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PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Resin duct characteristics associated with tree resistance to bark beetles across lodgepole and limber pines

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Abstract Bark beetles have recently killed billions of trees, yet conifer defenses are formidable and some trees resist attack. A primary anti-insect defense of pines is oleoresin from a system of resin ducts throughout the tree. Resin defense traits are heritable, and evidence suggests that resin duct characteristics are associated with resistance to insects. However, comparisons of resin ducts in trees killed by bark beetles to trees that resisted attack are unavailable. We compared vertical resin duct characteristics (number, density, and size) and growth rates from trees that were "resistant" (survived mass attack) versus "susceptible" (killed by attack) to bark beetles in lodgepole (*Pinus contorta*) and limber (*Pinus flexilis*) pines. Resistant trees of both species had significantly more resin ducts in recent growth than susceptible trees. Discriminant analysis (DA) correctly categorized 84 % of lodgepole and 92 % of limber pines as susceptible/resistant based on combinations of resin duct and growth characteristics from recent 5- through 20-year growth intervals. DA models using measures from only the most recent 5 years of growth correctly categorized 72 and 81 % of lodgepole and limber pines, respectively. Comparing resistant to susceptible trees independent of species identity led to the correct categorization of 82 % of trees based on factors from 5- to 20-year intervals, and 73 % of trees using only resin duct counts from the most recent 5 years. We conclude that resin duct characteristics can be used to assess tree resistance to bark beetles across pine species, and offer a metric for management to enhance pest resistance.

Keywords Bark beetles · *Dendroctonus ponderosae* · Plant defense · Resin ducts · Resistance mechanisms

Introduction

Native bark beetles (Curculionidae: Scolytinae) that attack conifers as part of their reproductive cycle are the most destructive forest insects worldwide and are a leading causes of recent tree mortality (Allen et al. 2010; Meddens et al. 2012). The mountain pine beetle (*Dendroctonus ponderosae*) alone has killed billions of pine trees (*Pinus* spp.) in recent epidemics from Alaska to northern Mexico (Meddens et al. 2012). While this extensive tree mortality suggests otherwise, conifer defenses against insects can be formidable (Franceschi et al. 2005), and some trees repel bark beetle attacks even during epidemics (Hodges et al. 1979; Strom et al. 2002). Plant secondary chemistry is widely considered the most important factor in plant resistance to insects (Mumm and Hilker 2006), and the role of secondary chemistry in anti-insect defense is well documented for many plants including coniferous trees (Mumm and Hilker 2006; Kolosova and Bohlmann 2012). However, a recent meta-analysis suggests that physical defense mechanisms (e.g., latex, trichomes) and life history traits (e.g., growth rate, phenology) exert greater influences on plant resistance to insects than secondary chemistry at

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broad scales (Carmona et al. 2011). The possibility that anatomical structures linked to physical defenses in coniferous trees, independent of defensive chemistry, might determine resistance to insects has support from studies linking increasing numbers or size of resin ducts to resistance against several destructive insects (O'Neill et al. 2002; Kane and Kolb 2010; King et al. 2011; Moreira et al. 2012; Gaylord et al. 2013). Collectively, these studies suggest that resin duct measures can be used to assess tree resistance to insects such as bark beetles. However, studies comparing resin duct characteristics of trees that were attacked and killed by bark beetles to trees that directly resisted bark beetles are not readily available in the literature. To investigate the importance of resin ducts for tree resistance to bark beetles, we compared bark beetle-resistant and -susceptible trees attacked over a 2-year period in a shared environment.

In pines, oleoresin (hereafter resin) is a primary defense against bark beetles. Resin is produced and stored in a system of vertical and horizontal ducts in a tree's phloem and xylem (Bannan 1936; Franceschi et al. 2005). Resin ducts function as a physical constitutive defense as they exist in a tree prior to insect attack, and as an inducible defense that produces resin in response to wounding or infection (Franceschi et al. 2005; Kolosova and Bohlmann 2012). Pine resin exudation can also be both a mechanical (physical) defense that repels or mires bark beetles and a chemical defense, as resin is typically laden with monoterpenes and diterpene acids which have been documented to reduce bark beetle fitness (Raffa et al. 2005; Faccoli and Schlyter 2007). Importantly, experimental evidence from pines and other conifers indicates that resin defense characteristics such as flow rates and chemistry 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).

Forest management to reduce the risk of bark beetle infestation is often based on the assertion that slow growing trees are "weaker" than fast growing trees and thus more likely to be killed by insects (e.g., Fettig et al. 2007). This assertion conflicts with documented patterns of greater herbivory and mortality rates in faster growing trees (Loehle 1988; Ruel and Whitham 2002; Wright et al. 2010). In addition, plant defense theory predicts that anti-insect defenses are costly to produce in terms of fitness, so fast growth should be accompanied by reduced allocation to defense (Herms and Mattson 1992; Heil and Baldwin 2002). Growth–defense tradeoffs have been documented for a range of defense mechanisms and plant species including coniferous trees (Koricheva 2002; Mooney et al. 2010; Zust et al. 2011). However, studies finding no evidence of growth–defense tradeoffs in conifers are also available, raising questions about how growth influences defense in coniferous trees (Hard 1985; McDowell et al. 2007; Gaylord et al. 2007). These conflicting reports could be due to variation in experimental designs, differing measures of growth rates (i.e. tree radial growth vs. basal area growth), or complicating effects of resource limitation or climate on plant physiology and defense (Koricheva 2002). Regardless, vertical resin ducts offer a long-term record of tree defense in relation to growth rates estimated from tree rings, and may also offer a way to assess tree resistance to insects prior to management efforts.

