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Research paper

Ponderosa pine resin defenses and growth: metrics matter

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Bark beetles (Coleoptera: Curculionidae, Scolytinae) cause widespread tree mortality in coniferous forests worldwide. Constitutive and induced host defenses are important factors in an individual tree's ability to survive an attack and in bottom-up regulation of bark beetle population dynamics, yet quantifying defense levels is often difficult. For example, in *Pinus* spp., resin flow is important for resistance to bark beetles but is extremely variable among individuals and within a season. While resin is produced and stored in resin ducts, the specific resin duct metrics that best correlate with resin flow remain unclear. The ability and timing of some pine species to produce induced resin is also not well understood. We investigated (i) the relationships between ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson) resin flow and axial resin duct characteristics, tree growth and physiological variables, and (ii) if mechanical wounding induces ponderosa pine resin flow and resin ducts in the absence of bark beetles. Resin flow increased later in the growing season under moderate water stress and was highest in faster growing trees. The best predictors of resin flow were nonstandardized measures of resin ducts, resin duct size and total resin duct area, both of which increased with tree growth. However, while faster growing trees tended to produce more resin, models of resin flow using only tree growth were not statistically significant. Further, the standardized measures of resin ducts, density and duct area relative to xylem area, decreased with tree growth rate, indicating that slower growing trees invested more in resin duct defenses per unit area of radial growth, despite a tendency to produce less resin overall. We also found that mechanical wounding induced ponderosa pine defenses, but this response was slow. Resin flow increased after 28 days, and resin duct production did not increase until the following year. These slow induced responses may allow unsuccessfully attacked or wounded trees to resist future bark beetle attacks. Forest management that encourages healthy, vigorously growing trees will also favor larger resin ducts, thereby conferring increased constitutive resistance to bark beetle attacks.

Keywords: constitutive defense, forest management, induced defense, resin canals.

Introduction

Conifers have an extensive system of constitutive and induced defenses to resist attacks from bark beetles (Coleoptera: Curculionidae, Scolytinae) and associated fungi (Berryman 1972, Franceschi et al. 2005). The primary defense is resin, a complex mixture of terpenoid compounds, which acts as both a physical and chemical defense against beetles (Keeling and Bohlmann 2006, Raffa 2014). In *Pinus* species, resin is synthesized and stored in an interconnected network of axial and radial resin ducts in the secondary phloem and xylem (Bannan 1936, Wu and Hu 1997). When bark beetles bore into a tree, they sever resin ducts, allowing resin to flow to the attack site. Constitutive resin traps and flushes attacking beetles, while induced resin deters the subsequent spread of beetles and symbiotic fungi inside the tree (Berryman 1972). Although critical, resin flow is extremely variable, depending on genetics, season, temperature, site fertility, age, tree growth, stand dominance, wounding and disturbance (Blanche et al. 1992, Lombardero et al. 2000, Knebel et al. 2008, Novick et al. 2012, Hood et al. 2015, Moreira et al. 2015). This variability complicates efforts to characterize the defensive potential of trees (Gaylord et al. 2007, 2011). Resin duct metrics may be better surrogates for defense potential because they represent the production and storage capacity of resin in the tree, they are recorded in the annual rings of trees, measurements are

repeatable and growth effects can be accounted for. Recent work in several *Pinus* species has consistently shown that trees with higher resin duct production and/or larger ducts are more likely to survive beetle attacks (Kane and Kolb 2010, Gaylord et al. 2013, 2015, Ferrenberg et al. 2014, Hood et al. 2015) and drought (Hereş et al. 2014). However, the relationships between resin flow, resin duct traits and growth rates are not straightforward, and the specific resin duct metric best suited to characterize defense potential remains unclear. If resin flow depends on resin ducts, this response may also be relevant for species where resin flow is inducible shortly after wounding and where slower induction responses include increases in resin ducts.

As with resin flow, resin duct production is also influenced by genetics, climate and environmental factors such as soil fertility and disturbance (Rigling et al. 2003, Rosner and Hannrup 2004, Hood et al. 2015, Moreira et al. 2015). Therefore, a tree's ability to produce resin and resin ducts is likely related to the degree of constraints imposed by genetic and environmental factors. Both resin and resin ducts are costly to produce (Gershenzon 1994). These costs could lead to trade-offs between growth and defense (Strauss et al. 2002) and help explain interspecific variation in investment to defense as a function of species-specific life-history strategies. For instance, the resource availability hypothesis (RAH) predicts that constitutive defenses are favored in slower growing species due to higher costs of replacing tissues, whereas inducible defenses are more common in fast growing species due to lower costs of tissue replacement (Endara and Coley 2011). Consequently, the RAH also predicts an inverse relationship between constitutive and inducible defenses. The growth-differentiation balance hypothesis (GDBH) allows predictions of costs of defense at the intraspecific level based on the degree to which stress affects growth vs carbon assimilation (Herms and Mattson 1992). The GDBH predicts that any resource limitation that reduces growth more than photosynthesis (e.g., water and nutrients) will increase carbohydrates available for defense with little-to-no trade-off to growth. Following this logic, resin flow is predicted to increase under moderate water stress, when growth is more limited than photosynthesis, but to decline under severe water stress, when carbon assimilation by photosynthesis is insufficient to meet carbon demands, including resin production. Therefore, the GDBH provides an intraspecific physiological explanation for both positive and negative correlations between growth and defense. Several studies in pines have found support for the GDBH (Lorio and Sommers 1986, Wilkens et al. 1998, Lombardero et al. 2000, Gaylord et al. 2007, 2013, Novick et al. 2012). Resin ducts also follow this pattern, forming primarily in the latewood when water stress limits growth and tracheid expansion (Rigling et al. 2003). Because resin and resin duct production may not coincide with growth demands due to growth limitations, no trade-off may occur. Therefore, vigorous, faster growing trees may be better defended, as higher photosynthetic rates during periods of moderate water stress are expected. This may explain why suppressed trees are less defended and preferentially attacked by beetles during endemic stages compared to larger trees (Boone et al. 2011). It may also explain why resin production tends to be higher at more productive sites where trees experience higher growth rates (McDowell et al. 2007).

