

Anatomical defences against bark beetles relate to degree of historical exposure between species and are allocated independently of chemical defences within trees

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Funding information

Natural Sciences and Engineering Research Council of Canada-Discovery Grant; UW-Madison Hatch funds to KFR; UW-Madison Graduate School, Department of Forest & Wildlife Ecology and College of Agricultural and Life Sciences to PAT and KFR

Abstract

Conifers possess chemical and anatomical defences against tree-killing bark beetles that feed in their phloem. Resins accumulating at attack sites can delay and entomb beetles while toxins reach lethal levels. Trees with high concentrations of metabolites active against bark beetle-microbial complexes, and more extensive resin ducts, achieve greater survival. It is unknown if and how conifers integrate chemical and anatomical components of defence or how these capabilities vary with historical exposure. We compared linkages between phloem chemistry and tree ring anatomy of two mountain pine beetle hosts. Lodgepole pine, a mid-elevation species, has had extensive, continual contact with this herbivore, whereas high-elevation whitebark pines have historically had intermittent exposure that is increasing with warming climate. Lodgepole pine had more and larger resin ducts. In both species, anatomical defences were positively related to tree growth and nutrients. Within-tree constitutive and induced concentrations of compounds bioactive against bark beetles and symbionts were largely unrelated to resin duct abundance and size. Fewer anatomical defences in the semi-naïve compared with the continually exposed host concurs with directional differences in chemical defences. Partially uncoupling chemical and morphological antiherbivore traits may enable trees to confront beetles with more diverse defence permutations that interact to resist attack.

KEYWORDS

carbohydrates, climate change, herbivory, lodgepole, minerals, phenolics, plant defence, resin ducts, terpenes, whitebark

1 | INTRODUCTION

Plant defences against herbivores often entail multiple modalities, rather than single attributes (Fürstenberg-Hägg, Zagrobelny, & Bak,

2013; Mithöfer & Boland, 2012; Schuman & Baldwin, 2016). These defences are commonly divided into broad categories such as anatomical, chemical, and indirect, with multiple specific traits operating within each category. However, we know relatively little about the patterns

and extents by which various defence modalities and their specific traits co-occur within species or individuals (Agrawal & Fishbein, 2006). Relationships among different modalities can influence plant allocation and fitness, because investment in each component incurs significant costs (Herms & Mattson, 1992; Huot, Yao, Montgomery, & He, 2014; Strauss, Rudgers, Lau, & Irwin, 2002). The effectiveness of various anatomical and chemical traits may be complementary, such as through transport of toxic chemicals, initiation of defence cascades, or trichomes that contain or exude toxins (Ambrósio et al., 2008; Glas et al., 2012; Peiffer, Tooker, Luthe, & Felton, 2009; Rasmann & Agrawal, 2009). Variability in plant defence traits can influence herbivore population dynamics, and whether herbivores can adapt to defences sequentially or must do so simultaneously (Alba, Bowers, Blumenthal, & Hufbauer, 2014). The sensitivity of plant–herbivore relationships to rapid environmental change may be particularly responsive to how defence components vary among and within species, such as when rising temperatures facilitate insect range expansions into new habitats (Anderegg et al., 2015; Bale et al., 2002; Parmesan, 2006).

Conifers are among the most widely distributed plants and often define expansive terrestrial biomes (Allen et al., 2010; Anderegg et al., 2015). The most important biotic threats confronting conifers are bark beetles (Curculionidae: Scolytinae) and their associated microbiota, which colonize mature trees (Meddens, Hicke, & Ferguson, 2012). Host colonization destroys vascular tissues and restricts water and nutrient transport, ultimately causing tree death.

Conifer defences against bark beetle–microbial complexes are multimodal and include both anatomical and chemical components (Erbilgin, 2018; Franceschi, Krokene, Christiansen, & Krokene, 2005; Krokene, 2015; Raffa, Aukema, Erbilgin, Klepzig, & Wallin, 2005). Upon injury, *Pinus* species exude oleoresin at the beetle entry site (Croteau, Gurdewitz, Johnson, & Fisk, 1987; Keeling & Bohlmann, 2006; Trapp & Croteau, 2001) and induce additional biosynthesis of terpenoid and phenolic components (Raffa et al., 2017). Resinous secretions consist of complex terpenoid mixtures and can physically expunge or entomb attacking beetles, prevent or mask the emission of pheromones beetles need to coordinate mass attacks, or delay beetle progress whereas induced toxins are synthesized (Franceschi et al., 2005; Raffa et al., 2005). Various terpenoid classes have different impacts on bark beetle–microbial complexes, with monoterpenes being toxic and repellent to beetles at high doses (Chiu, Keeling, & Bohlmann, 2017; Reid & Purcell, 2011; Reid, Sekhon, & LaFramboise, 2017; Wallin & Raffa, 2004), diterpene acids being strongly fungicidal (Kopper, Illman, Kersten, Klepzig, & Raffa, 2005; Mason et al., 2015), and sesquiterpenes having no known direct activities. Phloem tissues also contain phenolics (Erbilgin et al., 2017b), among which stilbenes are fungicidal, some are partially repellent, and most have no described defensive activity in this system (Bonello & Blodgett, 2003; Faccoli & Schlyter, 2007; Hammerbacher et al., 2011; Sherwood & Bonello, 2016; Wallis et al., 2008). High concentrations of constitutive and rapidly induced monoterpenes can increase the likelihood of tree survival under natural conditions (Erbilgin, Cale, Lusebrink, et al., 2017b; Raffa et al., 2005; Schiebe et al., 2012). Biosynthesis of terpenes is energetically demanding (Gershenzon, 1994), and induction relies on phloem carbohydrate resources (Goodsman, Lusebrink, Landhäuser, Erbilgin, & Lieffers, 2013; Raffa et al., 2017; Roth, Hussain, Cale, & Erbilgin, 2018).

In *Pinaceae*, oleoresin is synthesized and stored in an axially and radially connected network of specialized structures called resin cells and ducts, which possess plastid-enriched epithelial layers (Franceschi et al., 2005; Franceschi, Krokene, Krokene, & Christiansen, 2000). These resin ducts extend throughout the primary and secondary xylem and in the cortical tissues (Wu & Hu, 1997; Krokene & Nagy, 2012). Resin accumulates in these structures and is delivered to the point of beetle attack. Hence, xylem resin duct morphology is often related to the quantity of resin flow from an external wound (Blanche, Lorio, Sommers, Hodges, & Nebeker, 1992; Hood & Sala, 2015; Lombardero, Ayres, Lorio Jr, & Ruel, 2000; Rodríguez-García, López, Martín, Pinillos, & Gil, 2014; Westbrook et al., 2015). In some instances, resin ducts have been related to tree survival to bark beetles, with larger size and higher numbers having stronger relationships with survival (Ferrenberg, Kane, & Mitton, 2014; Hood, Sala, Heyerdahl, Boutin, & Raffa, 2015; Kane & Kolb, 2010). These processes have linkages, in that resin cells extend throughout the plant to produce and deliver chemicals in attacked tissues (Björkman, Larsson, & Gref, 1991; Zas et al., 2015). Although some studies examined both anatomical and chemical components of tree defence (Bentz, Hood, Hansen, Vandygriff, & Mock, 2016), or within-tree correlations within seedlings (Moreira, Zas, Solla, & Sampedro, 2015), to our knowledge, none have tested their relatedness within the same mature trees or compared relationships between species of different exposure histories.