Using two widespread tree species, lodgepole pine (*Pinus contorta,* subgenus *Pinus*) and limber pine (*Pinus flexilis,* subgenus *Strobus*), we compared bark beetle-resistant trees (survived mass attack) to bark beetle-susceptible trees (killed by mass attack) to assess the roles of vertical resin duct number, density, and size, along with tree growth rates in resistance to the mountain pine beetle (*D. ponderosae*). Both lodgepole and limber pine have suffered extensive mortality from the mountain pine beetle and often co-occur in high elevation forests. By comparing bark beetle resistant to susceptible trees in a common environment and using species representing long-diverged subgenera (~90 million years ago; He et al. 2012) our study is the first, to our knowledge, to quantify resin duct defenses in pines known to be bark beetle-resistant, and also the first study of resin duct defenses and bark beetle attack for the pine subgenus *Strobus* (five-needle pines). We hypothesized (1) that bark beetle-resistant trees of both species would have more vertical resin ducts, greater resin duct density (ducts/wood area), or larger resin ducts (cross-sectional area) in recent annual growth in the xylem, and (2) that resistant trees would exhibit growth-defense tradeoffs whereby their larger investment in resin duct defenses would result in slower growth. Ultimately, our goal was to determine if resin duct and/or growth rate characteristics could be used to differentiate among "resistant" and "susceptible" trees—our results supported this goal as models based primarily on resin duct counts accurately assessed tree-resistance both within and among the pine species studied.

Methods

Study site and tree sampling

We compared bark beetle-resistant to bark beetle-susceptible trees at the University of Colorado Mountain Research Station, 10 km east of the Continental Divide, Colorado, USA (40°1′49″N, 105°31′56″W; 2,900 m asl). The climate and soils of this site were described by Duhl, et al. (2013); monthly air temperatures from 1970 to 2008 were described by Mitton and Ferrenberg (2012). To minimize environmental and climatic differences across our study, we studied 102 trees (independent replicates) attacked by bark beetles; 25 resistant and 25 susceptible lodgepole pines and 26 resistant and 26 susceptible limber pines selected from a common 2.5-ha plot. Lodgepole pines in our study had a mean age (±1SE) of 117 (±6) years and a mean diameter at breast height (DBH, 1.37 m above ground level) of 22.0 (±0.7) cm. Limber pines had a mean age of 253 (±10) years and a mean DBH of 23.7 (±1.0) cm. All trees were mass-attacked by the mountain pine beetle (≥20 individual attacks on the lower 2 m of the trunk) between 2008 and 2010. Trees categorized as bark beetle-resistant survived for ≥2 years after attack, while trees categorized as susceptible died within 1 year of attack. We extracted a 12-mm-diameter increment core at DBH from each tree and mounted the cores on wooden blocks, sanded each with a progression of coarse to fine sandpaper, and created a flat cross-section for analysis, as in Kane and Kolb (2010).

Resin duct measures and tree growth

Using a stereo-microscope, we counted the number of vertical resin ducts in each of the most recent 20 growth rings (no. year^{-1}). Mean resin duct cross-sectional areas (mm^2) and variation in area (standard error of mean duct area) of each tree were calculated from all resin ducts in the most recent 5 years of growth rings using high resolution scans (9,600 dpi) and analysis tools in Photoshop CS2. We measured annual radial growth (mm year^{-1}) of the most recent 20 years using scans in CooRecorder 7.6 (Cybis Elektronik and Data, 2012). To simplify analyses, and to understand what intervals of tree growth are most important for insect resistance, we binned resin duct totals and densities, as well as growth measures for each tree into four overlapping time intervals that included the most recent 5-, 10-, 15-, and 20-year totals, and used the growth rate and resin duct totals of the entire interval to calculate resin duct density (resin ducts mm^{-2} of wood). We also used radial growth to calculate each tree's basal area increment (BAI) for the 5- through 20-year intervals of each tree. BAI in our study estimates tree growth as the percentage of a tree's total cross-sectional area (mm^2) comprised by a specific interval of growth.

Data analysis

For each species, we compared resin duct numbers, density, and size, and radial growth and BAI of resistant and susceptible trees for each time interval (5, 10, 15, and 20 years) via *t* tests on log-transformed data or Wilcoxon tests when assumptions of normality were not met by transformations. We examined relationships between tree growth and defenses within species via linear regression to relate radial growth or BAI for the 20-year interval (independent variables) to vertical resin duct counts and density (dependent variables) over the 20-year interval in each tree. Similarly, we used linear regression to examine the relationship between growth (radial and BAI) over the most recent 5 years to mean vertical resin duct size for each tree (dependent variable).

