

**Differential drivers of chemical defenses in three widespread pine species along a 1500-m elevational gradient: a test of the elevational gradient in plant defense hypothesis**

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## 1 Summary

- 2 1. The elevational gradient in plant defense (EGPD) hypothesis posits that natural enemy  
3 pressures increase positively alongside temperature across elevational climatic gradients,  
4 thereby selecting for enhanced defenses at lower elevations while leaving plants less defended  
5 at higher elevations. Phylogenetically constrained tests of this hypothesis in tree populations  
6 are exceedingly rare. Nevertheless, the presumed presence of poorly-defended trees has been  
7 invoked as an important driver of recent pest outbreaks at higher elevations than historically  
8 common.
- 9 2. Tree age, growth rate, and size have all been correlated with levels of tree defenses. Thus, we  
10 sought to disentangle the interacting influences of these properties from possible elevational  
11 climatic effects on monoterpene composition, concentrations, and diversity of constitutive  
12 resin within three widespread pines (*Pinus contorta*, *Pinus ponderosa*, and *Pinus flexilis*)  
13 across a 1532 m elevational transect in the Rocky Mountains, Colorado, USA.
- 14 3. Collectively, elevation and tree properties were relatively weak predictors of defenses in *P.*  
15 *ponderosa* and *P. flexilis*, but explained ~ 75% of variation in monoterpene concentration and  
16 ~ 50% of diversity in *P. contorta*.
- 17 4. Increasing tree age had the greatest positive influence on monoterpene concentration and  
18 diversity in *P. contorta*, while increasing tree size had a negative influence suggesting a  
19 potential lifelong tradeoff between tree growth and defense. Elevation had a significant,  
20 negative influence on monoterpene concentration but little to no effect on monoterpene  
21 diversity in *P. contorta*.
- 22 5. Overall, we found some support for the EGPD hypothesis within *P. contorta*, but no support  
23 within *P. ponderosa* or *P. flexilis*. Our results suggest the presence of divergent conifer defense  
24 allocation strategies and drivers even among congeners growing in shared environments. An  
25 improved understanding of the controls on tree defenses, particularly possible influences of  
26 climate-based drivers, is necessary for predicting forest pest dynamics under global change  
27 scenarios.

28

29 **Key words:** chemical ecology, climate, conifer, elevational gradient, monoterpenes, *Pinus*, plant  
30 defenses, resin

## 31 Introduction

32 Coniferous trees possess defense and resistance mechanisms that enable some individuals  
33 to avoid or withstand pest attack (Trapp & Croteau 2001; Franceschi et al. 2005; Mumm & Hilker  
34 2006). Tree defense phenotypes, while influenced by contemporary environmental conditions, are  
35 genetically controlled and have been shaped by abiotic and biotic selection pressures occurring at  
36 local and regional scales (Rosner & Hannrup 2004; Holeski, Kearsley & Whitham 2011; Sampedro  
37 et al. 2011; Moreira et al. 2015; Westbrook et al. 2015; Zinkgraf et al. 2015; Harper et al. 2016),  
38 as well as continental scales (Pearse & Hipp 2012; Moreira et al. 2014; Carrillo-Gavilán et al.  
39 2015; Wang et al. 2016). As a result, tree defense phenotypes can vary substantially within and  
40 among populations (Moreira et al. 2014; Ferrenberg, Kane & Langenhan 2015; Vogan & Schoettle  
41 2015; Zinkgraf et al. 2015; Harper et al. 2016). This spatial variation in tree defenses, particularly  
42 as it relates to landscape features, has important implications for pest outbreaks (Holdenrieder et  
43 al. 2004; Ferrenberg 2016).

44 Spatial variation in tree defense phenotypes can result from many dynamic, interacting  
45 mechanisms. At the scale of individual trees, defenses can increase or decrease across stages of  
46 ontogeny and phenology (Boege & Marquis 2005; Barton & Koricheva 2010). For example, in  
47 lodgepole pines (*Pinus contorta* Dougl. ex Loud.), older stem material has been reported to be less  
48 chemically defended than younger materials (Goodman et al. 2013). In contrast, chemical defenses  
49 of white spruce (*Picea glauca* Moench) needles increase with age (Quiring 1992). With respect to  
50 phenology, variations in tree size and growth rate over time have been linked to changes in  
51 defenses and risk of mortality (Ireland et al. 2014; Bigler 2016). For example, larger diameter  
52 limber pines (*Pinus flexilis* James) suffered higher densities of bark beetle attacks (beetle  
53 attacks/m<sup>2</sup> bark surface) than smaller trees (Ferrenberg & Mitton 2014) and larger diameter  
54 lodgepole pines (*Pinus contorta*) were attacked at higher frequencies than smaller trees in some  
55 locations (Björklund & Lindgren 2009). At the same time, slower growth rates correlate with  
56 reduced production of resin ducts (specialized cells where resins are produced and stored) in the

