. A. Hicke, R. A. Fisher, C. D. Allen, J. Aukema, B. Bentz, S. Hood, J. W. Lichstein, A. K. Macalady, N. McDowell, Y. Pan, K. Raffa, A. Sala, J. D. Shaw, N. L. Stephenson, C. Tague, and M. Zeppel. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. New Phytologist 208:674–683.

Aukema, B. H., A. L. Carroll, Y. Zheng, J. Zhu, K. F. Raffa, R. D. Moore, K. Stahl, and S. W. Taylor. 2008. Movement of outbreak populations of mountain pine beetle: influences of spatiotemporal patterns and climate. Ecography 31:348–358.

Bentz, B. J., J. Regniere, C. J. Fettig, E. M. Hansen, J. L. Hayes, J. A. Hicke, R. G. Kelsey, J. F. Negron, 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.

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-Revue Canadienne De Recherche Forestiere 41:1174–1188.

Bunn, A. G. 2010. Statistical and visual crossdating in R using the dplR library. Dendrochronologia 28:251–258.

Byun-McKay, A., K. A. Godard, M. Toudefallah, D. M. Martin, R. Alfaro, J. King, J. Bohlmann, and A. L. Plant. 2006. Wound-induced terpene synthase gene expression in sitka spruce that exhibit resistance or susceptibility to attack by the white pine weevil. Plant Physiology 140:1009–1021.

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.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science 230:895–899.

Conner, J. K. 2003. Artificial selection: A powerful tool for ecologists. Ecology 84:1650–1660.

Desprez-Loustau, M.-L., B. Marcais, L.-M. Nageleisen, D. Piou, and A. Vannini. 2006. Interactive effects of drought and pathogens in forest trees. Annals of Forest Science

63:597–612.

Dobbertin, M., B. Wermelinger, C. Bigler, M. Buergi, M. Carron, B. Forster, U. Gimmi, and A. Rigling. 2007. Linking increasing drought stress to Scots pine mortality and bark beetle infestations. Thescientificworldjournal 7:231–239.

Dunn, J. P., and L. Peter Jr. 1993. Modified water regimes affect photosynthesis, xylem water potential, cambial growth and resistance of juvenile Pinus taeda L. to Dendroctonus frontalis (Coleoptera: Scolytidae).

Erbilgin, N., J. S. Powell, and K. F. Raffa. 2003. Effect of varying monoterpene concentrations on the response of Ips pini (Coleoptera : Scolytidae) to its aggregation pheromone: implications for pest management and ecology of bark beetles. Agricultural and Forest Entomology 5:269–274.

Erbilgin, N., P. Krokene, E. Christiansen, G. Zeneli, and J. Gershenzon. 2006. Exogenous application of methyl jasmonate elicits defenses in Norway spruce (Picea abies) and reduces host colonization by the bark beetle Ips typographus. Oecologia 148:426–436.

Fahn, A., and E. Zamski. 1970. The influence of pressure, wind, wounding and growth substances on the rate of resin duct formation in Pinus halepensis wood. Israel Journal of Botany 19:429–446.

Faldt, J., D. Martin, B. Miller, S. Rawat, and J. Bohlmann. 2003. Traumatic resin defense in Norway spruce (Picea abies): Methyl jasmonate-induced terpene synthase gene expression, and cDNA cloning and functional characterization of (+)-3-carene synthase. Plant Molecular Biology 51:119–133.

Faraway, J. J. 2005. Extending the Linear Model with R. CRC Press.

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.

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.

Franklin, J. F., H. H. Shugart, and M. E. Harmon. 1987. Tree death as an ecological process. Bioscience 37:550–556.

Gaylord, M. L., T. E. Kolb, and N. G. McDowell. 2015. Mechanisms of pi<remove>on pine mortality after severe drought: a retrospective study of mature trees. Tree Physiology 35:806–816.

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-Revue Canadienne De Recherche Forestiere 37:1173–1183.