Using discriminant analysis (DA; a multivariate method that tests the ability of continuous variables to identify pre-assigned categorical groups), we attempted to correctly categorize bark beetle-resistant and -susceptible trees of each species based on the 5- through 20-year interval measures of resin duct characteristics (counts, density, size, and variation) and 5- through 20-year tree growth (radial and BAI). We created three DA models for lodgepole and limber pine, and three models for the two species combined that considered resistant and susceptible trees independent of species identity. For the first model (individual species or grouped), we used principal coordinates analysis (PCoA) to reduce the data from all resin duct and growth measures of the overlapping time intervals into axis scores—a method that allowed us to avoid violating assumptions regarding multiple collinearity in DA. The PCoA axis scores were then used to create a model that used the fewest axes to achieve the highest level of correct categorization of trees. The second DA models represented a best fit model constrained to only one time interval measure for each factor (i.e. each factors such as resin duct density could only be represented by one interval from the 5 through 20 years) to correctly categorize the largest % of trees. The third and final DA models were based only on the 5-year interval of each factor and used the fewest factors necessary to correctly categorize the largest % of trees.

Results

Resin ducts and bark beetle-resistance

Bark beetle-resistant trees for both lodgepole pine and limber pine had significantly more resin ducts in the most recent 5 and 10 years of growth measured in 12-mm-wide increment cores ($P < 0.05$; Fig. 1) than did susceptible (bark beetle-killed) trees. Specifically, bark beetle-resistant lodgepole pines had 23 % more resin ducts and resistant limber pines had 37 % more resin ducts in their most recent 5 years of radial growth (5 years of rings) on average than susceptible trees. Extended to the most recent 10 years of growth, resistant lodgepole pines had 21 % more, and resistant limber pines had 18 % more, resin ducts than bark beetle-susceptible trees. Over the 15- and 20-year growth interval, resistant trees of both species consistently had more resin ducts, but with no significant difference between groups ($P > 0.05$; Fig. 1).

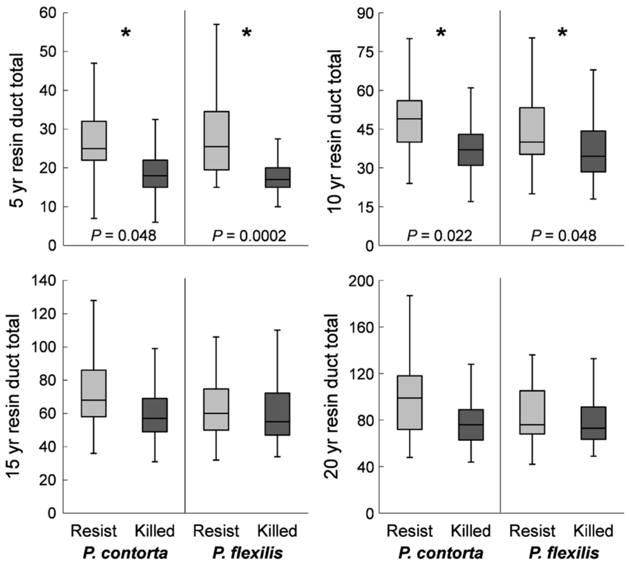

Fig. 1 Vertical resin duct counts in the most recent 5, 10, 15, and 20 years of growth rings from lodgepole (*Pinus contorta*) and limber (*P. flexilis*) pines that either resisted bark beetle attack or were killed (susceptible) by mass attack of mountain pine beetles. It is important to note that the values shown here are counts from 12-mm-wide increment cores; scaling these measures up to account for the whole tree stem would result in differences of many thousands of resin ducts between resistant and susceptible trees. Significant differences are indicated by an *asterisk*; *boxes* show medians and 1st and 3rd quartiles, and *whiskers* indicate 1.5 inter-quartile range or ~97 % of the variation in untransformed data

While mean resin duct density (vertical resin ducts/ mm^{-2} of wood growth) of bark beetle-resistant lodgepole pine trees was roughly 8 % greater than that of bark beetle-susceptible trees, resin duct density did not significantly differ between the groups for any interval of lodgepole annual growth rings ($P > 0.05$; Fig. 2). However, resin duct density in bark beetle-resistant limber pine trees was significantly greater than in bark beetle-susceptible trees over all the time intervals from 5 to 20 years ($P < 0.05$; Fig. 2). Resistant limber pines had 39, 37, 32, and 28 % greater resin duct densities than susceptible trees over the 5-, 10-, 15-, and 20-year growth intervals, respectively.

Resin duct size (mm^2 of cross-sectional area) did not differ significantly between bark beetle-resistant (0.018 ± 0.001) and susceptible (0.020 ± 0.001) lodgepole pine trees ($P > 0.05$), but was significantly different ($P < 0.05$) in limber pine with resistant trees having smaller resin ducts (0.014 ± 0.001) than susceptible trees (0.017 ± 0.001). Similar to resin duct size, variation in resin duct size (standard error of resin duct areas within a tree) did not significantly differ between bark beetle-resistant (0.0155 ± 0.0002) and susceptible (0.00143 ± 0.0001) lodgepole pine trees ($P > 0.05$), but did differ for limber pine with resistant trees having less variation in duct size (0.0008 ± 0.00007) than susceptible trees (0.0011 ± 0.00009) ($P < 0.05$).