57 stems of numerous pine species, which increases susceptibility to bark beetle attack and mortality  
58 (Kane & Kolb 2010; Gaylord et al. 2013; Ferrenberg, Kane & Mitton 2014; Moreira et al. 2015;  
59 Hood et al. 2015; Bentz et al. 2016). Importantly, Moreira et al. (2016) found that resin defenses  
60 of 17 pine species clustered into two divergent defensive-syndromes with slow-growing species  
61 (e.g., *Pinus contorta* and *Pinus ponderosa* Lawson) investing more heavily in constitutive defenses  
62 while fast growing species have greater inducible defenses regardless of phylogenetic relatedness.  
63 This finding suggests a hierarchy of influences on pine defenses, whereby an individual tree's  
64 defenses are influenced by age, size, and growth rate within the context of species-level adaptive  
65 strategies.

66 Beyond these influences, tree defenses are also hypothesized to have evolved in response  
67 to contemporary and historical climatic gradients (Moles et al. 2011a). For example, climatic  
68 suitability gradients for insects and pathogens are thought to influence the intensity of selection  
69 pressure placed upon plant defenses, shaping defenses as a function of time and biogeography  
70 (Schemske et al. 2009; Salazar & Marquis 2012; Anstett et al. 2016). This longstanding idea is  
71 encapsulated in the longstanding *latitudinal gradient in plant defenses* (LGPD) hypothesis (Moles  
72 et al. 2011a) and the related *elevational gradient in plant defenses* (EGPD) hypothesis (Rasman  
73 et al. 2014). These hypotheses posit that trees from lower latitudes and elevations (warmer regions)  
74 experience more consistent pest pressures than trees at higher latitudes and elevations (colder  
75 regions). Thus, in forest ecosystems, these selection gradients should result in greater defenses as  
76 a function of warmer temperatures (Moles et al. 2011a; Mitton & Ferrenberg 2012; Pearse & Hipp  
77 2012; Pellissier et al. 2012; Moreira et al. 2014; Wang et al. 2016). In general, the LGPD and  
78 EGPD hypotheses have received mixed support across a range of plants. This may be due in part  
79 to experimental designs that fail to control for phylogeny, variation in defense traits across tree  
80 species, and variable feeding strategies of different natural enemies (Anstett et al. 2016). The  
81 recent spread of pests from lower to higher elevation forests has rapidly increased interest in the  
82 EGPD hypothesis. In particular, this model might prove particularly applicable to coniferous tree

83 species that often occur along relatively large elevational climatic gradients (Mitton & Ferrenberg  
84 2012; Rasmann et al. 2014). Unfortunately, whether for conspecifics or congeners, comparisons  
85 of conifer defenses that examine a substantial elevational gradient are exceedingly rare (Hengxiao  
86 et al. 1999; Smith 2000). To our knowledge, there are no previous phylogenetically constrained  
87 studies of defenses across elevation that further parse the influences of elevation, tree age, and  
88 growth rate on defense patterns.