The mountain pine beetle (*Dendroctonus ponderosae*) kills more trees than any insect in North American conifer ecosystems. Historically, this native insect has interacted primarily with lodgepole pine, *Pinus contorta*, a mid-elevation species (Meddens et al., 2012). However, recent elevated temperatures have enabled beetle populations to survive at higher elevations. Now, it has frequent access to whitebark pine, *Pinus albicaulis*, a high-elevation species with which it previously interacted only occasionally during brief, intermittent warm spells (Logan, Macfarlane, & Willcox, 2010). Based on climatic models, *D. ponderosae* will be able to survive the temperatures in *P. albicaulis* habitat with high regularity across much of the United States by the end of this century (Buotte et al., 2016, 2017). *P. albicaulis* is widely recognized as a keystone species, fulfilling essential ecosystem services in hydrology, soil quality, succession, and wildlife habitat (Logan et al., 2010). Previous work has demonstrated substantial differences between the phloem chemistry of *P. contorta* and *P. albicaulis*, particularly in regard to the constitutive and induced concentrations of compounds active against *D. ponderosae* and its primary fungal symbiont *Grosmannia clavigera* (Raffa et al., 2017; Raffa, Powell, & Townsend, 2013).

We compared resin duct structures between the historically predominant host of *D. ponderosae*, *P. contorta*, and that of the semi-naïve host *P. albicaulis* (Figure 1). We also analysed constitutive and induced phloem chemistry and mineral content of both species along an elevation gradient. Our goals were to determine (a) if resin duct and tree ring growth characteristics differ between *P. contorta* and *P. albicaulis*, (b) relationships between tree ring characteristics and resin ducts within *P. contorta* and *P. albicaulis*, and (c) how tree morphological defences relate to constitutive and induced chemical defences and phloem mineral content. Understanding if and how resin ducts and defence chemistry relate can lead to a better understanding of defence syndromes in conifer–bark beetle interactions.

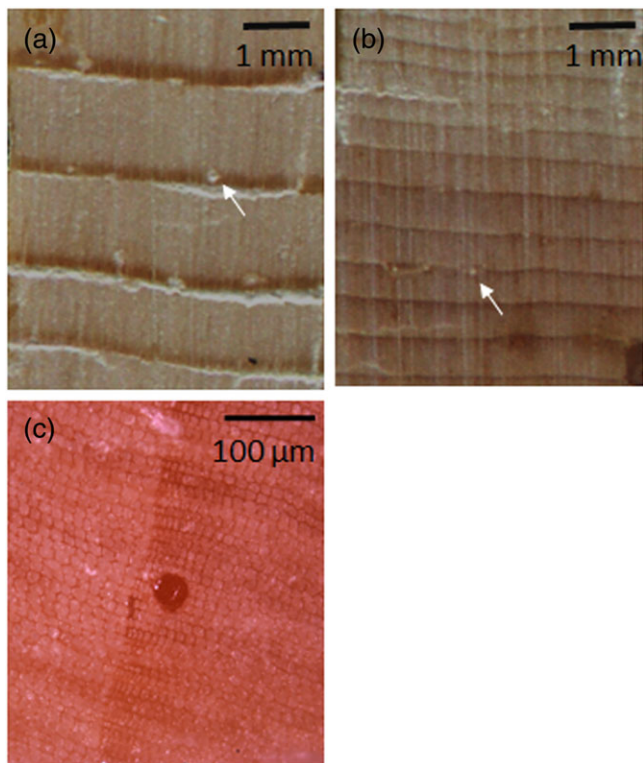


FIGURE 1 Increment cores illustrating tree rings and resin ducts of (a) *Pinus contorta* and (b,c) *Pinus albicaulis*. White arrows illustrate the resin ducts present in the increment cores [Colour figure can be viewed at wileyonlinelibrary.com]

2 | MATERIALS AND METHODS

2.1 | Site description, tree sampling, and chemistry

We identified six stands in the Gallatin National Forest near Cooke City, Montana, USA (45.05°N–109.92°W) within the Greater Yellowstone Ecosystem, as described in Raffa et al. (2017). This region is characterized by a mean annual temperature of 1°C, snowy winters and brief periods of summer suitable for beetle flight. Trees were located along an elevation gradient ranging from 2,662 to 2,931 m above sea level. Along this gradient, tree species composition changed, from nearly pure stands of *P. contorta* to monospecific *P. albicaulis*.

We selected 63 *P. contorta* and 118 *P. albicaulis* that had no apparent above ground signs or symptoms of biotic or physical injury. Trees were sampled in July 2014 for their resin duct morphology, radial growth, and constitutive phloem chemistry. Two perpendicular cores along cardinal directions were obtained, and tree diameter was measured at 1.3 m, from all trees over a 1-week period. Induced defences of a subset, 45 *P. contorta* and 82 *P. albicaulis*, were evaluated by simulating bark beetle attacks with a combination of mechanical wounding plus inoculation with the *D. ponderosae* fungal symbiont *G. clavigera*.

Simulated bark beetle attack was performed using methods and rationale described previously (Mason et al., 2017; Raffa et al., 2017). Briefly, a 1-cm diameter plug of phloem was removed, and actively growing *G. clavigera* was administered to the phloem–xylem interface. Phloem tissue was sampled prior to simulated attack and

resampled 1 week later and sent to the laboratory on dry ice. Constitutive and induced terpenoid, phenolic, sugar, and mineral chemistry were analysed. Terpenoid analyses were conducted by gas chromatography with flame ionization detector using the methods described in Keefover-Ring, Trowbridge, Mason, and Raffa (2016). Phenolics present in the ethanol extraction were analysed using ultra-high pressure liquid chromatography (UHPLC). Sugars and starches were extracted in 80% ethanol and analysed using methods of Chow and Landhäusser (2004). N levels were analysed by micro-Kjeldahl procedure, and other minerals were determined using an inductively coupled plasma emission spectrometer. Complete details of analytical methods and detailed chemical compositions are in Raffa et al. (2017). Here, we test for relationships between resin duct metrics and total concentrations of the major chemical groups.

2.2 | Increment core preparation and analysis

Individual cores were mounted in routered boards with wood glue and allowed to dry. Boards were machine-planed using an inverted jointer so that approximately half of the diameter of each core was removed, exposing the widest portion of each core (5.15 mm). Boards were then progressively sanded to 400 grit using a drum sander, and finished with 800 grit by hand.

Sanded boards were scanned at 1,200 dpi on a desktop scanning unit and analysed in the Fiji distribution of ImageJ (Schindelin et al., 2012; Schneider, Rasband, & Eliceiri, 2012). Characteristics for the most recent 30 years of growth were measured, including growth ring width, resin canal abundance, and resin canal area. Annual ring widths were manually delineated using the measure tool and resin duct areas estimated using the elliptical selection tool in ImageJ. All measurements were calibrated using the scanner resolution and verified with a ruler placed on each scan.

Several metrics of tree performance and defence were derived from the measurements in ImageJ. We calculated basal area increment (BAI; $\text{cm}^2 \text{ year}^{-1}$) and yearly radial growth (cm^2) using tree diameters. Metrics of tree investment into anatomical defence included four resin duct features (Kane & Kolb, 2010): resin duct production as a count of resin ducts per annual ring (no. year^{-1}), resin duct density as a factor of resin duct production per area of yearly growth (no. cm^{-2}), resin duct size as the average area of the resin ducts present in the tree ring (mm^2), and resin duct area as the percentage of resin duct area relative to radial growth (% of ring). Values were averaged over the two perpendicular cores and computed on a per year basis over the past 30 years. Resin duct production, density, and area were also binned into 5-, 15-, and 30-year growth intervals. Diameter breast height and age were quantified for each tree.

2.3 | Statistical analysis

Analysis of covariance and regression analyses were conducted in R Studio v. 1.0.143 (Team Rs, 2015) using R v. 3.2.1 (Team, 2013), and partial least squares regression (PLSR) were performed using SAS/STAT v. 9.4 (SAS Institute, Cary, NC). Residuals were assessed for assumptions of normality by visualization of the data. For tree

morphological measurements, data were averaged over 30-, 15- and 5-year intervals, and this binning followed identical trends for assessing normality. Normality was achieved using an $\ln(y + 1)$ transformation of the following tree characteristics: tree age, diameter at breast height (DBH), yearly growth, BAI, resin duct density, resin duct production, resin duct size, and resin duct area of ring. Induced monoterpenes and diterpene acids were \sqrt{y} transformed, and all other constitutive and induced chemicals were $\ln(y + 1)$ transformed. For all analyses, we included elevation as either an explanatory variable or as a covariate to take into account potential site gradient differences.