In addition to constitutive resin, inducible resin is also important for trees' ability to resist bark beetle attacks (Raffa and Berryman 1983, Paine et al. 1997, Wallin et al. 2008), particularly during epidemics (Boone et al. 2011). Rapid induction of resin chemistry and flow likely occurs via existing resin ducts. In addition, a slower, but long-term induced response can occur through the induction of resin duct production, which would increase the defense capacity of trees to resist future bark beetle attack. However, a trade-off can exist between constitutive and induced defense responses in plants (Kempel et al. 2011, Moreira et al. 2014). This trade-off is thought to be based on the probability and severity of an insect attack, with plants investing more in constitutive defenses when herbivory pressure is high or could cause plant death (Karban et al. 1999). Many species in the *Pinus* genus are highly susceptible to mortality from bark beetles and have the most developed constitutive defense structures among conifers (Bannan 1936, Wu and Hu 1997). Consistent with constitutive–induced defense trade-off theory, pines have relatively low induced responses compared with other conifer taxa (Lewinsohn et al. 1991). However, induced chemical and anatomical responses do occur (Sampedro et al. 2011, Moreira et al. 2012, 2014, 2015), although induction timing and extent is variable, species specific and herbivore specific (Ruel et al. 1998, Lombardero et al. 2006, Perrakis and Agee 2006, Knebel and Wentworth 2007, Cannac et al. 2009, Gaylord et al. 2011, Lombardero and Ayres 2011). Few studies exist on resin duct induction in pines, but these suggest that induction is possible after mechanical (Fahn and Zamski 1970, Rodríguez-García et al. 2014), chemical (Moreira et al. 2015) or fire-induced wounding (Hood et al. 2015). However, little information exists on the level of wounding required to induce resin ducts (but see Rodríguez-García et al. 2014, Hood et al. 2015) and the degree to which duct induction depends on growth rate. Gaylord et al. (2011) found no increase in induced resin flow in ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson) within a week after mechanical wounding. However, studies in other pine species have shown that induced resin responses may take several months to occur (Klepzig et al. 2005, Luchi et al. 2005, Knebel et al. 2008, Cannac et al. 2009).

While several studies have shown positive correlations between resin ducts and resin flow (Blanche et al. 1992, Lombardero et al. 2000, Rodríguez-García et al. 2014, Westbrook et al. 2015), there are numerous ways to quantify ducts (duct production, duct size, duct density, total duct area, etc.), and it is not yet clear which resin duct metrics best correlate with resin flow. For instance, genetic correlations between growth and resin ducts have yielded contradictory reporting of duct metrics and lack of

information as to how each specific metric relates to each result, which appears to be related to the use of different duct metrics. Positive correlations have been reported when using absolute resin duct metrics (Rosner and Hannrup 2004, Westbrook et al. 2015), while negative correlations have been reported when using relative metrics (Moreira et al. 2015). Inconsistencies in reporting duct metrics and lack of information as to how each specific metric relates to another and to resin flow often make it difficult to compare studies and to draw broader inferences about the effects of growth and climate on tree resistance. Better linkages between constitutive and induced resin duct metrics and growth are needed to more fully understand the complete defense potential of pines and its relationship with growth rate.

Here, we investigated the relationship of resin duct metrics and growth to constitutive resin flow in ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson var. *ponderosa* C. Lawson) and whether resin flow in this species is inducible. Ponderosa pine has a broad geographic distribution and is susceptible to attack from multiple bark beetle species, including mountain pine beetle (*Dendroctonus ponderosae* Hopkins) and western pine beetle (*Dendroctonus brevicomis* LeConte) in the northern part of its range. To explore the relationships between different resin duct metrics and growth and to determine how these variables relate to constitutive resin flow, we measured trees from two plantations of half-sibling families of the same age in western Montana, USA, with different stand densities. We then examined induced duct defense responses in these trees and, in a separate study area, the timing of induced resin flow due to mechanical wounding. We hypothesized that (i) resin duct production and size would be positively related to tree growth rate, but duct metrics standardized to wood production would be negatively related to growth; (ii) constitutive resin flow would be higher under moderate water stress when limited water availability reduced tree growth but allowed continued photosynthesis and that this response would be similar at the two sites; (iii) faster growing trees and trees in the lower density plantation would produce more constitutive resin than slower growing trees because trees with larger crown biomass would have greater carbon surplus later in the season to invest in resin and resin duct formation; (iv) constitutive resin flow would be more dependent on variables related to total duct area and size than to total number of ducts or duct density, as resin flow is related to duct diameter as described by Hagen Poiseuille's law (Schopmeyer et al. 1954); and (v) wounding would induce resin flow and the production of resin ducts, but response time would be slow (e.g., weeks to months).

Materials and methods

We used two experimental plantations of the same age to explore the relationships between resin duct metrics, growth and constitutive resin flow and inducible resin duct responses. Inducible resin flow responses were assessed in an adjacent, natural stand.

Factors relating to constitutive resin flow

Site description To minimize variability due to genetics, age and growing conditions (Roberds et al. 2003, Rosner and Hannrup 2004, Westbrook et al. 2015), we used two genetic field trials of ponderosa pine in western Montana, USA (Condon and Lubrecht; see Table 1 for site characteristics). Trials were established by the Inland Empire Tree Improvement Cooperative in 1974 in western Montana. Seeds were collected from open-pollinated trees in western Montana and Northern Idaho and reared at the USDA Forest Service tree nursery in Coeur d'Alene, Idaho. Both sites were level and cleared prior to planting 1-year-old bare root seedlings in 1974 on 3 × 3 m spacing, and containerized 2-year-old seedlings in 1975 to replace mortality during the first year. The Condon stand was thinned in 2003 to favor trees with higher growth and better form by removing one to two trees around each residual tree. The Lubrecht stand was not thinned.

Data collection and preparation In 2011, at each site, we randomly selected six half-sibling families with at least nine trees per family with no sign of insect attack. We randomly assigned three trees from each family to one of three sampling periods: 27 June, 27 July and 22 August (18 trees per sampling period). For trees assigned to the June sampling period, we also measured resin flow during the July and August periods (three times in total). Trees assigned to July and August periods were sampled once. We measured diameter at breast height (DBH; 1.37 m above ground), height (m) and live crown base height (m) for each tree in June. During each sampling period, we collected 24-h constitutive resin flow samples on the north and south aspects of each tree at ~1.37 m above ground using the methods of Ruel et al. (1998). We removed a 2.5-cm circular section of the bark and phloem using an arch punch and measured phloem thickness to the nearest 0.1 mm. We then created a funnel immediately below the tapping site with silicone and attached a 50-ml vial to the tree

Table 1. Site description and mean (range) of tree characteristics sampled in 2011 at Condon and Lubrecht, Inland Empire Tree Improvement Cooperative, Montana, USA.

Variable	Condon ($n = 54$)	Lubrecht ($n = 54$)
Longitude	–113.7182	–113.4753
Latitude	47.5437	46.8874
Elevation (m)	1123	1260
Year planted[1]	1974, 1975	1974, 1975
Year thinned[2]	2003	not thinned
Original spacing (m)	3 × 3	3 × 3
DBH (cm)	26.1 (17.3–37.1)	19.0 (10.9–33.0)
Tree height (m)	17.4 (12.5–21.3)	11.7 (8.2–18.9)
Live crown ratio (0–1)	0.6 (0.3–0.9)	0.6 (0.4–0.7)

[1]Bare root seedlings were planted in 1974, and 1-year-old seedlings from containers were planted in 1975, making all trees in the trial the same age.
[2]Thinning removed at least one tree from around each residual tree.

below the funnel to collect the resin. After 24 h, we measured the volume of resin to the nearest 0.25 ml.