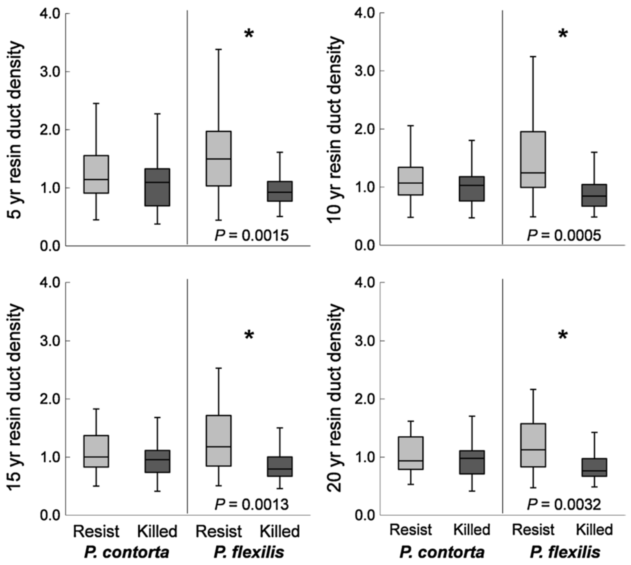

Fig. 2 Vertical resin duct density (ducts/mm^2 of wood) in the most recent 5, 10, 15, and 20 years of growth rings from lodgepole (*P. contorta*) and limber (*P. flexilis*) pines that either resisted bark beetle attack or were killed (susceptible) by beetles. Significant differences are indicated by an *asterisk*; *boxes* show medians and 1st and 3rd quartiles, and *whiskers* indicate 1.5 inter-quartile range or ~97 % of the variation in untransformed data

Table 1 Tree radial growth and basal area increment (*BAI*) over the most recent 5-, 10-, 15-, and 20-year growth intervals in lodgepole (*Pinus contorta*) and limber (*P. flexilis*) pines that either resisted bark beetle attack or were killed by bark beetles

Growth type	*P. contorta*		*P. flexilis*	
	Resist	Killed	Resist	Killed
5-year radial	1.91 (0.15)	1.68 (0.14)	1.80 (0.17)	1.83 (0.20)
10-year radial	3.96 (0.29)	3.61 (0.30)	3.10 (0.25)	4.00 (0.41)
15-year radial	6.23 (0.47)	5.94 (0.45)	4.84 (0.38)^{b}	6.70 (0.67)^{a}
20-year radial	8.65 (0.67)	8.06 (0.54)	6.89 (0.56)^{b}	9.10 (0.90)^{a}
5-year BAI	3.75 (0.29)^{a}	3.02 (0.28)^{b}	3.14 (0.38)	3.02 (0.62)
10-year BAI	7.73 (0.60)	6.47 (0.62)	5.48 (0.56)	6.41 (1.31)
15-year BAI	11.98 (0.93)	10.53 (0.92)	8.59 (0.77)	10.62 (2.17)
20-year BAI	16.39 (1.29)	14.15 (1.13)	12.17 (1.08)	14.29 (2.87)

Basal area increment (*BAI*) is a measure of the % of total stem area comprised by the specified interval. Trees that resisted bark beetles were compared to those killed within a species via *t* tests on log-transformed values; untransformed means with 1SE in parentheses are shown here; mean ± SE followed by different letters are significantly different ($P < 0.05$)

Tree growth and bark beetle-resistance

Tree radial growth (mm time $interval^{-1}$) in bark beetle-resistant lodgepole pines exceeded radial growth of susceptible trees by up to 12 %, but was not significantly greater for any of the 5- through 20-year growth intervals

($P > 0.05$; Table 1). An opposite trend was present in limber pines, where bark beetle-resistant trees had less total radial growth over the 5- and 10-year growth intervals and significantly less radial growth over the 15- and 20-year intervals ($P < 0.05$; Table 1). Specifically, resistant limber pines had 38 and 32 % less radial growth than susceptible limber pines over the most recent 15 and 20 years of growth respectively.

Tree growth considered as the basal area increment (BAI) also indicated that bark beetle-resistant lodgepole pine trees tended to have greater growth rates than bark beetle-susceptible trees, while resistant limber pine trees tended to have lower growth rates than susceptible trees (Table 1). BAI of resistant lodgepole pines was significantly greater (24 %) than in susceptible trees over the most recent 5 years of tree growth ($P < 0.05$; Table 1), but, for all other time intervals (10, 15, and 20 years), differences were non-significant ($P > 0.05$) despite resistant trees having a roughly 16 % greater BAI over time. While bark beetle-resistant limber pine trees had a BAI roughly 17 % less than bark beetle-susceptible trees over the 20 years of growth rings, the lower BAI in resistant trees was not significantly different from susceptible trees regardless of time interval ($P > 0.05$; Table 1).

Tree growth had a moderate, significantly positive relationship with resin duct defenses in both lodgepole pine and limber pine trees. In both species, tree radial growth over the most recent 20 years was positively related to resin duct counts ($r^2 = 0.21$ and 0.22 in lodgepole and limber pine, respectively; $P < 0.05$ for both) (Fig. 3). BAI was also significantly related to resin duct counts in lodgepole pine trees ($r^2 = 0.17$, $P < 0.05$; Fig. 3), but the relationship was weaker than that of radial growth and resin duct counts. BAI was not significantly related to resin duct counts in limber pine trees ($r^2 = 0.08$, $P > 0.05$; Fig. 3). Tree radial growth had a stronger positive relationship than BAI with resin duct size for both tree species ($r^2 = 0.36$ and 0.15 in lodgepole and limber pine, respectively; $P < 0.05$) (Fig. 4). BAI was also significantly related to resin duct size in both lodgepole ($r^2 = 0.11$, $P < 0.05$) and limber ($r^2 = 0.17$, $P < 0.05$; Fig. 4) pine trees.