Tree morphological traits were compared between *P. contorta* and *P. albicaulis* using analysis of covariance for 30-, 15- and 5-year means. Analyses were conducted in the base R package with the `aov()` command. Each trait of interest was implemented into the model as a response variable with tree species as an explanatory variable and elevation as the covariate. Pairwise comparisons were completed using `LSD.test()` in the “agricolae” package (De Mendiburu, 2014) using a Fisher-LSD test.

To test within-tree relationships of ring characteristics, we generated correlation coefficients for the morphological traits. Separate analyses were conducted for *P. contorta* and *P. albicaulis*. We used the 30-, 15- and 5-year means for each of the traits derived from resin duct measurements. We utilized these intervals rather than assessing all relationships across all years as a conservative approach to avoid false positives and spurious correlations that could arise from high sample sizes (i.e., $n = 1,630$ and $n = 3,540$ for *P. contorta* and *P. albicaulis*, respectively). Spearman correlation coefficients were produced using the `ppcor()` command in the R package “ppcor” (Kim, 2015) with elevation included as a covariate in the model. Minerals were analysed using Spearman correlation coefficients with 5-year means.

PLSR modelling (Wold, Ruhe, Wold, & Dunn, 1984) was used to test whether tree morphological and site characteristics were predictive of phloem chemistry. PLSR jointly transforms predictor and response variables to identify orthogonal latent vectors that maximize explanation of covariance (Singh, Serbin, McNeil, Kingdon, & Townsend, 2015). PLSR is designed for analyses in which sample size may be low relative to number of independent variables and in which independent variables may be highly correlated. Jackknifing is used to identify a parsimonious number of latent vectors that minimizes model overfitting. We employed PLSR rather than model selection because it enables comparison among dependent variables as all potential independent variables are retained in the analysis (Grossman et al., 1996). We employed the standardized PLSR coefficients and the variable importance of projection statistic (a weighted measure based on absolute coefficient size and partial R^2 of a predictor; Wold, 1994) to assess the predictors. PLSR models are reported for the number of latent vectors with the lowest PRESS (predicted residual sum of squares) on jackknifed observations, with failed models defined as those that do not outperform the mean of the dependent variable. For each tree species and induction status, models were generated using transformed values and included the following explanatory values for each chemical assessed: elevation, tree age, DBH, yearly growth, BAI, resin duct density, resin duct production, resin duct size, and resin duct area (%; at 30-, 15- and 5-year increments).

2.4 | Data accessibility

Raw resin duct anatomy and phloem chemistry data have been deposited publicly to figshare under DOI 10.6084/m9.figshare.7092977.

3 | RESULTS

3.1 | Differences in tree ring anatomy between *P. contorta* and *P. albicaulis*

The 30-year mean annual growth of *P. contorta* was 1.8× greater than that of *P. albicaulis*, and the 30-year mean BAI of *P. contorta* was 1.1× greater (Figure 2). The 15- and 5-year intervals exhibited similar trends, but mean BAI did not differ between species. *P. contorta* had consistently higher yearly growth, whereas BAI was higher in *P. contorta* from 1984 to 2003 before becoming equivalent from 2004 to 2014. *P. albicaulis* DBH was ~1.6× greater than *P. contorta* ($p < 0.001$; Figure 2).

P. contorta had larger resin ducts and greater yearly production of resin ducts than *P. albicaulis* (Figure 3). The 30-year mean size of *P. contorta* resin ducts was 1.5× that of *P. albicaulis*, with 15- and 5-year means following similar patterns. The 30-year mean resin duct production was 1.7× greater in *P. contorta* than *P. albicaulis*, with the 15- and 5-year means being 1.5× and 1.3× greater, respectively. The two species differed in total area of the resin ducts present within tree rings for the 30-year mean, but these differences were not present among 15- and 5-year means. The two species had generally similar densities (Figure 3a). This pattern was consistent among the 30-, 15-, and 5-year means. Resin duct morphologies exhibited considerable year-to-year variability in both *P. contorta* and *P. albicaulis* (Figure 3b).

3.2 | Within-tree relationships between ring growth and resin ducts

Multiple ring anatomy and resin duct traits correlated with each other within *P. contorta* (Table 1). These generally followed similar trends among the 30-, 15-, and 5-year interval groupings (Table S1). Within *P. contorta*, tree size (DBH) was not related to yearly growth and BAI, and yearly growth and BAI were highly correlated with each other.

Resin duct production, resin duct size, and resin duct area (%) were positively related to the yearly growth and BAI of *P. contorta* (Table 1). Resin duct density was inversely related to yearly growth and BAI. All resin duct characteristics were positively related to each other, except for resin duct density that was unrelated to resin duct size.

As in *P. contorta*, resin duct production, resin duct size, and the percent resin duct area of the ring of *P. albicaulis* were positively related to yearly growth and BAI (Table 1). As well, like *P. contorta*, resin duct density was inversely related to yearly growth and BAI. The positive relationships between resin duct characteristics and tree growth were also always stronger than the negative relationships observed with resin duct density. Unlike *P. contorta*, the DBH of *P. albicaulis* was positively related to the resin duct area of the ring