We collected additional data at Condon to determine how physiological status influenced resin flow. We measured AM water potential (07:00–09:00 h) with a Model 1000 pressure chamber (PMS Instrument Company, Albany, OR, USA), photosynthesis and stomatal conductance to quantify water stress and gas exchange at each 2011 sampling period. Gas exchange measurements were done with a LiCor 6400 (LI-COR, Lincoln, NE, USA), at 1000 μmol m^{-2} s^{-1} light provided by an LED light source. Temperature and CO_2 concentration were maintained at 25 °C and 380 μmol mol^{-1}. Branches from the lower third of the crown were collected using a pruning pole and 1-year-old needles were used for measurements. We were not able to reach the crown for some trees; therefore, sample size was 15 in June and July and 14 in August.

For tree growth and resin duct production measures, we extracted one, 4.5-mm-wide increment core from each tree in July 2013 at Condon and October 2013 at Lubrecht using a manual increment borer to obtain a cross section of wood containing annual rings from 2013 to the tree pith. Cores were collected at ~1.37 m above the ground, below and within 3–6 cm of the resin-flow-tapping site. In addition, we collected cores centered directly in the tapping locations on a portion of trees sampled three times for resin flow at each site in 2012. Because we removed the cambium when sampling resin flow, growth ceased on the sampling date. This allowed us to determine when the trees began transitioning to latewood xylem production.

We prepared cores using standard techniques (mounted and sanded until cellular structure was visible through a binocular microscope). For the cores used to quantify annual growth and resin duct production, we assigned the correct calendar year to each tree ring (i.e., crossdated) (Grissino-Mayer 2001). We scanned all cores using an Epson platform scanner at 2400 d.p.i. and measured ring widths to the nearest 0.001 mm using CooRecorder v7.7 (Cybis Elekronik & Data AB, Saltsjöbaden, Sweden). We measured resin ducts in ImageJ (version 1.46r, National Institutes of Health, Bethesda, MD, USA) to the nearest 1×10^{-7} mm^2 using the ellipse tool and assigned the calendar year in which each duct formed. We measured ducts formed from 2001 until 2013 for Lubrecht and from 2001 until 2012 for Condon, as these cores were collected while the trees were still growing at this site.

We calculated five resin duct metrics for each core (Table 2). Three of these metrics were used to capture annual absolute investment in resin ducts, unadjusted for ring area: (i) mean duct size, (ii) duct production and (iii) total duct area. The other two metrics capture the annual investment in resin ducts standardized to ring area: (iv) duct density and (v) relative duct area.

We used raw ring width values and basal area increment (BAI) as annual growth metrics. We observed no age-related decline in the ring width for the section of the chronology we used for analyses (the most recent 13 years); therefore, we did not detrend raw ring width. We calculated BAI (cross-sectional area of secondary xylem produced per year) from ring widths and the tree diameter inside bark using dplR package v. 1.6.0 in R v. 3.0.1 (Bunn 2008).

Data analysis **Relationships between resin duct and tree growth metrics** We performed correlation analyses of resin duct and growth variables for the last 10 years of growth prior to resin flow sampling in 2011 (2001–10) by site and also pooled for both sites to determine the relationships between the duct and growth metrics. Mean values were calculated for each core (n = 54 at each site) for the analyses.

Seasonal patterns of resin flow, water stress and tree growth We used separate general linear mixed models (GLMM) to examine the seasonal pattern of resin flow, water stress and carbon acquisition at Condon (SAS Institute v. 9.3, Cary, NC, USA). Family nested within month was used as a random effect, with individual trees used as the experimental unit. Repeated resin flow measurements in July and August from the trees sampled in June were excluded from the analysis. Water potential, photosynthesis and stomatal conductance were normally distributed, but we specified a lognormal link function for resin flow to stabilize residuals. Pairwise differences in sampling period LS means were tested using Tukey's post hoc test (significance level $\alpha = 0.05$).

Resin flow relationships to site, tree growth and resin duct metrics Resin ducts can remain functional for numerous years (Lewinsohn et al. 1991), thereby potentially contributing to resin flow long after the ducts were formed. To investigate the contribution of previous years' resin ducts and growth to resin flow, we created 5- and 10-year independent variables from the resin duct

Table 2. Resin duct variable name and description.

Variable	Type[1]	Description
Duct size (mm^2)	Unstandardized	Mean size of all ducts per annual ring
Duct production (no. $year^{-1}$)	Unstandardized	Total number of ducts per annual ring
Total duct area (mm^2 $year^{-1}$)	Unstandardized	Sum of duct area per annual ring
Duct density (no. mm^{-2} $year^{-1}$)	Standardized	Total number of ducts per annual ring divided by ring area[2]
Relative duct area (% annual ring)	Standardized	Total duct area divided by ring area[2] × 100

[1]Standardized = adjusted for growth rate; unstandardized = unadjusted for growth rate.
[2]Ring area = ring width × core diameter.

and annual growth variables. We chose 5 and 10 years based on preliminary correlation analysis that showed resin flow was related to total duct area in a given year for 9 of the last 10 years, and also based on previous studies that pooled duct data over numerous years to determine either resin flow or resistance to attack (Blanche et al. 1992, Kane and Kolb 2010, Ferrenberg et al. 2014). For duct production, total duct area, ring width and BAI, we summed the values for (i) 5 years (2006–10) and (ii) 10 years (2001–10) prior to resin flow sampling. We then calculated 5- and 10-year resin duct density and relative duct area from these summed values. For duct size, we calculated the mean duct size for the 5- and 10-year period. We used average resin flow calculated from the north and south aspects of each tree, as aspect did not influence resin flow at either site ($F_{1,193} = 1.18$, $P = 0.2793$) (see Figure S1 available as Supplementary Data at *Tree Physiology* Online).

We used separate GLMMs to examine the relationship of average resin flow to each duct and growth metric, site and sampling period, using family as a random effect, maximum likelihood to estimate model parameters and differences in Akaike Information Criterion (ΔAIC) as a comparative metric across morphological variables. For all models, we specified a lognormal link function to stabilize residuals. One tree at Lubrecht died in July, and resin leaked out of sample vials on another four trees; these were excluded from all analyses of resin flow. We applied a Bonferroni correction factor to account for multiple comparisons for each 5- and 10-year metric (significance level P-value <0.025); for all other variables, we used a P-value <0.05 to determine significance. We also used the Benjamini–Hochberg procedure as an additional precaution against Type 1 errors due to multiple comparisons (Benjamini and Hochberg 1995), with the total number of tests equal to 18 and false discovery rate equal to 0.1. Both methods identified the same morphological variables as statistically relating to resin flow.