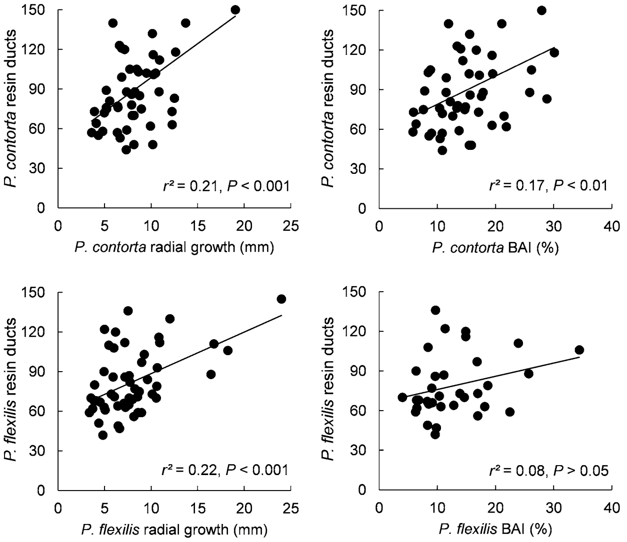

Fig. 3 Relationship between tree radial growth (mm per last 20 years) or basal area increment (*BAI*; % of total tree basal area comprised by the last 20 years of growth) and resin duct counts over the last 20 years in lodgepole (*P. contorta*) and limber (*P. flexilis*) pines

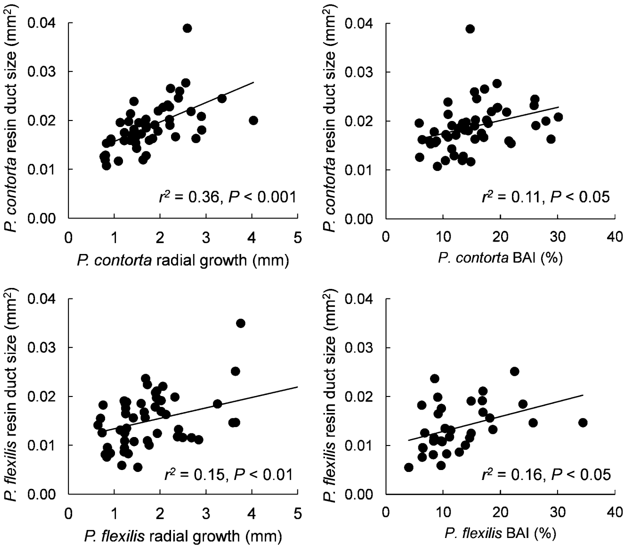

Fig. 4 Relationship between tree radial growth (mm per last 20 years) or basal area increment (*BAI*; % of total tree basal area comprised by the last 20 years of growth) and resin duct size (mm^2) in lodgepole (*P. contorta*) and limber (*P. flexilis*) pines

Predicting tree resistance and susceptibility

Discriminant analysis (DA) was consistently able to better categorize limber pine trees as resistant or susceptible than lodgepole pines. While the best fit DA models (explaining the greatest amount of variation with the fewest variables) for both pine species included resin duct counts and density, as well as radial growth and BAI measures, the two species differed in the importance of resin duct size for categorizing trees—i.e., the best fit model for limber pine did not include measures of resin duct size or variation in resin duct size, while resin duct size was included in the best fit model for lodgepole pine. Overall, models based on multivariate combinations of resin duct measures and tree growth reduced into PCoA axis scores were able to correctly categorize 84.0 % of lodgepole pines and 92.3 % of limber pines as resistant (survived bark beetle attacks) or susceptible (killed by bark beetle attacks) (Table 2). Both DA models resulted in an area under the receiver operating characteristic (ROC) curve ≥ 0.93 (a value of 1.0 indicates a perfect test, a value of 0.5 indicates a worthless test) indicating that both models are strong tests for discriminating

Table 2 Discriminant analysis classifications of bark beetle-resistant versus bark beetle-susceptible (killed) trees based on vertical resin duct totals, vertical resin duct mean area and standard errors, tree radial growth, and tree basal area increment characterized over 5-, 10-, 15-, and 20-year intervals in lodgepole (*P. contorta*) and limber (*P. flexilis*) pines