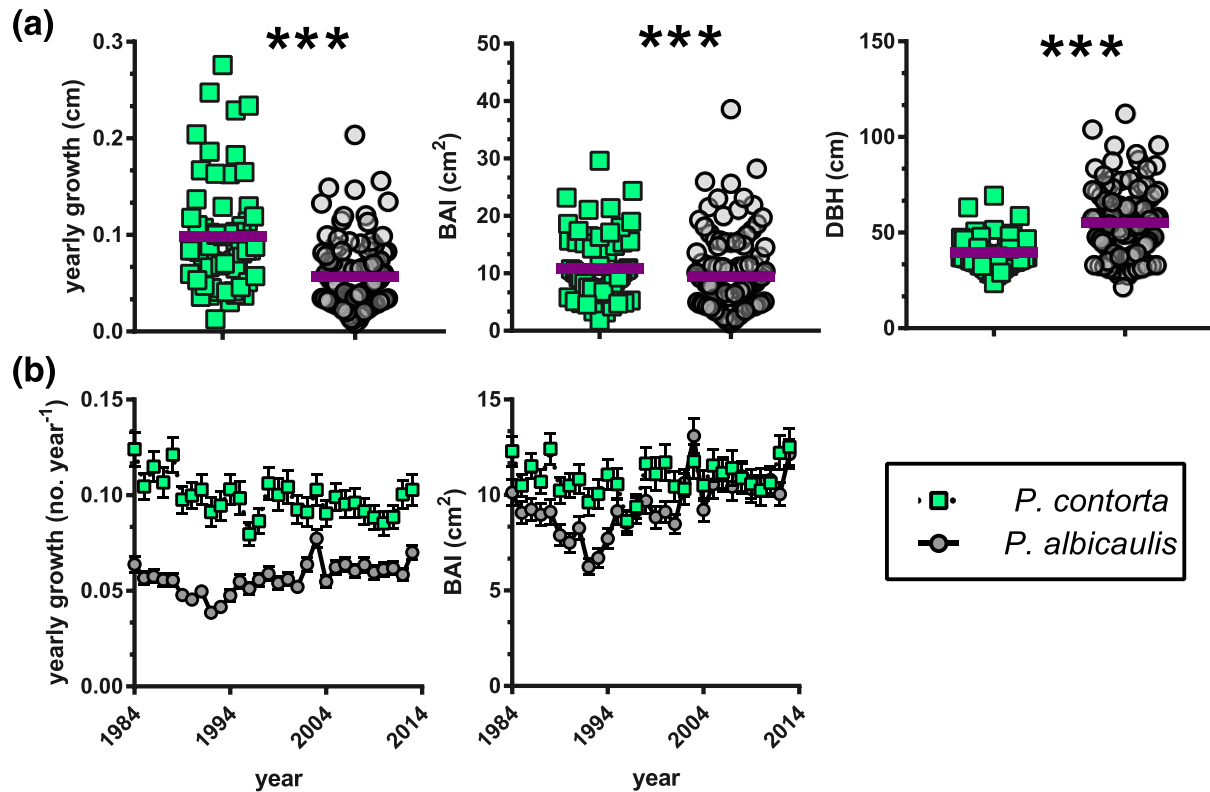


FIGURE 2 Growth trends of *Pinus contorta* and *Pinus albicaulis*. (a) 30-year mean yearly growth, basal area increment (BAI), and tree diameter at breast height (DBH). Each symbol represents means for a single tree. A single point in panel (a) represents the mean value of a single tree over 30 years. Bars represent means and asterisks denote significant differences using $\ln(y + 1)$ transformed values ($*** < 0.001$). (b) Yearly growth and BAI over a 30-year period. In panel (b), a single point represents the mean value for all trees at that particular year, with standard errors. *P. contorta* $n = 62$; *P. albicaulis* $n = 117$ [Colour figure can be viewed at wileyonlinelibrary.com]

and BAI, and resin duct density was not related to percent resin duct area. These relationships were generally consistent over the 30, 15, and 5-year intervals, as in *P. contorta* (Table S2).

3.3 | Phloem chemical composition in trees

The quantitative secondary, carbohydrate, and mineral chemistry on which we performed our analyses are presented and discussed in Raffa et al. (2017). All data are summarized in Tables S3–S5. In general, the chemical profiles differed both between induced and constitutive states, and between *P. contorta* and *P. albicaulis*. In both tree species, the terpenoids increased in response to simulated attack (Tables S3 and S4), but the composition of the classes and the extent to which groups responded differed between species. Localized carbohydrate pools decreased during induction in both species (Table S4). In both species, many of the phenolics decreased in response to simulated bark beetle attack, with the exception of the stilbenes. Constitutive mineral contents also varied between species, with *P. contorta* having greater Ca and Al, and *P. albicaulis* having higher N, P, and K.

3.4 | Within-tree relationships between ring anatomy and constitutive minerals

The yearly growth of *P. contorta* was positively related to phloem N, P, K, Mg, S, Fe, and Al, with the other minerals being unrelated (Table 2).

The strongest relationships were with N, P, and Al. Similarly, BAI was positively correlated with all minerals, with the exception of Zn and Mn, which were unrelated.

Resin duct production in *P. contorta* was positively correlated with N, P, K, Mg, S, and Al. Percent resin duct area followed similar trends as resin duct production but was also correlated with B. Resin duct size was positively correlated with N, P, K, Mg, Ca, S, Fe, B, and Al. Overall, resin duct size and percent resin duct area were more strongly related to phloem mineral content than was resin duct production.

Yearly growth of *P. albicaulis* was positively related to all phloem minerals except Zn, for which there was no relationship (Table 2). BAI was positively related to all minerals surveyed. The strongest relationships between tree growth and phloem mineral content were with N, P, Al, and K. Diameter at breast height was positively correlated with N, P, K, Mg, and Cu and unrelated to other phloem minerals.

Resin duct production was positively related to all minerals in *P. albicaulis*, except Mn and Cu, for which there was no relationship. Similarly, resin duct area was positively related to all phloem minerals, and resin duct size was positively related to all but Zn, Mn, and S, with which there were no relationships. Resin duct density was unrelated to all phloem minerals except P, with which there was an inverse relationship. Overall, the positive relationships between resin duct characters and tree growth metrics were stronger than this negative relationship.

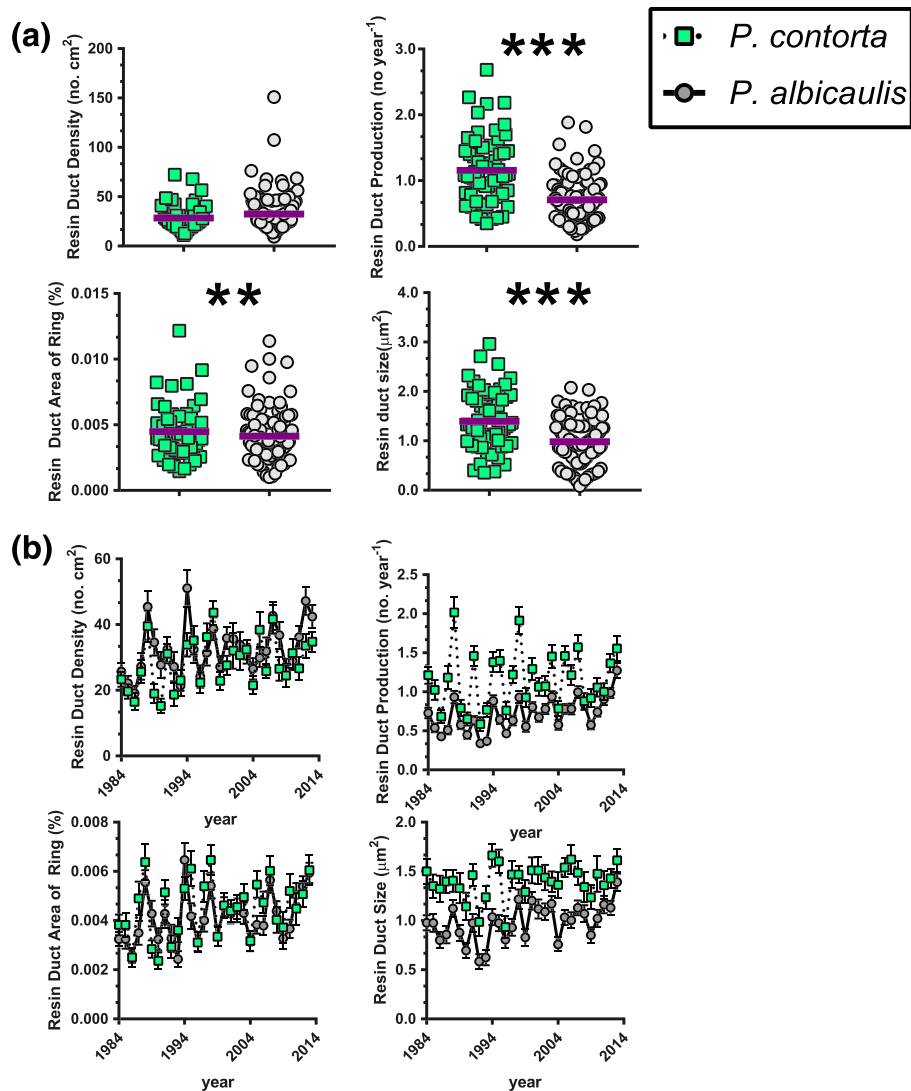


FIGURE 3 Resin duct characteristics of *Pinus contorta* and *Pinus albicaulis*. (a) 30-year mean and (b) annual responses of resin duct production, resin duct density, resin duct area, and resin duct size. A single point in panel (a) represents the mean value of a single tree over 30 years. In panel (b), a single point represents the mean value for all trees at that particular year, with standard errors. Bars represent means and asterisks denote significant differences using $\ln(y + 1)$ transformed values for all metrics except for resin duct size, which was \sqrt{y} transformed. *P. contorta* $n = 62$, *P. albicaulis* $n = 117$ (** < 0.01 ; *** < 0.001) [Colour figure can be viewed at wileyonlinelibrary.com]

3.5 | Relationships between tree ring morphology and constitutive and induced phloem chemistry

Due to the large number of pairwise correlations between compound classes and resin ducts (Tables S6–S9), we used PLSR to determine which, if any, anatomical characteristics relate to phloem chemistry. The summary of PLSR results and significant model fits are displayed in Table 3. Overall, about half of the possible tree tissue–phloem chemistry pairings yielded a successful model of some degree. In *P. contorta* constitutive phloem, significant regressions were developed for two classes of terpenes (monoterpenes and sesquiterpenes), four classes of phenolics (flavonoids, hydroxycinnamic acids, phenylpropanoids, and lignans), and one class of carbohydrate (sugars). In *P. contorta* induced phloem, successful models only emerged for vanilloids, hydroxycinnamic acids, and lignans. In *P. albicaulis* constitutive phloem, successful predictions were emerged for monoterpenes, diterpene acids, hydroxycinnamic acids, phenylpropanoids, sugars,

and starches. In *P. albicaulis* induced phloem, successful models emerged for vanilloids, lignans, stilbenes, sugars, and starches.