Inducibility of ponderosa pine defenses

Site description We examined induced resin flow responses for 60 days after mechanical wounding. We randomly selected 15 dominant or codominant trees between 15 and 23 cm DBH growing in a naturally established ponderosa pine-dominated stand also on the Lubrecht Experimental Forest in June 2011. This second site was necessary because we could not mechanically wound trees tapped for resin flow in the Condon and Lubrecht genetic trials, as these trees are part of a long-term research program.

In addition, we also used trees sampled for resin flow three times during the season in the genetic trials (Condon and Lubrecht; see above) to investigate whether the minor wounding caused by sampling induces resin flow later in the season. We used the cores collected from all trees in the genetic trials to determine whether tapping trees to sample resin flow changed resin duct production in the year after tapping.

Treatment description In the natural stand, we randomly assigned trees to one of two treatments: control ($n = 6$) and wounding ($n = 9$). Trees in the wounding treatment were then randomly assigned an aspect for the wounding (north or south). Wounding was designed to drain the area of constitutive resin in order to measure induced resin production. We generally followed the methods used by Ruel et al. (1998) and Gaylord et al. (2011). We predicted that the unwounded side of the trees could serve as a control to the wounded side due to limited connectivity of the ducts on opposite sides of the tree. Our sampling design allowed us to test this hypothesis by comparing resin flow between wounded and unwounded trees and also between the wounded side and control side of wounded trees.

To begin the experiment, we tapped all trees for constitutive resin on 6 July 2011 on the north and south aspects of each tree using the same methods as described above. The following day (7 July 2011; Day 0), 24 ± 1 h after tapping, we measured the amount of constitutive resin to the nearest 0.25 ml. We then wounded the trees in the wounding treatment on the assigned aspect by using a chisel to remove 1.5 cm tall strips of bark and phloem 20 cm above and below the constitutive resin flow site. Strips were 40% of the circumference of the tree bole, centered from the resin flow site (see Figure S2 available as Supplementary Data at *Tree Physiology* Online). After wounding, we tapped all trees to measure resin flow ~3 cm to one side of the first tapping site. On 8 July 2011 (Day 1), we measured resin flow from the second tapping site (8 July 2011) and rewounded trees in the wounding treatment by expanding each strip an additional 1.5 cm to equal 3 cm. We tapped all trees to measure 24-h resin flow three additional times: 15 July 2011 (Day 8), 4 August 2011 (Day 28) and 6 September 2011 (Day 61). Tapping sites were always positioned successively in a horizontal line, each within 3 cm of the previous tapping site. We assumed that resin flow from trees in the control and wounded-control side (unwounded sides of mechanically wounded trees) treatments was constitutive resin for all dates, and resin collected from trees in the wounded-wound side treatment (the wounded side of mechanically wounded trees) after wounding (Day 1 and after) was induced resin.

Data analysis **Inducibility of resin flow** To determine the extent to which mechanical wounding increases resin flow, we compared resin flow between control trees, wounded-control side and wounded side of wounded treatments based on day after wounding using a GLMM with day, treatment and day × treatment as fixed effects and tree as a random effect. We then compared resin flow between trees sampled once vs multiple times at Condon and Lubrecht, using sampling period, sample class (sampled once vs sampled multiple times) and sampling period × sample class as fixed effects and tree as a random effect. All models specified a lognormal link function to stabilize residuals.

Inducibility of resin ducts We compared annual resin duct and growth metrics between the 10 years prior to sampling resin flow and 1 year after sampling at Condon and for 2 years after sampling at Lubrecht to determine whether minor wounding induces duct production. A preliminary analysis showed that the number of times sampled (1 vs 3 times) did not affect any measured resin duct or growth variable in the years following tapping ($P > 0.1247$ for all variables; Table S1 available as Supplementary Data at *Tree Physiology* Online). Therefore, all trees were included in the analyses and number of times sampled was not included in the final models. Separate analyses by site and metric were performed, with duct production, total duct area, duct size and BAI as dependent variables; site, period (before = 10-year average prior to sampling; after = 2012) and site × period as independent, fixed effects and tree as a random effect (Tukey's post hoc test for pairwise differences reported with significance level $\alpha = 0.05$). Duct size was normally distributed, but we used a lognormal distribution for all other variables to stabilize residuals. We back-transformed values for reporting means.

Results

Relationships between resin duct and tree growth metrics

Duct production, duct size and total duct area were positively correlated with ring width and BAI at both sites (Table 3). Resin duct variables standardized to ring area (resin duct density and relative duct area) were negatively correlated with annual growth variables at Condon. At Lubrecht, only resin duct density, but not relative duct area, was significantly negatively correlated with growth. These standardized measures were much more weakly related to growth (Table 3; $r = -0.32$ to -0.45) than the non-standardized variables (Table 3; $r = 0.57$–0.84). Total duct area had the strongest relationship to both ring width ($r = 0.84$, $P < 0.0001$) and BAI ($r = 0.84$, $P < 0.0001$) of all resin duct variables measured. Duct size and duct production were positively correlated at Lubrecht ($r = 0.34$, $P = 0.0110$), but not at Condon (Table 3). Duct size and duct production were positively correlated with total duct area at both sites, but the relationship was stronger for number of ducts ($r = 0.91$, $P < 0.0001$) than duct size ($r = 0.79$, $P < 0.0001$). Relative duct area was only correlated with duct production and duct size at Lubrecht.

Seasonal patterns of resin flow, water stress and tree growth

Resin flow varied among months ($F_{2,87.42} = 19.86$, $P < 0.001$). As predicted from the GDBH, both sites exhibited the same seasonal pattern, with the highest flow in August when water stress presumably began limiting growth (Figure 1; Figure S3 available as Supplementary Data at *Tree Physiology* Online). While resin flow was lowest in July relative to August (Condon: $P = 0.0092$; Lubrecht: $P < 0.0001$), June values did not differ from July or August. At Condon, where we collected additional physiological

Table 3. Correlation matrices of mean annual constitutive resin duct and growth variables by site and pooled based previous 10 years (2001–10) and 4.5 mm core diameter. Significant correlations indicated by *$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$. Duct production = mean number of resin ducts per ring; duct size = mean duct size (mm^2); total duct area (mm^2) = sum of annual resin duct area; duct density (no. ducts mm^{-2}) = number of ducts per ring/ring area; relative duct area (%) = total duct area per ring/ring area × 100; ring width (mm) = annual ring width.