Gaylord, M. L., T. E. Kolb, W. T. Pockman, J. A. Plaut, E. A. Yepez, 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.

Gershenzon, J. 1994. Metabolic costs of terpenoid accumulation in higher plants. Journal of Chemical Ecology 20:1281–1328.

Grissino-Mayer, H. D. 2001. Evaluating Crossdating Accuracy: A Manual and Tutorial for the Computer Program COFECHA. Tree-ring research.

Hamilton, J. G., A. R. Zangerl, E. H. DeLucia, and M. R. Berenbaum. 2001. The carbon-nutrient balance hypothesis: its rise and fall. Ecology Letters 4:86–95.

Haukioja, E., V. Ossipov, J. Koricheva, T. Honkanen, S. Larsson, and K. X. S. Lempa. 1998. Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization? Chemoecology 8:133–139.

Heil, M., and I. T. Baldwin. 2002. Fitness costs of induced resistance: emerging experimental support for a slippery concept. Trends in Plant Science 7:61–67.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. Quarterly Review of Biology:283–335.

Hodges, J. D., W. W. Elam, W. F. Watson, and T. E. Nebeker. 1979. Oleoresin characteristics and susceptibility of four southern pines to southern pine beetle (Coleoptera: Scolytidae) attacks. The Canadian Entomologist 111:889–896.

Hood, S., A. Sala, E. K. Heyerdahl, and M. Boutin. 2015. Low-severity fire increases tree defense against bark beetle attacks. Ecology 96:1846–1855.

Hood, S., and A. Sala. 2015. Ponderosa pine resin defenses and growth: metrics matter. Tree Physiology 35:1223–1235.

Huber, D. P. W., R. N. Philippe, L. L. Madilao, R. N. Sturrock, and J. Bohlmann. 2005. Changes in anatomy and terpene chemistry in roots of Douglas-fir seedlings following treatment with methyl jasmonate. Tree Physiology 25:1075–1083.

Kaiser, K. E., B. L. McGlynn, and R. E. Emanuel. 2013. Ecohydrology of an outbreak: mountain pine beetle impacts trees in drier landscape positions first. Ecohydrology 6:444–454.

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.

Knebel, L., D. J. Robison, T. R. Wentworth, and K. D. Klepzig. 2008. Resin flow responses to

fertilization, wounding and fungal inoculation in loblolly pine (Pinus taeda) in North Carolina. Tree Physiology 28:847–853.

Kolosova, N., and J. Bohlmann. 2012. Conifer Defense Against Insects and Fungal Pathogens. Pages 85–109 *in* Ecological Studies; The productivity and sustainability of southern forest ecosystems in a changing environment. Springer Berlin Heidelberg, Berlin, Heidelberg.

Litton, C. M., J. W. Raich, and M. G. Ryan. 2007. Carbon allocation in forest ecosystems. Global Change Biology 13:2089–2109.

Lombardero, M. J., M. P. Ayres, P. L. Lorio, and J. J. Ruel. 2000. Environmental effects on constitutive and inducible resin defences of Pinus taeda. Ecology Letters 3:329–339.

Lorio, P. L. J., and R. A. Sommers. 1986. Evidence of competition for photosynthates between growth processes and oleoresin synthesis in Pinus taeda L. Tree Physiology 2:301–306.

Marini, L., M. P. Ayres, A. Battisti, and M. Faccoli. 2012. Climate affects severity and altitudinal distribution of outbreaks in an eruptive bark beetle. Climatic Change 115:327–341.

Martin, D., D. Tholl, J. Gershenzon, and J. Bohlmann. 2002. Methyl jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. Plant Physiology 129:1003–1018.

Mattson, W. J., and R. A. Haack. 1987. The role of drought in outbreaks of plant-eating insects. Bioscience 37:110–118.

McDowell, N. G., and S. Sevanto. 2010. The mechanisms of carbon starvation: how, when, or does it even occur at all? New Phytologist 186:264–266.