The extent and direction to which tree ring characteristics helped explain *P. contorta* constitutive and induced phloem chemicals varied between the particular classes of compounds (Table 4). Overall, yearly growth and BAI were more consistent predictors than resin duct anatomy. For constitutive monoterpenes, resin duct area was the only positive anatomical characteristic associated with concentration. Similarly, resin duct production was the only positively associated anatomical trait associated with constitutive sesquiterpenes. The various phenolic compounds were more variable but generally had a negative association with resin duct characteristics. Induced vanilloids were the only exception, which had positive associations with 5-year mean resin duct production and 30- and 5-year mean resin duct density and resin duct size. Note that vanilloids were present at relatively low concentrations relative to other compounds ($> 2.0 \text{ mg g}^{-1}$) and decreased in concentration in induced tissues (Raffa et al., 2017).

TABLE 1 Spearman correlation coefficients of tree ring growth and resin duct anatomical measurements of *Pinus contorta* and *Pinus albicaulis*

<i>Pinus contorta</i>	DBH	Yearly growth	BAI	Resin duct density	Resin duct production	Resin duct size
Yearly growth	-0.20					
BAI	0.16	0.91				
resin duct density	0.24	-0.33	-0.30			
resin duct production	0.05	0.58	0.60	0.53		
resin duct size	-0.10	0.69	0.68	0.06	0.71	
percent resin duct area of ring	0.10	0.59	0.65	0.43	0.92	0.83
<i>Pinus albicaulis</i>	DBH	Yearly growth	BAI	Resin Duct Density	Resin Duct Production	Resin Duct Size
yearly growth	-0.10					
BAI	0.38	0.87				
resin duct density	0.07	-0.45	-0.40			
resin duct production	-0.01	0.59	0.54	0.36		
resin duct size	0.02	0.70	0.66	-0.05	0.56	
percent resin duct area of ring	0.35	0.61	0.79	0.15	0.86	0.77

Note. Correlations were conducted using tree elevation as a covariate. Bold values indicate significant correlations ($p < 0.05$). Correlations were conducted on metrics averaged over 5-year periods. Five-year averages followed similar trends as 15- and 30-year averages for both tree species (Tables S1 and S2). BAI: basal area increment; DBH: diameter breast height.

TABLE 2 Relationships between phloem mineral content (mg cm^{-3}) and tree growth and anatomy (5-year interval)

Mineral	<i>Pinus contorta</i>						
	DBH	Yearly growth	BAI	Resin duct density	Resin duct production	Percent resin duct area	Resin duct size
N	-0.02	0.56	0.65	-0.17	0.40	0.51	0.53
P	0.02	0.55	0.63	-0.18	0.36	0.47	0.50
K	-0.02	0.48	0.56	-0.17	0.35	0.46	0.51
Mg	-0.01	0.48	0.59	-0.11	0.40	0.49	0.51
Ca	0.01	0.28	0.38	-0.01	0.29	0.36	0.30
S	0.20	0.42	0.57	-0.13	0.34	0.42	0.40
Zn	0.08	0.15	0.26	-0.08	0.15	0.14	0.14
Mn	0.01	-0.23	-0.20	0.06	-0.14	-0.15	-0.20
Cu	0.18	0.30	0.42	-0.18	0.19	0.26	0.28
Fe	-0.02	0.44	0.49	-0.24	0.20	0.29	0.36
B	0.12	0.26	0.40	-0.13	0.18	0.32	0.35
Al	-0.17	0.56	0.61	-0.14	0.47	0.57	0.62
Mineral	<i>Pinus albicaulis</i>						
	DBH	Yearly growth	BAI	Resin duct density	Resin duct production	Percent resin duct area	Resin duct size
N	0.26	0.46	0.50	-0.18	0.32	0.44	0.29
P	0.26	0.50	0.54	-0.25	0.27	0.42	0.31
K	0.32	0.40	0.49	-0.11	0.32	0.47	0.29
Mg	0.24	0.36	0.41	-0.09	0.34	0.43	0.27
Ca	0.19	0.29	0.34	0.01	0.32	0.40	0.27
S	0.13	0.47	0.43	-0.17	0.33	0.35	0.23
Zn	0.09	0.22	0.23	-0.08	0.30	0.25	0.15
Mn	0.17	0.28	0.32	-0.10	0.21	0.28	0.14
Cu	0.22	0.24	0.28	-0.07	0.18	0.28	0.13
Fe	0.06	0.32	0.30	-0.06	0.33	0.33	0.22
B	0.21	0.36	0.38	-0.01	0.37	0.45	0.26
Al	0.02	0.41	0.33	-0.10	0.36	0.24	0.19

Note. Bolded values represent statistically significant correlations. Spearman correlations were generated using elevation as a covariate. BAI: basal area increment; DBH: diameter breast height.

TABLE 3 Significant models achieved for the *Pinus contorta* and *Pinus albicaulis* phloem metabolite concentration ($\text{mg}\cdot\text{g}^{-1}\cdot\text{dw}$) through partial least squares regression

	<i>Pinus contorta</i>		<i>Pinus albicaulis</i>	
	Constitutive	Induced	Constitutive	Induced
monoterpenes	0.18	NS	0.09	NS
sesquiterpenes	0.08	NS	NS	NS
diterpene acids	NS	NS	0.04	NS
vanilloids	NS	0.77	NS	0.28
flavonoids	0.28	NS	0.42	NS
hydroxycinnamic acids	0.06	0.24	0.35	NS
phenylpropanoids	0.09	NS	0.14	NS
lignans	0.11	0.24	NS	NS
stilbenes	NS	NS	NS	0.14
starch	0.09	NS	NS	0.32
sugar	NS	NS	0.10	0.14

Note. Values indicate significant models and correspond to the amount of variance explained. NS: non-significant PLSR output.

Similar to *P. contorta*, *P. albicaulis* had variable relationships between growth and resin duct anatomy with phloem chemical components (Table 5). Like *P. contorta*, BAI and yearly growth were significantly associated with terpenoids, carbohydrates, and phenolics, but the extent to which they were positive or negative varied. The only exception was induced stilbenes, which did not have a significant association. Also like *P. contorta*, anatomical defences were variable predictors of phloem chemistry. Constitutive starches were positively associated with resin duct production, resin duct area, and resin duct size, whereas induced starches were only positively associated with 5-year resin duct production and area. Induced sugars were positively associated with resin duct area and resin duct size. Constitutive monoterpenes were negatively related to resin duct density but positively associated with resin duct size. Constitutive diterpene resin acids were only positively associated with resin duct area. Resin duct area was a generally positive predictor of constitutive phenolics but a negative predictor of induced phenolics. Resin duct size was likewise variable. Induced stilbenes were positively associated with resin duct density and negatively associated with resin duct area.