Species	Model[a]	Variables included[b]	Classified (%)	Area under ROC	Log likelihood	Wilks' λ	*F*	*df*	*P*
Pinus contorta	All measures	PCoA axis scores (8 axes included)	84.0	0.92	35.7	0.50	5.1	8, 41	<0.001
	Best fit	RDT$_{(20)}$, RDD$_{(20)}$, Mean RDA, RG$_{(10)}$, BAI$_{(20)}$	80.0	0.78	29.7	0.82	1.9	5, 44	0.11
	5-year only	RDT$_{(5)}$	72.0	0.72	32.6	0.92	4.1	1, 48	<0.05
Pinus flexilis	All measures	PCoA axis scores (6 axes included)	92.3	0.95	26.9	0.40	11.2	6, 45	<0.001
	Best fit	RDT$_{(5)}$, RDD$_{(5)}$, RG$_{(10)}$, BAI$_{(20)}$	88.5	0.92	17.6	0.45	14.1	4, 47	<0.001
	5-year only	RDT$_{(5)}$, RDD$_{(5)}$, RG$_{(5)}$, BAI$_{(5)}$	80.8	0.88	22.3	0.57	9.0	4, 47	<0.001
Species combined	All measures	PCoA axis scores (6 axes included)	82.3	0.89	87.1	0.59	11.0	6, 95	<0.001
	Best fit	RDT$_{(5)}$, RDD$_{(5)}$, RG$_{(15)}$, BAI$_{(5)}$	76.5	0.83	52.2	0.69	10.8	4, 97	<0.001
	5-year only	RDT$_{(5)}$	72.6	0.75	62.4	0.85	17.5	1, 100	<0.001

[a] Best fit models are those that correctly categorize the largest % of trees using the fewest total variables combined from any time interval (5-, 10-, 15-, or 20-year values); best 5-year only models correctly categorize the largest % of trees using the fewest variable from only the 5-year interval

[b] Variables included: resin duct totals (*RDT*), resin duct density (*RDD*), mean resin duct area (*RDA*), standard error of RDA (*SE RDA*), tree radial growth (*RG*), and basal area increment (*BAI*)

between resistant and susceptible tree groups. Simplifying the DA models to best fit using one time interval (either 5-, 10-, 15-, or 20-year values) for resin duct total, density, size, or duct size variation, and radial growth and BAI resulted in the correct categorization of 80.0 % of lodgepole (area under ROC = 0.78) and 88.5 % of limber pine (area under ROC = 0.92) trees as resistant or susceptible (Table 2). Finally, best fit DA models using only measures from the most recent 5 years of annual growth rings correctly categorized 72.0 % (area under ROC = 0.72) and 80.8 % (area under ROC = 0.88) of lodgepole and limber pine trees, respectively (Table 2).

Combining the two species into one best fit DA model, independent of species identity, correctly categorized 82.3 % of trees as resistant or susceptible (area under ROC = 0.89) (Table 2; Fig. 5). A best fit simple DA model, using only one time interval (either 5-, 10-, 15-, or 20-year values) for resin duct total and density, and radial growth and BAI resulted in the correct categorization of 76.5 % of trees (area under ROC = 0.83), while a final DA model based only on the most recent 5-year resin duct totals from each tree correctly categorized 72.6 % of trees from the two species combined as resistant or susceptible (area under ROC = 0.75) (Table 2).

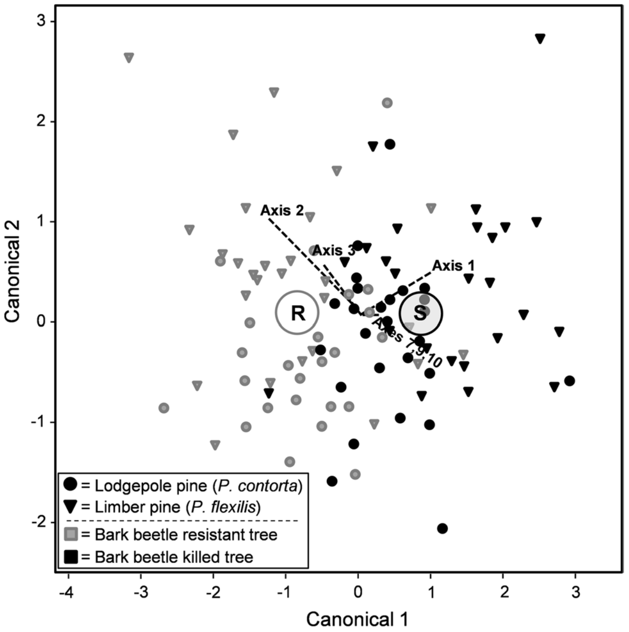

Fig. 5 Canonical correlation plot illustrating categorization of bark beetle-resistant (*gray* markers) and susceptible (killed) trees (*black* markers) via discriminant analysis (DA). DA was performed on the tree species combined; for reference lodgepole pines (*P. contorta*) are shown as *circles* and limber pines (*P. flexilis*) as *triangles*. *Open circles* denoted by *R* and *K* show the central ordination point for resistant and susceptible trees, respectively; *vectors* indicate the strength and directional influence of PCoA axis scores used for data reduction