McDowell, N., W. T. Pockman, C. D. Allen, D. D. Breshears, N. Cobb, T. Kolb, J. Plaut, J. Sperry, A. West, D. G. Williams, and E. A. Yepez. 2008. Mechanisms of plant survival

and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist 178:719–739.

McKay, S. A. B., W. L. Hunter, K.-A. Godard, S. X. Wang, D. M. Martin, J. Bohlmann, and A. L. Plant. 2003. Insect attack and wounding induce traumatic resin duct development and gene expression of (-)-pinene synthase in Sitka spruce. Plant Physiology 133:368–378.

Meddens, A. J. H., J. A. Hicke, and C. A. Ferguson. 2012. Spatiotemporal patterns of observed bark beetle-caused tree mortality in British Columbia and the western United States. Ecological Applications 22:1876–1891.

Messina, F. J. 1993. Heritability and“evolvability”of fitness components in Callosobruchus maculatus. Heredity 71:623–629.

Messina, F. J., and C. W. Fox. 2001. Offspring size and number. Pages 113–127 (C. W. Fox, D. A. Roff, and D. J. Fairbairn, Eds.) Evolutionary ecology: concepts and case studies.

Miller, B., L. L. Madilao, S. Ralph, and J. Bohlmann. 2005. Insect-induced conifer defense. White pine weevil and methyl jasmonate induce traumatic resinosis, de novo formed volatile emissions, and accumulation of terpenoid synthase and putative octadecanoid pathway transcripts in Sitka spruce. Plant Physiology 137:369–382.

Mitton, J. B., and S. M. Ferrenberg. 2012. Mountain pine beetle develops an unprecedented summer generation in response to climate warming. The American naturalist 179:E163–71.

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.

Moreira, X., R. Zas, and L. Sampedro. 2012. Differential Allocation of Constitutive and

Induced Chemical Defenses in Pine Tree Juveniles: A Test of the Optimal Defense Theory. PloS One 7.

Netherer, S., B. Matthews, K. Katzensteiner, E. Blackwell, P. Henschke, P. Hietz, J. Pennerstorfer, S. Rosner, S. Kikuta, H. Schume, and A. Schopf. 2015. Do water-limiting conditions predispose Norway spruce to bark beetle attack? New Phytologist 205:1128–1141.

Novick, K. A., G. G. Katul, H. R. McCarthy, and R. Oren. 2012. Increased resin flow in mature pine trees growing under elevated CO2 and moderate soil fertility. Tree Physiology 32:752–763.

Powell, J. A., and B. J. Bentz. 2009. Connecting phenological predictions with population growth rates for mountain pine beetle, an outbreak insect. Landscape Ecology 24:657–672.

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., and E. B. Smalley. 1995. Interaction of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle-fungal complexes. Oecologia 102:285–295.

Raffa, K. F., B. H. Aukema, B. J. Bentz, A. L. Carroll, J. A. Hicke, M. G. Turner, and W. H. Romme. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: The dynamics of bark beetle eruptions. Bioscience 58:501–517.

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 Sciences of the United States of America 110:2193–2198.

Regniere, J., and V. G. Nealis. 2007. Ecological mechanisms of population change during outbreaks of the spruce budworm. Ecological Entomology 32:461–477.

Reid, R. W., and J. A. Watson. 1966. Sizes, distributions, and numbers of vertical resin ducts in lodgepole pine. Canadian Journal of Botany-Revue Canadienne De Botanique 44:519–525.

Roff, D. A., S. Mostowy, and D. J. Fairbairn. 2002. The evolution of trade-offs: Testing predictions on response to selection and environmental variation. Evolution 56:84–95.

Rose, M. R., and B. Charlesworth. 1981. Genetics of life history in Drosophila melanogaster. II. Exploratory selection experiments. Genetics 97:187–196.

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.