89         The possible interacting influences of tree growth rate, size, age, and climatic gradients  
90 inspired us to examine conifer defenses along a 1532 m elevational transect in the Rocky  
91 Mountains, Colorado, USA. We characterized the defenses of three widespread and ecologically  
92 important pine species of western North America: *Pinus ponderosa* var. *scopulorum* (Engelm),  
93 *Pinus contorta* var. *latifolia* (Engelm), and *Pinus flexilis* (James). Like all conifers, these species  
94 use resin as a primary defense against pests (Franceschi et al. 2005; Mumm & Hilker 2006),  
95 investing significantly in constitutive resin defenses, particularly in stem material targeted by  
96 specialized bark and wood-boring insects that can cause substantial pine mortality rates during  
97 outbreaks and droughts (Ferrenberg et al. 2014). Pine resin consists of a suite of secondary  
98 metabolites, primarily diterpene acids dissolved in sesquiterpenes and monoterpenes. Variation in  
99 tree monoterpene phenotypes strongly influences host selection and reproductive success of insect  
100 pests, as well as tree survival during pest outbreaks (Seybold et al. 2006; Raffa, Powell &  
101 Townsend 2013; Taft, Najir & Erbilgin 2015; Taft et al. 2015). Thus, in this study, we focused on  
102 the monoterpene components of constitutive stem resin. We hypothesized that monoterpene  
103 concentrations and diversity would be positively related to tree growth rates, and would decline  
104 with increasing tree age, size (stem diameter), and elevation. Our *a priori* prediction was that  
105 relatively faster-growing, younger trees at lower elevations would be the most defended within our  
106 sampling region, while slower-growing and older trees at higher elevations would be the least  
107 defended. However, given potential tradeoffs among tree defenses and growth (or other  
108 physiological processes) under optimal resource conditions, we considered an alternate hypothesis

109 that higher growth rates would lead to decreased concentration of defenses as predicted by several  
110 plant defense theories (Stamp et al. 2003).

111

## 112 **Materials and Methods**

113 We tested our hypothesis regarding the influence of tree age, size, and growth on tree  
114 defenses along climatic gradients by sampling monoterpene chemistry of the constitutive resin of  
115 *P. ponderosa*, *P. contorta*, and *P. flexilis* across an elevational transect covering a total range of  
116 1532 meters from the lowest to highest sampling sites. The highest sampling site was located at  
117 3330 m above sea level (asl) (40.0375, -105.5739; -0.3°C mean annual air temperature) and the  
118 lowest sampling site at 1795 m asl (40.1113, -105.3076; 11.1°C mean annual air temperature). The  
119 highest, western-most site (located within the Niwot Ridge LTER, Boulder County, Colorado,  
120 USA) and the lowest, eastern-most site (located on private property near the city of Boulder,  
121 Colorado, USA) were separated by approximately 20 km of planar distance. The majority of  
122 sampling sites for intermediate elevations were located within a 10 km wide transect (North to  
123 South) lying between the high and low points of the East to West transect. Sampling sites were  
124 restricted to low and moderately sloping ridge-tops and benches with a south-southeast aspect in  
125 locations where access was permitted by private landowners, the Niwot Ridge LTER, the  
126 University of Colorado's Mountain Research Station, the U.S Forest Service, and Boulder County  
127 Parks and Open Space. Previous studies along or near this elevation gradient have revealed a  
128 decreasing stand disturbance frequency as elevation increases (Schuster et al. 1995) leading to a  
129 higher proportion of trees >100 years for all three species in higher elevation stands. However,  
130 selective harvesting in the late 19<sup>th</sup> and early 20<sup>th</sup> century has led to a more homogenous age  
131 distribution in *P. contorta* compared to other conifers (Knowles & Grant 1983).

132 The species used here have large, often overlapping geographic ranges, yet have elevation  
133 ranges partitioned by climate. For instance, while all three species can be found growing together  
134 between 2579 m asl and 2870 m asl, *P. ponderosa* dominates at lower elevations while *P. contorta*

135 and *P. flexilis* are more common at higher elevations. We attempted to sample each pine species  
136 across the majority of their elevational distributions, with *P. ponderosa* sampled from 1795 to  
137 2854 m asl, *P. contorta* sampled from 2347 to 3330 m asl, and *P. flexilis* sampled from 2579 to  
138 3330 m asl. Trees of each species were sampled between 2011 and 2014 from five or more  
139 locations along the elevational transect, with a diverse range of tree sizes included from along  
140 randomly oriented transects within each sampling site. The goal was to collect data on defenses  
141 from across a range of tree ages and growth rates while also effectively assessing  
142 elevational/climatic influences. In addition to characterizations of constitutive monoterpene  
143 chemistry, we measured tree diameter at breast height (DBH, 1.4 m above the ground surface) and  
144 collected an increment core from the majority of sampled trees with a 12 mm diameter borer  
145 (several trees of each species were lost after resin sampling but before physical measurements were  
146 taken due to wildfires or harvest). Increment cores were used to estimate tree age at breast height  
147 from annual ring counts and annual radial growth ( $\text{mm}\cdot\text{yr}^{-1}$ ) of the most recent 5 years which were  
148 measured from high resolution scans (9600 dpi) in *Image J*. Radial growth rates were then used to  
149 calculate each tree's basal area increment (BAI) growth for the 5 year xylem ring interval. BAI in  
150 this study was calculated as the percentage of a tree's total cross-sectional area ( $\text{mm}^2$ ) comprised  
151 by of the most recent five years of xylem growth.