	Duct size (mm^2)	Total duct area (mm^2)	Duct density (no. ducts $year^{-1}$)	Relative duct area (%)	Ring width (mm)	BAI (mm^2)
Condon ($n = 54$)						
Duct production	0.27	0.89***	0.11	0.23	0.68***	0.63***
Duct size		0.66***	–0.45**	–0.17	0.58***	0.58***
Total duct area			–0.16	0.06	0.81***	0.77***
Duct density				0.94***	–0.51***	–0.45**
Relative duct area					–0.40*	–0.35*
Ring width						0.93***
Lubrecht ($n = 54$)						
Duct production	0.34*	0.91***	0.17	0.35**	0.71***	0.67***
Duct size		0.69***	–0.30*	0.07	0.66***	0.70***
Total duct area			–0.02	0.27*	0.85***	0.84***
Duct density				0.92***	–0.41**	–0.32*
Relative duct area					–0.19	–0.09
Ring width						0.95***
Both sites pooled ($n = 108$)						
Duct production	0.50***	0.91***	0.14	0.39***	0.74***	0.71***
Duct size		0.79***	–0.28**	0.15	0.68***	0.72***
Total duct area			–0.06	0.30**	0.84***	0.84***
Duct density				0.90***	–0.42***	–0.34**
Relative duct area					–0.15	–0.06
Ring width						0.94***

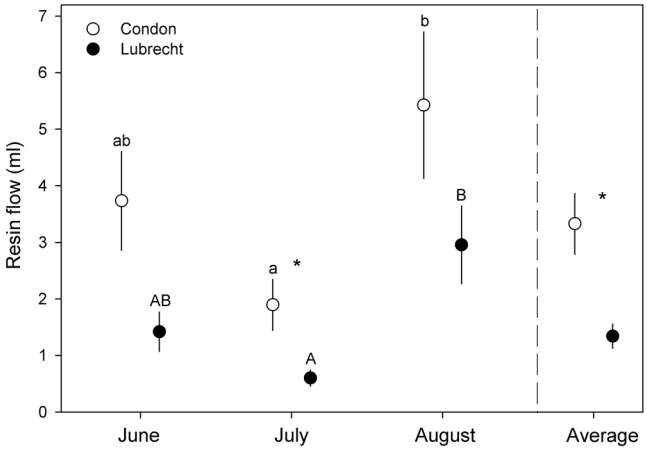

Figure 1. Monthly and seasonal 2011 average constitutive resin flow (±SE) for Condon and Lubrecht Inland Empire Tree Improvement Cooperative Sites. Only trees sampled once or for the first time (June sample trees) are included in the analyses ($n = 103$). Asterisks denote significant differences ($\alpha = 0.05$) between sites within a given month and for season average. Different letters denote significant differences between months within a site; lowercase letters indicate Condon and uppercase letters indicate Lubrecht.

measurements, water potential, photosynthesis and conductance were significantly lower in August than in either June or July (see Figure S3 available as Supplementary Data at *Tree Physiology* Online). Based on the cores collected directly from the monthly tapping sites, the trees began transitioning to latewood between the July and August sampling periods, indicating that seasonal water stress increased in August.

Trees at Condon had higher growth rates and longer crown lengths than at Lubrecht, but similar phloem thickness and crown ratio (Table 4). Following our predictions that faster growing trees should produce more resin, resin flow was higher at Condon compared with Lubrecht (3.3 vs 1.3 ml; $F_{1,9.803} = 15.87$, $P = 0.0027$; Figure 1; Table 4).

Resin flow relationships to site, tree growth and resin duct metrics

Resin flow was significantly higher at the site with faster growing trees (Condon), but growth metrics (e.g., DBH, height and BAI) did not predict resin flow (Table 5). Phloem thickness was the only non-duct variable that was significantly and positively related to resin flow (Table 5). Phloem thickness was also positively correlated with DBH ($r = 0.55$, $P < 0.0001$). Site was a significant factor influencing resin flow, with higher resin flow at Condon than Lubrecht for a given growth value.

Of the resin duct variables, only duct size and total duct area were related to resin flow, and these relationships did not depend on site (Table 5). Site was significant for all other resin duct variables, with higher resin flow at Condon than Lubrecht for a given duct or growth value (Figure 2). Mean duct size and total duct area for both the previous 5 years and previous 10 years prior to resin flow sampling was positively related to resin flow (Table 5). Month was a significant factor in resin flow in all models.

Table 4. Mean (SE) tree growth and resin flow of trees samples at Condon and Lubrecht, Inland Empire Tree Improvement Cooperative, Montana, USA. Different letters within rows indicate significant site differences ($\alpha = 0.05$).

Variable	Condon ($n = 54$)	Lubrecht ($n = 54$)
DBH (cm)	26.1 (0.71)a	19.0 (0.71)b
Tree height (m)	17.4 (0.31)a	11.7 (0.31)b
Live crown length (m)	9.6 (0.27)a	6.7 (0.27)b
Live crown ratio (0–1)	0.55 (0.01)a	0.57 (0.01)a
Phloem thickness (mm)	2.58 (0.11)a	2.28 (0.10)a
BAI (cm^2)[1]	11.2 (0.84)a	6.9 (0.51)b
Resin flow (ml)	3.3 (0.54)a	1.4 (0.22)b

[1]Average BAI for most recent 20 years of growth.

These patterns were similar when examined over average monthly resin flow for trees sampled three times. Mean 5-year duct size and total duct area were positively related to resin flow (Figure 2). However, phloem thickness was only weakly positively related to average monthly flow ($F_{1,10} = 4.75$; $P = 0.0543$, Figure 2d). Resin flow did not differ as a function of site in any of the models of average monthly resin flow ($P > 0.05$, Figure 2).

Inducibility of resin flow

Induced resin flow changed by day ($F_{4,122} = 16.82$, $P < 0.0001$), treatment ($F_{2,30.63} = 4.61$, $P = 0.0178$) and by day × treatment ($F_{8,122} = 2.17$, $P = 0.0346$). There were no differences in resin flow prior to wounding (Day 0, constitutive flow; Figure 3). After wounding, there was no difference in resin flow between treatments through Day 8. Resin flow in the wounded-wound side treatment, however, was higher on Day 28 compared with the wounded-control side treatment (3.0 vs 0.08 ml; $P = 0.0064$) but was not different from the control treatment (3.0 vs 1.4 ml; $P = 0.7789$). On Day 61, resin flow generally increased in the wounded treatment, but this increase was not statistically different from the control and wounded-control treatments due to large variation in flow. Resin flow increased throughout the season in all treatments (Figure 3; Table S2 available as Supplementary Data at *Tree Physiology* Online). Tapping trees once vs three times had no effect on resin flow within the same year at either site (sample time—Lubrecht: $F_{1,47.69} = 1.78$, $P = 0.1887$; Condon: $F_{1,50.02} = 0.25$, $P = 0.6221$; Figure S4 available as Supplementary Data at *Tree Physiology* Online).

Inducibility of resin ducts

Sampling for constitutive resin flow increased duct production 1 year later at both sites, resulting in higher duct production in 2012 (Condon: 5.7 ± 0.3 standard error (SE); Lubrecht: 6.0 ± 0.4 SE) compared with the previous 10-year average (Condon: 4.0 ± 0.2 SE; Lubrecht: 2.9 ± 0.2 SE) (Figure 4, $F_{1,99} = 111.51$, $P < 0.0001$). The magnitude of increase differed by site ($F_{1,99} = 13.90$, $P = 0.0003$). Prior to tapping, resin duct

Table 5. Relationship of ponderosa pine constitutive resin flow with morphological variable, site and month as indicated by *F*-values (*P*-values) and ΔAIC from GLMM analyses by morphological variable. *F*-values in bold indicate significant relationship between variable and resin flow ($P < 0.05$ and a Bonferroni correction factor $P < 0.025$ for variables based on 5- and 10-year averages). Only trees sampled once or for the first time (June sample trees) are included in the analyses ($n = 103$).