Discussion

Resin ducts and bark beetle-resistance

Bark beetle epidemics have renewed interest in forest pest dynamics, and much work has explored the role of tree secondary chemistry and mechanical resin flow on bark beetle host selection (Byers 1995; Raffa et al. 2005; Boone et al. 2011; Kolosova and Bohlmann 2012). Less work has focused on pine tree anatomical characteristics that might be associated with resistance to bark beetles such as bark traits or the expression of resin ducts, yet available studies suggest strong influences of anatomical characters on bark beetle attack densities and tree mortality (Kane and Kolb 2010; Gaylord et al. 2013). Importantly, a recent meta-analysis indicates that plant life history and physical traits are often stronger influences on plant resistance than chemical traits (Carmona et al. 2011). While we did not measure tree secondary chemistry, our results offer additional support for a strong role of a physical trait—i.e. vertical resin duct production—in determining pine tree resistance to bark beetles. We found support for our first hypothesis that resistant trees would have greater numbers of resin ducts, as both lodgepole and limber pines that resisted bark beetles had significantly more vertical resin ducts over the last decade of growth than trees killed by bark beetle attacks (Fig. 1). More resin ducts should translate into greater resin production, storage, and flow during insect attack, likely explaining the differences in resin duct numbers between resistant and susceptible trees. Through de novo formation of traumatic resin ducts, conifers can increase the number of vertical resin ducts when attacked by insects or infected by fungal pathogens. However, these traumatic resin ducts form in the developing secondary xylem (the woody portion of a developing growth ring) and not in the previously developed xylem of earlier growth rings (Martin et al. 2002; Hudgins and Franceschi 2004). The formation of additional resin ducts in a portion of only one growth ring in trees used in our study is possible, but cannot be the driver of the results we present here, given that the pattern of greater resin duct numbers and density in resistant trees than in susceptible trees is present over the full two-decade period (Figs. 1, 2). Also, significant differences in resin duct counts between resistant and susceptible trees were found over a decade of growth rings in both tree species (Fig. 1)—a result that is retained (but not shown here to avoid redundancy) when the growth rings with the greatest number of resin ducts are excluded from each tree's total count and the data are reanalyzed.

Also, using discriminant analysis, we were able to correctly categorize 84 % of lodgepole and 92.3 % of limber pines as bark beetle-resistant or -susceptible based on combinations of resin duct and growth rate measures (Table 2). Considering both species together, we were still able to correctly categorize 82.3 % of trees using combinations of factors and 72.6 % of trees using just the resin duct counts from the last 5 years of growth (Table 2).

Our models had reasonably high success categorizing trees as resistant and susceptible without measures of secondary chemistry for these two pine species (from long-diverged subgenera) that vastly differ in their respective constitutive and inducible secondary chemistry (Smith 2000). While our study is the first to compare vertical resin ducts in the xylem of pines that resisted bark beetle attacks to pines that died from attacks, the role of resin ducts in tree resistance to insects is not without some precedent. For example, greater vertical resin duct density was shown to increase resistance of Sitka spruce (*Picea sitchensis*) to white pine weevils (*Pissodes strobi*) (O'Neill et al. 2002; King et al. 2011; Moreira et al. 2012), while smaller resin duct size was linked to higher densities of the piñon *Ips* beetle (*Ips confuses*) on piñon pines (*P. edulis*) (Gaylord et al. 2013). Also, a combination of resin duct density and size was highly successful at separating trees selected and killed by bark beetles from neighboring trees that were not attacked by bark beetles in logistic models following a bark beetle infestation in ponderosa pines (*P. ponderosa* var. *scopulorum*) (Kane and Kolb 2010).

Tree growth and bark beetle-resistance

We found evidence that tree growth rates may influence anti-insect defense in conflicting ways for lodgepole and limber pines, leading to mixed support for our second hypothesis, derived from the growth–differentiation balance hypothesis (Lerdau et al. 1994), that greater resin duct defenses would result in less growth. Results from lodgpole pines countered this hypothesis, as susceptible trees had smaller basal area increment (BAI) growth over the last 5 years of their lives (as well as fewer resin ducts) than resistant lodgepole pines, with no significant difference in radial growth between groups. Meanwhile, results for limber pines supported our hypothesis, as limber pines killed by bark beetles had greater radial growth over the past 15–20 years of their lives (along with fewer resin ducts, suggesting a growth–defense trade-off) than resistant limber pines, but with no significant difference in BAI between groups. Interestingly, we found a positive relationship between both growth measures and total resin ducts for lodgepole pine, and between radial growth and total resin ducts in limber pine (Fig. 3), as well as positive relationships of growth measures and resin duct size for both species (Fig. 4). These concomitant increases in growth and defense seem to run counter to plant defense theory as well as reports of growth–defense trade-offs in other plant species (Herms and Mattson 1992; Heil and Baldwin 2002; Mooney et al. 2010; Zust et al. 2011; Sampedro et al. 2011; Moreira et al. 2012). However, an absence of growth–defense trade-offs has been noted for systems characterized by resource limitation and plants with terpenoid-based defenses, including other coniferous forest types and trees (Hard 1985; Christiansen et al. 1987; Koricheva 2002; McDowell et al. 2007), and neither radial growth nor BAI are complete measures of tree growth, since they consider only changes in stem diameter or area. Nevertheless, if all trees had equal energy budgets to be apportioned between growth and defense, then trade-offs would likely become apparent (Koricheva 2002). However, conifers are among the most genetically variable groups of organisms known, with substantial variability in photosynthetic capacity, respiration and growth rates, water potential, and resin duct density within and among species, and many of these traits are heritable, or at least influenced by genetics (Linhart and Mitton 1985; Cornelius 1994; Neale and Savolainen 2004; Chhatre et al. 2013; Benkman and Parchman 2013). Therefore, the energy budgets of trees in a stand can vary, and some trees, by virtue of greater ability to fix carbon, can allocate to both growth and defense with no immediate trade-off.