152

### 153 *Resin chemistry*

154 Constitutive monoterpene types and concentrations were measured from resin collected in  
155 glass scintillation vials placed into a 65 mm diameter bore hole drilled at an upward 45° angle and  
156 approximately 25 mm deep into each tree's xylem. Vials were tightly inserted to reduce contact  
157 between resin and the atmosphere, left in place for 48 hours, and then capped and stored at -30°C  
158 until analysis. For laboratory analysis, resin samples were allowed to warm to room temperature  
159 before an aliquot of each sample was transferred to a 3 dram glass vial which was promptly capped  
160 and weighed. Each sample was then dissolved into methyl tert-butyl ether (MTBE,  $\text{C}_5\text{H}_{12}\text{O}$ )

161 containing either 5 mM isobutylbenzene (C<sub>10</sub>H<sub>14</sub>) or 5 mM cyclohexylbenzene (C<sub>12</sub>H<sub>16</sub>) as an  
162 internal standard. A Hewlett-Packard HP6890 Series GC/MS equipped with a Restek Rtx-5Sil MS  
163 column was used to quantify monoterpene amounts. After split injection (2  $\mu$ L, 34:1 ratio, 220  
164 °C), the oven temperature increased from an initial value of 40 °C at 10 °C/min until 200 °C was  
165 reached (5 min hold). During this time, helium flow rate was maintained at 1.6 mL/min.  
166 Identification of peaks was accomplished by comparing retention times to those of authentic  
167 standards. Calibration curves for quantification for the following monoterpenes were generated  
168 using authentic (standard)  $\alpha$ -pinene (Fluka),  $\beta$ -pinene (Fluka),  $\alpha$ -phellandrene (TCI),  $\beta$ -  
169 phellandrene (supplied by Dr. Ken Keefover-Ring, University of Wisconsin),  $\Delta^3$ -carene (Aldrich),  
170 limonene (Fluka), myrcene (Acros), sabinene (Indofine), and terpinolene (TCI).

171

#### 172 *Statistical analyses*

173 Relationships among tree age and size (DBH) were examined for each tree species with  
174 linear regression. DBH for each species was log-transformed to meet the assumptions of normality  
175 prior to regression analysis. Monoterpene concentration (mg monoterpenes / mg resin) and  
176 diversity ( $\alpha$ -diversity) calculated as the Shannon diversity index ( $H'$ ) based on the composition  
177 and abundance of monoterpenes in each sample were compared among the three species using  
178 Kruskal-Wallis nonparametric tests followed by Steel-Dwass comparisons (a nonparametric  
179 multiple means comparison with sequential correction). Differences in the mean pair-wise  
180 dissimilarity of monoterpene diversity ( $\beta$ -diversity) calculated as the Bray-Curtis dissimilarity  
181 index of each species were compared to mean differences obtained by calculating all possible  
182 values of the test statistic under rearrangements of the labels on the observed data points, otherwise  
183 known as a permutation resample test. This approach determines whether observed differences in  
184 mean monoterpene dissimilarity among species differs from random differences; we used this  
185 approach to avoid violating assumptions of sample independence associated with other possible  
186 tests. In addition to simple assessments of monoterpene concentration and diversity, we treated



187 monoterpene composition and abundances in each tree's resin sample as 'communities' which  
188 were compared via multi-response permutation procedure (MRPP, a non-parametric method for  
189 comparing the hypothesis of no difference among groups/communities). We also used a stepwise  
190 logistic regression model to determine what proportion of resin samples could be correctly  
191 categorize to their species of derivation via monoterpene measures, with the best-fit logistic  
192 regression model determined via the Bayesian information criterion (BIC).