Rouault, G., J.-N. Candau, F. Lieutier, L.-M. Nageleisen, J.-C. Martin, and N. Warzee. 2006. Effects of drought and heat on forest insect populations in relation to the 2003 drought in Western Europe. Annals of Forest Science 63:613–624.

Ryan, M. G. 2011. Tree responses to drought. Tree Physiology 31:237–239.

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.

Sampedro, L. 2014. Physiological trade-offs in the complexity of pine tree defensive chemistry. Tree Physiology 34:915–918.

Sampedro, L., X. Moreira, and R. Zas. 2011. Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. Journal of Ecology 99:818–827.

Schaller, A. 2008. Induced Plant Resistance to Herbivory. (A. Schaller, Ed.). Springer Science & Business Media, Dordrecht.

Schluter, D., T. D. Price, and L. Rowe. 1991. Conflicting selection pressures and life history trade-offs. Proceedings of the Royal Society B-Biological Sciences 246:11–17.

Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H.-P. Huang, N. Harnik, A. Leetmaa, N.-C. Lau, C. Li, J. Velez, and N. Naik. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science 316:1181–1184.

Seidl, R., M.-J. Schelhaas, and M. J. Lexer. 2011. Unraveling the drivers of intensifying forest disturbance regimes in Europe. Global Change Biology 17:2842–2852.

Stearns, S. C. 1992. The evolution of life histories 249.

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.

Team, R. 2013. R Development Core Team. R: A Language and Environment for Statistical Computing 55:275–286.

van Mantgem, P. J., N. L. Stephenson, L. S. Mutch, V. G. Johnson, A. M. Esperanza, and D. J. Parsons. 2003. Growth rate predicts mortality of Abies concolor in both burned and unburned stands. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 33:1029–1038.

Wallin, K. F., and K. F. Raffa. 2000. Influences of host chemicals and internal physiology on the multiple steps of postlanding host acceptance behavior of Ips pini (Coleoptera : Scolytidae). Environmental Entomology 29:442–453.

Wallin, K. F., and K. F. Raffa. 2004. Feedback between individual host selection behavior and

population dynamics in an eruptive herbivore. Ecological Monographs 74:101–116.

Weed, A. S., M. P. Ayres, and J. A. Hicke. 2013. Consequences of climate change for biotic disturbances in North American forests. Ecological Monographs 83:441–470.

Westbrook, J. W., A. R. Walker, L. G. Neves, P. Munoz, M. F. R. J. Resende, 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.

Westbrook, J. W., M. F. R. J. Resende, P. Munoz, A. R. Walker, J. L. Wegrzyn, C. D. Nelson, D. B. Neale, M. Kirst, D. A. Huber, S. A. Gezan, G. F. Peter, and J. M. Davis. 2013. Association genetics of oleoresin flow in loblolly pine: discovering genes and predicting phenotype for improved resistance to bark beetles and bioenergy potential. New Phytologist 199:89–100.

Wilkens, R. T., M. P. Ayres, P. L. Lorio, and J. D. Hodges. 1998. Environmental Effects on Pine Tree Carbon Budgets and Resistance to Bark Beetles. Pages 591–616 *in* Ecological Studies; The productivity and sustainability of southern forest ecosystems in a changing environment. Springer New York, New York, NY.

Williams, A. P., C. D. Allen, A. K. Macalady, D. Griffin, C. A. Woodhouse, D. M. Meko, T. W. Swetnam, S. A. Rauscher, R. Seager, H. D. Grissino-Mayer, J. S. Dean, E. R. Cook, C. Gangodagamage, M. Cai, and N. G. McDowell. 2013. Temperature as a potent driver of regional forest drought stress and tree mortality. Nature Climate Change 3:292–297.

Wimmer, R., and M. Grabner. 1997. Effects of climate on vertical resin duct density and radial growth of Norway spruce Picea abies (L) Karst. Trees-Structure and Function 11:271–276.

Wood, D. L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Annual Review of Entomology 27:411–446.