Morphological variable[1]		Site	Month	ΔAIC
Duct size 5	**11.90** (0.0009)	2.91 (0.0915)	**24.38** (<0.0001)	0
Duct size 10	**7.26** (0.0085)	5.17 (0.0254)	**22.42** (<0.0001)	4.21
Total duct area 5	**6.77** (0.0109)	4.26 (0.0420)	**22.59** (<0.0001)	4.67
Total duct area 10	**5.49** (0.0214)	**6.40** (0.0132)	**21.79** (<0.0001)	5.88
Duct production 5	3.75 (0.0561)	**7.96** (0.0059)	**21.54** (<0.0001)	7.54
Duct production 10	3.20 (0.0771)	**10.88** (0.0014)	**21.30** (<0.0001)	8.07
Duct density 5	0.02 (0.8807)	**18.87** (<0.0001)	**20.58** (<0.0001)	11.2
Duct density 10	0.04 (0.8389)	**18.84** (<0.0001)	**20.61** (<0.0001)	11.18
Relative duct area 5	1.91 (0.1703)	**13.83** (0.0004)	**20.95** (<0.0001)	9.33
Relative duct area 10	2.95 (0.0893)	**9.90** (0.0023)	**20.70** (<0.0001)	8.32
BAI 5	1.92 (0.1689)	**9.96** (0.0022)	**21.21** (<0.0001)	9.32
BAI 10	1.73 (0.1918)	**11.83** (0.0009)	**20.98** (<0.0001)	9.5
Ring width 5	1.35 (0.2483)	**11.00** (0.0013)	**21.25** (<0.0001)	9.88
Ring width 10	1.30 (0.2572)	**13.72** (0.0004)	**21.12** (<0.0001)	9.93
DBH	3.12 (0.0808)	**8.71** (0.0041)	**21.15** (<0.0001)	8.21
Height	0.77 (0.3827)	**5.96** (0.0167)	**20.77** (<0.0001)	10.48
Live crown ratio	0.66 (0.4170)	**18.10** (<0.0001)	**20.52** (<0.0001)	10.58
Phloem thickness	**5.20** (0.0250)	**13.60** (0.0004)	**22.92** (<0.0001)	6.22

[1]Morphological variables: 5 indicates that variable is based on most recent 5 years (2006–10) and 10 the most recent 10 years (2001–10) based on a 4.5-mm core diameter. Duct production = sum of resin ducts; duct size = mean duct size (mm^2); total duct area (mm^2) = sum of resin duct area; duct density (no. ducts mm^{-2}) = number of ducts/ring area; relative duct area (%) = total duct area/ring area × 100; ring width (mm) = sum of ring width; BAI (mm^2) = sum of cross-sectional area of secondary xylem produced; DBH (cm) = diameter at breast height, 1.37 m above ground; height (m) = tree height; live crown ratio (0–1) = (total height − height to base of tree crown)/total height; phloem thickness (mm) = average phloem thickness at site of resin flow collection.

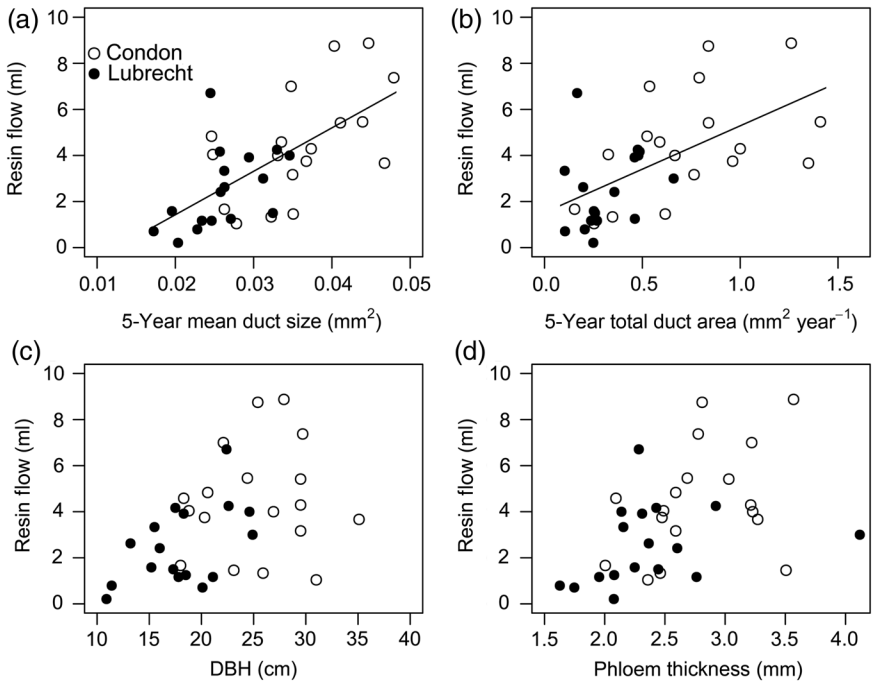

Figure 2. Average monthly constitutive resin flow (ml) as a function of (a) 5-year mean duct size ($F_{1,10} = 12.47$, $P = 0.0054$; site: $F_{1,10} = 0.04$, $P = 0.8388$), (b) 5-year total duct area ($F_{1,10} = 7.38$, $P = 0.0217$; site: $F_{1,10} = 0.49$, $P = 0.5002$), (c) DBH ($F_{1,10} = 3.31$, $P = 0.0988$; site: $F_{1,10} = 0.87$, $P = 0.3734$) and (d) phloem thickness ($F_{1,10} = 4.75$, $P = 0.0543$; site: $F_{1,10} = 2.44$, $P = 0.1496$) by site for trees sampled three times total in 2011 ($n = 35$).

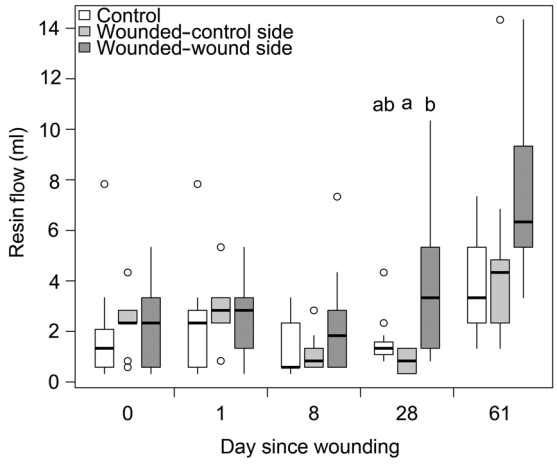

Figure 3. Ponderosa pine 24-h resin flow by wounding treatment at Lubrecht Experimental Forest, MT. Trees were wounded on Day 0 after measuring constitutive resin flow. Control = tree with no mechanical wounding; wounded-control side = opposite side of tree with mechanical wounding; wounded-wound side = side of tree with mechanical wounding (see Treatment description for additional details). Different letters indicate resin flow is significantly different between treatments within a time step ($\alpha = 0.05$). Boxes denote first and third quartiles, lines the median and whiskers the 1.5 interquartile range (IQR). Circles indicate outliers that are >1.5× IQR.