193 We examined hypothesized relationships of total monoterpene concentration, diversity,  
194 dissimilarity, and individual monoterpene concentrations with elevation, tree age, size, growth rate  
195 (entered into each model as radial growth and BAI of the most recent five years from tree  
196 increment cores) via generalized regression models. Generalized regressions were completed in  
197 JMP Pro 12.0.1 (2015) using the adaptive lasso estimation method, an approach that computes  
198 parameter estimates by penalizing a weighted sum of the absolute values of the regression  
199 coefficients (Zou 2006). Best-fit models were validated using the Corrected Akaike Information  
200 Criterion (AIC<sub>C</sub>). Concentration, diversity, and dissimilarity data had variable distributions across  
201 the three species. Thus, *P. contorta* monoterpene concentration (untransformed) and diversity  
202 (cube-root transformed) were modeled as normal distributions, while monoterpene dissimilarity  
203 was modeled as a gamma distribution. For *P. ponderosa* and *P. flexilis* diversity and dissimilarity  
204 were modeled as a gamma distribution and monoterpene concentrations were modelled as a  
205 Cauchy-Lorentz distribution due to the larger number of outliers observed.

206 We used Structural Equation Modeling (SEM; Grace 2006) to parameterize and evaluate  
207 the fit of hypothetical, causal models linking the monoterpene concentration and monoterpene  
208 diversity of constitutive resin to factors found to be significant in generalized regressions (i.e., tree  
209 age, size, growth rate, and elevation). Similar to generalized regressions, monoterpene measures  
210 were only weakly explained at best by tree properties and elevation for *P. ponderosa* and *P. flexilis*  
211 resulting in poorly supported SEMs for these species, leading us to discard models for these  
212 species. SEMs were performed in R version 3.1.2 (R Core Development Team 2014) with the

213 *lavaan* package (Rosseel 2012). With the exception of monoterpene concentration (normally  
214 distributed without transformation in *P. contorta*) most variables used in the model were not  
215 normally distributed so we used maximum likelihood parameter estimates (MLM) and a mean  
216 adjusted Satorra-Bentler  $\chi^2$  (Satorra & Bentler 1988) to fit and test model fit, respectively. Because  
217 data used in the model were comprised of measures with various units (i.e., monoterpene  
218 concentration per mg of resin, tree age in years, tree size/growth in mm, and meters above sea  
219 level) we calculated standardized coefficients as indicators of the magnitudes of the various paths  
220 in our SEM.

221

## 222 Results

223 We attempted to collect samples of constitutive resin from 106 individual trees of *P.*  
224 *contorta*, 118 individuals of *P. ponderosa*, and 106 individuals of *P. flexilis* across the elevational  
225 gradient. Of these trees, 60 *P. contorta* (57%), 105 *P. ponderosa* (89%), and 106 *P. flexilis* (100%)  
226 yielded a sufficient quantity of resin for further analysis. For trees yielding samples, the mean age  
227 of *P. contorta* was 104 years with a range of 23 to 186, *P. ponderosa* mean age was 135 years with  
228 a range of 21 to 424, and *P. flexilis* mean age was 118 with a range of 29 to 375. Mean tree size,  
229 measured as diameter at breast height (DBH) in *P. contorta* was 20 cm with a range of 11.3 to  
230 36.1, in *P. ponderosa* mean DBH was 20.2 with a range of 10.1 to 43.8, and in *P. flexilis* mean  
231 DBH was 16.5 with a range of 3.7 to 39.2. Tree size was negatively related to tree age for *P.*  
232 *contorta* ( $R^2 = 0.14$ ,  $P = 0.009$ ), but positively related to tree age in *P. ponderosa* ( $R^2 = 0.14$ ,  $P =$   
233  $0.0004$ ) and *P. flexilis* ( $R^2 = 0.22$ ,  $P < 0.0001$ ) (Fig. S1).

234 Regardless of tree properties (age, growth rate, or size) or sample elevation, monoterpene  
235 concentration within constitutive resin (mg monoterpene / mg resin) was significantly greater in  
236 *P. contorta* and *P. ponderosa* than in *P. flexilis* ( $H = 58.8$ , d.f. = 2,  $P < 0.0001$ ; Fig. 1a). Total  
237 monoterpene ‘communities’ of constitutive resin significantly differed among all three species  
238 (MRPP  $T = -142.5$ ,  $A = 0.38$ ,  $P < 0.0001$ ; Fig. 2). Logistic regression with a subset of six

239 monoterpene concentrations (in order of their log worth for categorizing the pine species:  $\beta$ -  
240 phellandrene, limonene, sabinene,  $\beta$ -pinene,  $\Delta^3$ -carene, and terpinolene) correctly categorized 98.2  
241 % of resin samples by their tree species or origin (Generalized  $R^2 = 0.99$ ,  $X^2 = 557.5$ ,  $P = 0.0001$ ;  
242 Fig. 3) with an area under the receiver operating characteristic (ROC) curve  $\geq 0.99$  for all three  
243 species (a value of 1.0 indicates a perfect grouping test, a value of 0.5 indicates a poor test that  
244 performs no better than random assignment).