The increase in resin duct size with larger growth rates we found here, regardless of underlying cause, could explain why we did not find resin duct density to be significantly different for bark beetle-resistant and -susceptible lodgepole pines. Resistant lodgepoles had a greater BAI and resin duct counts than trees killed by bark beetles. The associated increase of resin duct size with increasing growth could lead to greater defense without increased duct density, thereby minimizing the effect of total wood area on defense (Fig. 2). Yet, we found that resistant and susceptible lodgepole pines did not differ with respect to resin duct sizes, and that resistant limber pine trees had smaller resin ducts than susceptible trees, calling into question the importance of resin duct size in defense against insects for the pine species studied here.

Predicting tree resistance and susceptibility

Anti-insect defenses of plants are influenced by genotype, phenology, physiology, and location on the plant itself (e.g., roots vs. leaves) among other factors (Barton and Koricheva 2010; Muola et al. 2010; Erbilgin and Colgan 2012) which can lead to difficulty in characterizing plant defenses under natural conditions. Additionally, plant defenses can be plastic in response to biotic and abiotic factors that can vary over time (Huberty and Denno 2004; Heil 2010; Hart et al. 2013). As a result, characterizing anti-insect defenses of long-lived trees based on secondary chemistry may accurately assess current status, but fail to capture trends in tree defense. Our results indicate that relatively short-term trends in tree defenses (5–10 years) are sufficient to characterize defense against bark beetles. Nevertheless, vertical resin ducts of lodgepole pine can remain active for 25 years (Reid et al. 1967) and are responsive to inter-annual climate factors in Norway spruce (Wimmer and Grabner 1997), suggesting that tree defense structures might operate on, or be influenced by conditions, over long temporal and spatial scales. However, insect life-histories could influence the importance of older structures for tree resistance. For example, primary bark beetles (e.g., the mountain pine beetles) are active beneath the tree bark near newer growth, while wood-boring beetles (Coleoptera: Buprestidae and Cerambycidae) burrow deeper into a tree's xylem, possibly contacting older resin ducts.

We found the inclusion of measures such as resin duct size (mm^2), variation in resin duct size (standard error of resin duct areas), and tree growth rates in discriminant analysis can lead to a greater percentage of trees being correctly classified as "resistant" or "susceptible". However, using just resin duct counts from 5 years of tree growth led to the correct categorization of >72 % of all trees across species. Thus, reasonable levels of accuracy for characterizing pine tree resistance to bark beetles can be achieved without time-consuming measures. Also, while we and others have pointed out the possibility that physical defenses outweigh chemical defenses of pines in their effect on insect-resistance (e.g., Strom et al. 2002), a role of tree secondary chemistry in defense against bark beetles has been shown in numerous studies (e.g., Christiansen et al. 1987; Mumm and Hilker 2006; Raffa et al. 2013). Our results indicate that resin duct assessments are more accurate for categorizing limber pine trees as opposed to lodgepole pine—a result that is possibly due to the "stronger" anti-insect defensive chemistry of lodgepole pine compared to white pine species (subgenus *Strobus*) such as limber pine (Smith 2000; Raffa et al. 2013). Thus, the effort placed on characterization of pine defenses should be guided by pine evolutionary history and experimental design.

Our results suggest that vertical resin ducts in coniferous tree xylem can be used to asses insect resistance for ecological and evolutionary biology studies and also for forest management. While measuring resin duct characteristics in forest stands that have not undergone recent bark beetle infestations complicates the identification of characteristics associated with trees predisposed to bark beetle mortality, vertical resin ducts in recent xylem could still be used to characterize "mean resistance" of trees. This assessment of variation in resin duct characters might then enable "resistance-based thinning", where harvesting efforts target trees with lower resin duct counts until the desired tree density is attained. While tree survival if attacked by bark beetles or other insects is not ensured by this method, overall levels of resistance against insects should increase across the managed forest as per capita levels of resin defense increase.

Conclusions

Tree mortality from bark beetles during recent epidemics has renewed interest in predicting the spatial and temporal population dynamics of these destructive forest insects. However, detailed consideration for tree anti-insect defenses in bark beetle risk models remains rare (e.g., Fuentealba et al. 2013), in part due to difficulty in finding a consistent metric for assessing resistance/susceptibility across tree species. We found bark beetle-resistant trees had more resin ducts than -susceptible trees, and that resin duct characteristics from recent growth can be used with high accuracy to differentiate among resistant and susceptible trees both within and across pine species—even for pine species belonging to long-diverged subgenera used in this study. Combined with work by Kane and Kolb (2010) that showed bark beetles preferentially attacked pine trees with fewer resin ducts and avoided trees with greater numbers of resin ducts, our results suggest that selection during insect epidemics favors trees with larger numbers of vertical resin ducts—either by reducing the likelihood of insect attack or by conferring greater resistance after attack. Because resin defense traits (e.g., resin production, flow, chemical content) are heritable genetic traits in pines and other conifers (Rosner and Hannrup 2004; Sampedro et al. 2011; Westbrook et al. 2013; Chhatre et al. 2013), forest managers could use selective removal of "susceptible" trees as part of a strategy to select for long-term, tree resistance to bark beetles.

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