4 | DISCUSSION

These results demonstrate that the semi-naïve host plant *P. albicaulis* possesses quantitatively less substantial anatomical defences against a tree-killing herbivore, *D. ponderosae*, than the historically more continuously exposed host, *P. contorta*. Specifically, *P. contorta* produce more and larger constitutive resin ducts than does *P. albicaulis*. These particular metrics have been associated with greater likelihoods of survival during bark beetle outbreaks in several pine species in the western US (Ferrenberg et al., 2014; Kane & Kolb, 2010). The higher investment in morphological defence structures by *P. contorta* appears to complement its higher levels of constitutive and induced defence compounds against *D. ponderosae* and its microbial associates, and *P. contorta*'s relatively lower proportions of the host compounds that *D. ponderosae* exploits to coordinate mass attacks during pheromone

signalling (Erbilgin et al., 2017a; Raffa et al., 2017, 2013). Thus, these higher resin-based defences may contribute to the greater impediment that *P. contorta* poses, relative to *P. albicaulis*, to the initiation of beetle aggregation (Bentz, Boone, & Raffa, 2015).

Because warming temperatures are increasing the proportions of high elevation stands that will be thermally suitable for *D. ponderosae* (Buotte et al., 2016, 2017), our results suggest that *P. albicaulis* is among other high-elevation species such as *Pinus flexilis* (Eidson, Mock, & Bentz, 2017; Ferrenberg, Langenhan, Loskot, Rozal & Mitton, 2017) that require particular attention with regard to conservation (Logan et al., 2010). In contrast, some other high-elevation pine species such as Great Basin bristlecone pine (*Pinus longaeva*) appear to be outside the suitable host range of *D. ponderosae* (Eidson et al., 2017) and so are less threatened. Additional work is needed to better understand how historical exposure to *D. ponderosa* influences defence capability, because in addition to opportunities for coadaptation, *P. contorta* and *P. albicaulis* differ in their life histories and phylogenetic position within the genus *Pinus*.

We did not observe trade-offs between tree growth and resin duct structures. Instead, most relationships were positive. The only exception was a negative correlation between resin duct density and growth (BAI, yearly growth), in both species. The negative correlation between density and growth appears to reflect a spatial dilution effect rather than a trade-off in structural resources, because trees with larger diameters actually had more resin ducts. These results are consistent with other studies in *P. contorta* and other *Pinus* spp. (Blanche et al., 1992; Ferrenberg et al., 2014; Hood et al., 2015; Kane & Kolb, 2010). Furthermore, our observation that resin ducts were directly related to tree growth rate supports the view of Björkman et al. (1991) that production of these structures is limited by the same resources that limit plant growth in general.

We likewise did not observe consistent trade-offs between tree growth and defence chemistry. In most cases, there was no relationship, but where present, terpenes were positively related to growth rate, in agreement with Villari, Faccoli, Battisti, Bonello, and Marini (2014), and phenolics were inversely related to growth, in agreement with Glynn, Herms, Egawa, Hansen, and Mattson (2003), supporting the view that such trade-offs vary among classes of secondary compounds (Massad, Dyer, & Vega, 2012).

Chemical and mechanical defences did not show inverse within-plant relationships in these two pine species. These results agree with a recent meta-analysis that found such trade-offs to be relatively uncommon across a broad range of plants, despite their appearance in some systems (Moles et al., 2013). A more common trend is that relationships between physical and defence components change during plant ontogeny (Hanley, Lamont, Fairbanks, & Rafferty, 2007; Kariño-Betancourt, Agrawal, Halitschke, & Núñez-Farfán, 2015; Quintero & Bowers, 2011). This may be particularly relevant to bark beetles such as *D. ponderosae* that are restricted to larger trees. Resin duct traits were generally poor predictors of chemical defences against bark beetles, in both host species. This included terpenoids, both constitutive and induced, of which resin is comprised. Similar results were obtained by Zas et al. (2015) with *Pinus pinaster*. Constitutive and induced starches, sugars, and phenolics were highly variable and likewise did not exhibit clear patterns.

TABLE 4 Partial least squares regression results between chemical concentration (mg·g⁻¹·dw) and ring anatomy for *Pinus contorta*

	Constitutive						Induced					
	Starch	Monoterpenes	Sesquiterpenes	Flavonoids	Phenylpropanoids	Hydroxycinnamic acids	Lignans	Hydroxycinnamic acids	Lignans	Vanilloids		
Elevation	0.17 (0.004)	0.84 (0.026)	0.87 (0.017)	1.46 (0.278)	0.92 (0.021)	1.19 (0.023)	0.08 (0.002)	1.03 (0.035)	0.95 (0.032)	1.34 (0.178)		
Tree age	1.52 (-0.032)	0.85 (0.026)	1.07 (0.011)	0.75 (-0.274)	0.57 (-0.014)	1.35 (-0.003)	0.89 (-0.006)	1.35 (0.036)	1.17 (0.020)	1.28 (0.827)		
DBH	1.37 (-0.035)	0.85 (-0.026)	0.55 (-0.021)	1.45 (-0.090)	0.63 (0.013)	0.15 (0.026)	0.26 (0.021)	1.07 (0.046)	0.61 (0.039)	1.30 (-1.232)		
BAI-30 year	0.82 (0.019)	1.57 (0.048)	1.65 (0.033)	0.82 (-0.033)	1.18 (-0.026)	1.25 (-0.024)	1.19 (-0.028)	1.06 (-0.036)	1.06 (-0.036)	0.66 (-0.663)		
BAI-15 year	0.95 (0.022)	1.52 (0.047)	1.37 (0.027)	0.80 (0.002)	0.93 (-0.021)	1.09 (-0.021)	1.18 (-0.028)	0.93 (-0.031)	0.96 (-0.032)	0.63 (0.505)		
BAI-5 year	0.88 (0.020)	1.44 (0.044)	1.22 (0.024)	0.94 (-0.059)	0.91 (-0.02)	1.52 (-0.029)	1.40 (-0.033)	0.87 (-0.029)	0.79 (-0.027)	0.81 (0.634)		
YG-30	1.36 (0.032)	1.09 (0.033)	1.42 (0.028)	0.85 (-0.002)	1.22 (-0.027)	1.25 (-0.024)	1.23 (-0.029)	1.38 (-0.047)	1.34 (-0.045)	0.70 (-0.958)		
YG-15	1.44 (0.033)	1.03 (0.032)	1.11 (0.022)	0.83 (0.022)	1.04 (-0.023)	1.09 (-0.021)	1.24 (-0.029)	1.28 (-0.043)	1.30 (-0.044)	0.59 (-0.321)		
YG-5	1.30 (0.030)	0.89 (0.027)	0.97 (0.019)	0.97 (-0.045)	1.05 (-0.023)	1.44 (-0.027)	1.41 (-0.033)	1.26 (-0.043)	1.18 (-0.040)	0.79 (0.080)		
RDP-30 year	0.58 (0.013)	0.9 (0.027)	1.09 (0.022)	0.87 (0.114)	0.67 (-0.015)	0.95 (-0.018)	0.8 (-0.019)	1.05 (-0.036)	1.00 (-0.034)	0.75 (-0.454)		
RDP-15 year	0.59 (0.014)	0.6 (0.018)	0.95 (0.019)	0.77 (-0.035)	1.07 (-0.024)	0.69 (-0.013)	0.75 (-0.018)	0.89 (-0.03)	0.95 (-0.032)	0.94 (-0.153)		
RDP-5 year	1.00 (0.023)	0.71 (0.022)	0.78 (0.015)	1.07 (-0.115)	1.51 (-0.034)	0.83 (-0.016)	1.26 (-0.030)	0.73 (-0.025)	0.68 (-0.023)	1.11 (1.116)		
RDD-30	1.39 (-0.032)	0.79 (-0.024)	0.97 (-0.019)	1.09 (0.093)	1.15 (0.026)	0.83 (0.016)	0.93 (0.022)	0.69 (0.023)	0.64 (0.022)	1.09 (0.045)		
RDD-15	1.42 (-0.033)	0.98 (-0.03)	0.66 (-0.013)	1.03 (-0.127)	0.26 (0.006)	0.83 (0.016)	0.88 (0.021)	0.40 (0.014)	0.33 (0.011)	1.07 (-1.020)		
RDD-5	0.32 (-0.007)	0.45 (-0.014)	0.19 (-0.004)	1.23 (-0.111)	0.66 (-0.015)	0.82 (0.016)	0.18 (0.004)	0.21 (0.007)	0.18 (0.006)	1.30 (0.558)		
RD Area-30	0.00 (0.008)	1.12 (0.028)	0.77 (0.019)	0.90 (-0.060)	0.82 (-0.029)	0.64 (-0.012)	0.79 (-0.022)	1.12 (-0.035)	1.30 (-0.045)	0.91 (-0.912)		
RD Area-15	0.36 (0.000)	0.91 (0.034)	0.94 (0.015)	0.94 (0.040)	1.28 (-0.018)	0.64 (-0.012)	0.95 (-0.019)	1.03 (-0.038)	1.34 (-0.044)	0.99 (-0.511)		
RD Area-5	0.72 (0.017)	1.06 (0.032)	0.77 (0.015)	1.01 (-0.033)	1.24 (-0.028)	0.62 (-0.012)	1.22 (-0.029)	0.81 (-0.027)	0.99 (-0.033)	1.05 (-1.119)		
Resin duct size	0.65 (0.015)	0.55 (0.017)	0.58 (0.012)	0.82 (0.064)	1.06 (-0.024)	0.70 (-0.013)	0.91 (-0.021)	1.01 (-0.034)	1.20 (-0.040)	1.14 (1.860)		