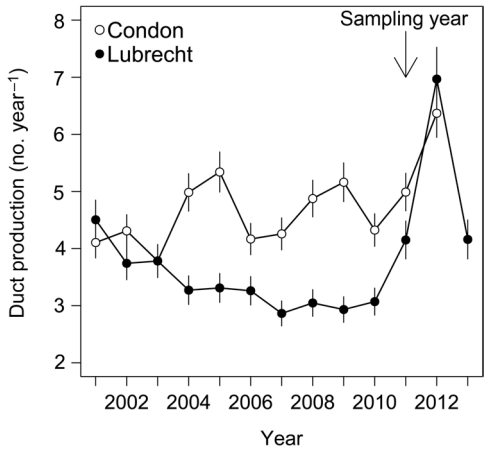

Figure 4. Annual duct production for trees at Condon and Lubrecht. Arrow indicates year of resin flow sampling (2011) when localized wounding occurred. Trees sampled once vs three times included ($n = 103$).

production was higher at Condon than at Lubrecht ($P = 0.0003$), but there was no difference between sites 1 year after tapping ($P = 0.9265$). At the slower growing Lubrecht site, duct production increased an average of 3.15 ducts per 4.5 mm wide core ($P < 0.0001$), while Condon duct production increased 1.7 ducts per 4.5 mm wide core ($P < 0.0001$). Resin flow sampling once vs three times did not affect the magnitude of any resin duct or growth metric (data not shown; $P > 0.05$).

Discussion

Constitutive resin flow in pines is the first line of defense against bark beetle attacks, and induced resin flow is significant for minimizing attack effects by compartmentalizing fungal growth and the attacking beetles. However, predicting resin flow as a function of tree size, growth and site characteristics is difficult due to large variability and has resulted in inconsistency among previous studies (see below). Here, we show that the best predictor of resin flow is resin duct size and total duct area. We also show that mechanical wounding induced ponderosa pine defenses, although this effect was slow (i.e., 28 days for resin flow to 1 year for resin ducts). Therefore, resin duct metrics have the potential to influence both constitutive and inducible resin flow.

Relationships between resin duct and tree growth metrics

Many studies of pines have reported increased resin duct production with tree growth, as we found (Blanche et al. 1992, Rigling et al. 2003, Kane and Kolb 2010, Ferrenberg et al. 2014, Hereş et al. 2014, Rodríguez-García et al. 2014, Westbrook et al. 2015). Few studies have quantified resin duct size, but Ferrenberg et al. (2014) also found a strong positive relationship between duct size and radial growth. The negative correlation between growth variables and the standardized metrics of resin duct density and relative duct area (Table 3) is consistent with other studies (Blanche et al. 1992, Rigling et al. 2003, Kane and Kolb 2010, Moreira et al. 2015, Westbrook et al. 2015) and indicates that while faster growing trees produce more and bigger ducts relative to slower growing trees, overall they invest proportionally less in resin ducts relative to xylem production. These results also highlight one of the difficulties in testing defense theories (sensu Stamp 2003) because defenses could be either positively or negatively correlated with growth depending on the metric used. For example, if only relative defense measures were included, we would have reported a negative correlation between constitutive defense and growth, which supports the RAH. However, absolute measures of constitutive defense show a positive correlation with growth, which contradicts the RAH.

Seasonal patterns of resin flow, water stress and tree growth

Perhaps the most consistent pattern reported in the literature is an increase of resin flow during periods of moderate drought, as predicted by the GDBH (Blanche et al. 1992, Lombardero et al. 2000, Gaylord et al. 2007). Our results also support this pattern. Under moderate water stress, carbon demand for growth decreases but photosynthesis continues, presumably causing a shift of carbohydrate allocation from growth to defense (resin production and resin duct formation). Most ducts occurred in the latewood portion of the annual ring, which began forming in August at both sites, consistent with other studies of resin duct formation (Wimmer and Grabner 1997, Rigling et al. 2003, Westbrook et al. 2015). At both sites, resin flow was significantly greater in August than July. Overall, these results suggest that a trade-off between growth and defense may only occur during periods of active growth, but that this trade-off is relaxed

as water stress restricts growth more than photosynthesis. However, severe drought should restrict both growth and defense (Dunn and Lorio 1993, Gaylord et al. 2013). Increased resin flow may also be related to the initial formation of the current year's resin ducts. Blanche et al. (1992) reported resin ducts in *Pinus taeda* L. began forming 12 weeks after cambial activation and estimated that the ducts were not fully functional until 6 weeks after formation. Therefore, resin flow may increase later in the growing season as a function of both water stress and the addition of new resin ducts that have little to no xylem between the ducts and cambium (Blanche et al. 1992, Lorio et al. 1995).

Resin flow relationships to site, tree growth and resin duct metrics

As predicted, we found higher resin flow at Condon, the thinned site with faster growth rates (i.e., greater BAI), where lower competition likely resulted in more resources for both growth and defense. Across stands, McDowell et al. (2007) also found that the best predictor of ponderosa pine resin flow was BAI. However, stand-level effects on resin flow are conflicting, with some studies reporting reduced resin flow after thinning (Lombardero et al. 2000, Zausen et al. 2005) and mixed effects in response to fertilization (see Novick et al. 2012 for summary).

At the individual tree level, however, neither tree size, radial growth nor crown ratio (an indicator of tree vigor) explained resin flow (Table 5). Results reported in the literature on the relationship between tree size or growth and constitutive resin flow are also mixed, with some studies finding no relationship (Blanche et al. 1992, Kytö et al. 1998) and others reporting a positive relationship (Kolb et al. 1998, Kyto et al. 1999, Gaylord et al. 2007, Rodríguez-García et al. 2014). In contrast to our results, Davis et al. (2012) and Rodríguez-García et al. (2014) reported a positive relationship between crown ratio and resin flow.

The only growth-related variable with a positive effect on resin flow was phloem thickness (Table 5). Phloem, as the main food source for bark beetles, affects host suitability and brood production (Amman 1972). Consistent with other studies (Shrimpton and Thomson 1985, Zausen et al. 2005, Davis and Hofstetter 2014), we found that phloem thickness was positively related to tree growth. Again, results in the literature on the relationship between resin flow and phloem thickness are mixed, with both positive (Davis and Hofstetter 2014) and negative (Zausen et al. 2005) relationships reported. Overall, the conflicting results on the relationship between resin flow and tree and stand-level characteristics suggest that resin production is the integrated effect of many factors that likely vary depending on resource availability, competition and species-specific responses to these conditions. A metric that captures such integrated effects would, therefore, provide a more reliable estimate of defense potential than resin flow.