Note. Values are VIP statistics and respective standardized coefficients in parentheses. Values greater than 1.0 are bolded and indicate significant components of the models. Positive coefficients indicate positive relationships, and negative coefficients indicate negative relationships. BAI: basal area increment; RD Area: resin duct area (proportion); RDD: resin duct density; RDP: resin duct production; YG: yearly growth.

TABLE 5 Partial least squares regression results between chemical concentration (mg·g⁻¹·dw) and ring anatomy for *Pinus albicaulis*

	Constitutive										Induced			
	Sugar	Monoterpenes	Diterpene acids	Hydroxycinnamic acids	Flavonoids	Phenylpropanoids	Starch	Sugar	Vanilloids	Lignans	Stilbenes			
Elevation	0.61 (0.014)	0.38 (0.009)	1.5 (0.024)	1.49 (0.286)	1.18 (0.193)	1.34 (0.038)	1.14 (-0.026)	0.79 (0.022)	1.23 (-0.045)	1.07 (-0.044)	0.40 (-0.031)			
Tree age	0.14 (0.009)	0.44 (-0.004)	0.97 (0.002)	0.95 (-0.298)	1.05 (0.221)	0.41 (0.028)	1.33 (0.115)	1.04 (0.042)	0.15 (-0.047)	0.05 (-0.048)	2.47 (-0.030)			
DBH	0.39 (-0.003)	0.17 (0.010)	0.12 (0.015)	0.96 (-0.067)	1.37 (-0.241)	0.99 (-0.011)	1.13 (0.345)	1.51 (0.029)	1.28 (-0.005)	1.16 (0.002)	0.86 (-0.036)			
BAI-30 year	1.16 (0.027)	0.99 (0.022)	1.07 (0.017)	1.07 (0.454)	1.26 (0.117)	1.43 (0.04)	1.07 (-0.001)	1.19 (0.033)	1.25 (-0.046)	1.27 (-0.052)	0.30 (-0.029)			
BAI-15 year	1.23 (0.029)	1.19 (0.027)	1.13 (0.018)	0.98 (0.286)	1.19 (0.084)	1.3 (0.036)	1.25 (0.014)	1.10 (0.030)	1.17 (-0.043)	1.23 (-0.051)	0.37 (-0.034)			
BAI-5 year	1.30 (0.030)	1.23 (0.028)	1.24 (0.020)	0.97 (0.146)	1.20 (0.105)	1.27 (0.036)	1.42 (0.009)	1.06 (0.029)	1.15 (-0.042)	1.19 (-0.049)	0.36 (-0.038)			
YG-30	1.11 (0.026)	1.31 (0.030)	1.24 (0.020)	0.96 (0.154)	1.08 (0.035)	1.25 (0.035)	0.97 (-0.073)	0.79 (0.022)	1.14 (-0.042)	1.2 (-0.049)	0.64 (-0.026)			
YG-15	1.13 (0.026)	1.47 (0.033)	1.18 (0.019)	0.99 (-0.354)	1.06 (0.012)	1.13 (0.032)	1.12 (-0.05)	0.61 (0.017)	0.98 (-0.036)	1.08 (-0.044)	0.62 (-0.030)			
YG-5	1.19 (0.028)	1.49 (0.034)	1.28 (0.02)	1.27 (-0.817)	1.04 (0.010)	1.09 (0.031)	1.33 (-0.066)	0.58 (0.016)	1.04 (-0.038)	1.08 (-0.044)	0.67 (-0.036)			
RDP-30 year	1.25 (0.029)	0.74 (0.017)	0.68 (0.011)	0.94 (0.173)	0.89 (0.113)	0.88 (0.025)	0.60 (-0.119)	0.69 (0.019)	0.63 (-0.023)	0.59 (-0.024)	0.79 (-0.016)			
RDP-15 year	1.07 (0.025)	0.77 (0.017)	0.80 (0.013)	0.87 (0.118)	0.87 (0.050)	1.08 (0.030)	0.86 (0.031)	0.94 (0.026)	0.53 (-0.019)	0.6 (-0.024)	0.48 (-0.023)			
RDP-5 year	1.06 (0.025)	0.76 (0.017)	0.74 (0.012)	0.92 (0.080)	0.75 (0.052)	0.69 (0.019)	1.02 (0.083)	0.82 (0.023)	0.46 (-0.017)	0.57 (-0.023)	1.05 (-0.028)			
RDD-30	0.48 (-0.011)	0.95 (-0.022)	0.86 (-0.014)	0.80 (-0.246)	0.69 (0.016)	0.71 (-0.020)	0.64 (-0.007)	0.35 (-0.010)	1.10 (0.040)	1.15 (0.047)	0.70 (0.017)			
RDD-15	0.61 (-0.014)	1.28 (-0.029)	0.92 (-0.015)	0.79 (0.160)	0.63 (0.049)	0.33 (-0.009)	0.24 (0.113)	0.36 (0.010)	0.65 (0.024)	0.74 (0.03)	1.53 (0.006)			
RDD-5	0.39 (-0.009)	0.85 (-0.019)	0.68 (-0.011)	0.86 (-0.318)	0.45 (0.040)	0.39 (-0.011)	0.13 (0.140)	0.29 (0.008)	0.19 (0.007)	0.23 (0.009)	1.70 (0.003)			
RD Area-30	1.17 (0.027)	0.96 (0.022)	1.00 (0.018)	0.97 (-0.412)	0.97 (-0.041)	1.00 (0.033)	0.87 (0.061)	1.30 (0.04)	1.37 (-0.043)	1.25 (-0.046)	0.37 (-0.027)			
RD Area-15	1.18 (0.027)	0.95 (0.022)	1.11 (0.016)	0.98 (-0.001)	1.01 (0.011)	1.18 (0.028)	0.99 (-0.04)	1.44 (0.036)	1.18 (-0.05)	1.12 (-0.051)	0.06 (-0.023)			
RD Area-5	1.14 (0.027)	0.81 (0.018)	0.91 (0.014)	1.00 (0.141)	0.94 (-0.085)	0.78 (0.022)	1.15 (0.159)	1.67 (0.046)	1.11 (-0.041)	1.12 (-0.046)	1.28 (-0.031)			
Resin Duct Size	1.15 (0.027)	1.07 (0.024)	0.69 (0.011)	1.00 (0.330)	0.89 (-0.006)	0.72 (0.020)	0.48 (-0.121)	1.00 (0.028)	1.07 (-0.039)	1.09 (-0.045)	0.79 (-0.013)			

Note. Values are VIP statistics and respective standardized coefficients in parentheses. Values greater than 1.0 are bolded and indicate significant components of the models. Positive coefficients indicate positive relationships, and negative coefficients indicate negative relationships. BAI: basal area increment; RD Area: resin duct area (proportion); RDD: resin duct density; RDP: resin duct production; YG: yearly growth.