In our study, the best predictors of resin flow were duct size and total duct area over the past 5 years. Consistent with results in *Pinus sylvestris* Aiton (Kyto et al. 1999), resin duct production (number of resin ducts), density and relative duct area did not predict resin flow (Table 5). It appears that duct size and total duct area are better predictors of resin flow as resin duct production may (Westbrook et al. 2015) or may not (Lombardero et al. 2000) relate to constitutive resin flow. The important role of resin duct size and total area is further reflected in our results showing that growth *per se* is not a good predictor of resin flow despite the fact that resin duct production, size and total duct area increased with tree growth (Table 3). Although faster growing trees with more and larger resin ducts tended to produce more resin as expected, the relationship between growth and resin flow was not statistically significant (Table 5; Figure 2c). The influence of duct size on resin flow in our study is clear in our models on the effect of morphological traits, site and month, where the site effect (higher resin flow at Condon, the faster growth site) is not significant after accounting for resin duct size. That is, resin flow at Condon is greater (Figure 1) because duct size is greater at this site (Figure 2a). The strong effect of resin duct size on resin flow is consistent with theory: flow is proportional to the fourth power of the duct diameter following Hagen–Poiseuille's law for liquid laminar flow within a tube (Schopmeyer et al. 1954). Therefore, for a given total duct area, fewer, larger ducts relative to more, smaller ducts should result in higher resin flow.

The mixed responses of resin production as a function of specific resin duct traits and individual tree- and stand-level factors may be explained by how these factors affect resin duct size and total area. Because ducts synthesize, store and deliver resin, resin duct size and total duct area are likely better predictors than the investment in resin ducts relative to xylem area. Thus, as our results and those of others suggest, a small tree with a high relative resin duct investment (i.e., duct density or relative duct area) is likely to produce less resin than a larger tree with lower relative resin duct investment because the absolute number of ducts, size and area is larger due to strong correlations with growth. In addition to the existing duct infrastructure to produce resin, producing resin requires resources and the mixed resin flow responses may also reflect the degree to which resources can be allocated to resin production (or the formation of new resin ducts) rather than to growth. This, in turn, depends on the site, species and, as predicted by the GDBH, the degree to which environmental variables influence growth vs photosynthesis. We note that we focused only on axial ducts, and future studies may reveal that radial resin duct area also contributes to resin flow in ponderosa pine as has been shown in other pines (Rodríguez-García et al. 2014).

The length of time resin ducts remain functional and contribute to resin flow is hard to measure, but our results suggest that it could be for at least 10 years, as indicated by the models predicting resin flow from the 10-year total duct area and size

(Table 5). Previous studies of resin ducts and flow have used a wide variety of methods to collect and quantify resin ducts. However, numerous studies have shown that resin duct production from the previous 3–30 years is associated with tree resistance to bark beetle attacks (Kane and Kolb 2010, Gaylord et al. 2013, Ferrenberg et al. 2014, Hood et al. 2015). This body of work investigating the role of resin in resistance to bark beetles supports our findings that resin ducts are long-term investments in a tree's defense system.

Inducibility of resin flow and resin ducts

We found that both resin flow and resin ducts are inducible in ponderosa pine, although the response time was relatively slow (Figures 3 and 4). The slow response in induced resin flow (28 days after wounding) is consistent with that in *Pinus nigra* Arnold (Luchi et al. 2005) and with the lack of induced resin flow shortly after wounding (7 days) in ponderosa pine (Gaylord et al. 2011). Both the degree and timing of resin flow induction in pines appear to be highly variable, even within a single species, as found in *P. taeda* (Ruel et al. 1998, Lombardero et al. 2000, Klepzig et al. 2005, Knebel et al. 2008). Wounding by low-severity fire has also been shown to induce resin flow, but again the response time was slow, taking months to increase above unburned tree levels (Lombardero et al. 2006, Perrakis and Agee 2006, Cannac et al. 2009). These slow responses follow trade-off theory between constitutive and induced resistance (Kempel et al. 2011), as pines generally have high levels of constitutive defenses (Lewinsohn et al. 1991, Moreira et al. 2014). Although induced resin flow occurred 28 days after wounding, induced resin duct production did not occur until the following year. Production of induced anatomical defenses, such as resin ducts, is an inherently slower process compared with production of chemical defenses, which can occur as rapidly as a few days (Bonello et al. 2006). Resin duct induction has also been shown after mechanical wounding (Rodríguez-García et al. 2014) and methyl jasmonate application (Moreira et al. 2015) in *Pinus pinaster* and in ponderosa pine after fire (Hood et al. 2015). In contradiction to RAH predictions, induced duct production after resin flow sampling was greater at the site with slower growing trees (Lubrecht). These results are consistent with Kempel et al. (2011), which found a negative correlation with induced resistance and growth. Resin flow and resin duct induction are slow, but these long-lasting induced defenses could affect resistance of surviving trees in the event of an attack in subsequent years.

Conclusion

Constitutive and induced host defenses are important in the ability of an individual tree to survive an insect attack and in bottom-up regulation of bark beetle population dynamics (Raffa et al. 2008). We show here that unstandardized resin duct metrics of total resin duct area and average duct size, but not tree growth variables or relative measures of resin ducts, were predictive of resin flow. Faster growing trees had larger ducts and higher total duct area, which generally led to higher resin flow than slower growing trees. Therefore, although slower growing trees invested more in resin duct defenses per unit area of radial growth (i.e., duct density and relative duct area), faster growing trees had larger ducts and tended to produce more resin.

The positive effects of tree growth on total resin duct area and size, which also were associated with increased resin flow, suggest that forest management that encourages healthy, vigorously growing trees should, therefore, also increase constitutive resin defenses to bark beetles. These positive correlations also suggest that pine constitutive resin defenses could be further increased through artificial selection that improves radial growth, due to genetic correlations (Roberds et al. 2003, Westbrook et al. 2015). However, our results also suggest that faster growth may limit induced duct production responses. These results are consistent with our ongoing work on the genetic correlation between growth rate and bark beetle-caused mortality (R. de la Mata et al., unpublished results). While constitutive resin flow is critical to flush and deter attacking beetles, overall defense responses are very complex and likely due to a combination of constitutive and induced defenses. Additional research is needed to more thoroughly investigate genetic and environmental effects on both constitutive and induced defenses, how these defenses vary with growth rate and, ultimately, to characterize actual resistance from endemic to epidemic bark beetle population levels. Understanding these complex interactions is necessary to improve predictions of bark beetle population dynamics and how climate change may alter forest resistance to bark beetles.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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