These results illustrate that allocation to anatomical components of defence is unrelated to allocation to chemical defences in these two conifer species. This lack of constraint imposed by anatomical structures on defence chemistry may allow conifers to be highly plastic in their defence syndromes, thereby confronting herbivores with a wide array of quantitative permutations in physical and chemical defence components. These permutations may pose particular challenges to bark beetles because physical and chemical defence components can enhance each other's efficacy against herbivores, as occurs in some trichome-based systems (Alba et al., 2014).

Understanding these absences of correlations between anatomical and chemical defences can also provide guidance to conservation programmes, because they can be targeted separately or jointly as desired. This is a particularly useful consideration with *P. albicaulis* being threatened by a combination of climate-driven expansion of the native *D. ponderosae*, climate-driven encroachment by competing plant species, and invasion by the exotic fungal pathogen *Cronartium ribicola* (Logan et al., 2010; Tomback & Achuff, 2010).

Although resin ducts and chemical defences were unrelated to ring growth, we cannot infer that these traits are not costly. Terpenoid defences are well-documented as being energetically demanding (Gershenzon, 1994) and deplete phloem carbohydrate reserves (Goodsman et al., 2013; Raffa et al., 2017; Wiley, Rogers, Hodgkinson, & Landhäusser, 2016). Trade-offs between growth and defence have been observed in pine seedlings (Moreira et al., 2014), although our understanding of relationships in mature trees is more limited. The costs of induced defences against bark beetles are also lacking. However, they are likely reduced by their highly localized allocation of defence compounds (Mason et al., 2017).

In addition to constitutive resin ducts, conifers also produce traumatic resin ducts (TRD) in response to mechanical and biotic wounds (Lombardero et al., 2000). These are particularly prominent in *Picea* (DeRose, Bekker, & Long, 2017; Franceschi et al., 2005) but also occur in the response of *Pinus nigra* to infection by pathogens such as *Diplodia sapinea* (syn. *Sphaeropsis sapinea*) and *Diplodia scrobiculata* (Luchi, Ma, Capretti, & Bonello, 2005). TRD can also be retained in old rings following bark beetle outbreaks but are rare during endemic periods (DeRose et al., 2017). There was extensive *D. ponderosae* activity in the Gallatin National Forest during 2007–2009, but most other years within the range of our sampling had low populations (Gannon & Sontag, 2010; Hayes, 2015). Our experiment was not designed to study and quantify the formation of TRDs, as we concurrently collected core samples, sampled constitutive phloem and inoculated fungi to test whether resin duct metrics predict chemical defences. Therefore, future work into mechanisms of induction should test whether within-tree post-elicitation anatomical (TRDs) and chemical changes are correlated or unrelated and thus further adding to this complexity.

In general, both the *P. contorta* and *P. albicaulis* in our study region had smaller and lower production of resin ducts than those observed in some other systems. For example, *P. contorta* trees that resisted *D. ponderosae* attacks at a similar elevation in Colorado had resin duct sizes that averaged 0.018 mm² (0.001 s.e.) and production of ~6 ducts per year (Ferrenberg et al., 2014), compared with 0.014 mm² (0.001 s.e.) and ~1.4 per year in our stands. Likewise, the *P. albicaulis* in our

study region had, on average, smaller resin duct sizes (0.010 mm²) and less production (~0.8 ducts per year) than other *Pinus* species in the subgenus *Strobus*, including *P. flexilis* (0.014–0.017 mm²; ~2.5 ducts per year), *P. longaeva* (0.015 mm²; ~2 ducts per year), and *P. balfouriana* (0.030 mm²; ~5 ducts per year; Bentz et al., 2016; Ferrenberg et al., 2014). However, the range of sizes and production of resin ducts in both species at our sites overlapped with these other populations, indicating substantial between-site variability due to potential environmental, genetic, or interacting factors (Ferrenberg et al., 2014; Hood & Sala, 2015).

The strong, predominantly positive relationships between phloem mineral content and the numbers and size of resin ducts, in both *P. contorta* and *P. albicaulis*, suggest soil nutritional characteristics directly affect production of anatomical defence traits (Cook, Carroll, Kimsey, & Shaw, 2015). For example, fertilization can increase tree growth and resin flow (Knebel, Robison, Wentworth, & Klepzig, 2008; Lombardero et al., 2000), and some aspects of resin ducts may be manipulated genetically and environmentally (Hood et al., 2015; Rosner & Hannrup, 2004; Westbrook et al., 2015). In contrast, *P. pinaster* seedlings in low-P soil had a higher density of resin ducts than fertilized seedlings (Moreira et al., 2015). Experiments assessing the incorporation of soil quality on resin duct production, the underlying heritability, and their interactions could provide important tools for the preservation of *P. albicaulis*.

This work improves our understanding of the nexus between climate change and plant allocation to multiple defence modalities. As temperature and precipitation patterns change, herbivores will encounter new host species upon range expansion, exploit intermittently encountered species with increased regularity, and interact with historical species under different stress conditions and disturbance regimes. Our study illustrates how a diversity of plant defence attributes relate to each other such that coevolved syndromes may provide historically exposed host species with more integrated defence mechanisms. For example, interactions between tree-killing bark beetles and conifers are largely determined by the opposing rates of pheromone-mediated aggregation vs. activated defences. Based on this and our previous studies on this system (Raffa et al., 2017, 2013), historically more exposed species possess both greater anatomical and chemical properties that inhibit pheromone communication, higher constitutive and induced concentrations of insecticidal and fungicidal compounds, and fewer trade-offs between insecticidal and antifungal compounds. However, as with invasion ecology, there will likewise be novel species that pose full or largely impenetrable barriers based on features of herbivore behaviour, nonhost resistance, or environmental conditions. A better understanding of the relatedness among defence components will improve our ability to predict and ameliorate the most threatened systems.

ACKNOWLEDGEMENTS

Keith Konen (USDA-FS) facilitated site location. Jun Zhu (UW-Madison Statistics) provided recommendations regarding statistical analyses. Hannah Hubanks, Ryan Sword, Will Sass, Nichole Boyea, and Erin McMahan conducted field sampling. Grady Munroe managed the data, including tree ring analyses, with assistance from Alex Goke.

Funding was provided by the UW-Madison Graduate School, Department of Forest & Wildlife Ecology and College of Agricultural and Life Sciences to PAT and KFR, UW-Madison Hatch funds to KFR, and a Natural Sciences and Engineering Research Council of Canada-Discovery Grant to NE. We would like to thank the editor and two anonymous reviewers for comments that helped improve our manuscript.

AUTHOR CONTRIBUTIONS

P.A.T., K.F.R., and C.J.M. designed the research; P.A.T. led the fieldwork and analysis of tree-ring data; C.J.M., C.V., P.B., N.E., S.C., K.K.R., J.G.K., and P.A.T. analysed the sample data; C.J.M. and P.A.T. performed the quantitative analyses and interpretations. All authors contributed to the writing of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Mason CJ, Keefover-Ring K, Villari C, et al. Anatomical defences against bark beetles relate to degree of historical exposure between species and are allocated independently of chemical defences within trees. *Plant Cell Environ.* 2018;1–14. <https://doi.org/10.1111/pce.13449>