245 The diversity of resin monoterpenes (calculated as Shannon's  $H'$ , a measure of within  
246 sample diversity or  $\alpha$ -diversity) significantly differed among all three species (Kruskal-Wallis  $H$   
247 = 101.3, d.f. = 2,  $P < 0.0001$ ; Fig. 1b), with the highest diversity found in *P. flexilis* ( $1.57 \pm 0.03$ ),  
248 followed by *P. ponderosa* ( $1.31 \pm 0.03$ ) and *P. contorta* ( $1.09 \pm 0.04$ ). Mean pair-wise dissimilarity  
249 in monoterpene diversity (a measure of average differences in monoterpene composition among  
250 samples or  $\beta$ -diversity) was also significantly different among the three species ( $P < 0.0001$ ; Fig.  
251 1c), with the lowest level of monoterpene dissimilarity found in *P. ponderosa* ( $0.48 \pm 0.007$ ),  
252 followed by *P. flexilis* ( $0.51 \pm 0.009$ ), and *P. contorta* ( $0.63 \pm 0.010$ ).

253 Generalized regressions using the adaptive lasso estimation method revealed that the  
254 combination of elevation and tree properties (age, size, and growth rates) were strong determinants  
255 of monoterpene concentration ( $R^2 = 0.76$ ) and diversity ( $R^2 = 0.50$ ) in *P. contorta* (Table 1).  
256 However, these factors are very weak influences on monoterpene concentration and diversity in  
257 *P. ponderosa* ( $R^2 = 0.08$  and  $R^2 < 0.01$ , respectively) and moderate to weak influences on  
258 concentration and diversity in *P. flexilis* ( $R^2 = 0.17$  and  $R^2 = 0.11$ , respectively) (Table 1).  
259 Monoterpene dissimilarity among trees was not influenced by tree properties or elevation for *P.*  
260 *contorta* ( $R^2 = 0.01$ ) but was weakly influenced by tree properties (primarily age) in *P. ponderosa*  
261 ( $R^2 = 0.12$ ), and by tree age and elevation in *P. flexilis* ( $R^2 = 0.10$ ).

262 Generalized regressions were also used to test the relationships of concentrations of  
263 individual monoterpenes (as opposed to total monoterpene concentration described above) with  
264 elevation and tree properties. Overall, generalized regressions suggested that variation in the

265 concentrations of 7 of the 10 identified monoterpenes were significantly linked to one or more tree  
266 properties in *P. contorta*, with a significant influence of elevation apparent for only two  
267 monoterpenes ( $P < 0.05$ ; Table S1). Importantly, tree age was the most influential factor in the  
268 variation of three of the four most abundant monoterpenes on average found in *P. contorta* (Table  
269 S1). Full generalized regression models (i.e., age plus all other factors retained by adaptive lasso  
270 estimation) resulted in an  $R^2 = 0.71$  for  $\beta$ -phellendrene (the most abundant monoterpene of *P.*  
271 *contorta*),  $R^2 = 0.14$  for  $\beta$ -pinene (the third most abundant monoterpene in *P. contorta*), and  $R^2 =$   
272  $0.58$  for  $\alpha$ -pinene (the fourth most abundant monoterpene in *P. contorta*); with fits ranging from  
273  $R^2 = 0.69$  to  $0.10$  for the remaining less abundant monoterpenes in *P. contorta* (Fig. 2, Table S1).  
274 For *P. ponderosa*, tree properties and elevation were poor predictors of concentrations for any of  
275 the individual monoterpenes; the model explaining the most variation in any monoterpene yielded  
276 an  $R^2 = 0.08$  (camphene), while models for the four most abundant monoterpenes of *P. ponderosa*  
277 ( $\delta^3$ -carene,  $\beta$ -pinene,  $\alpha$ -pinene, and terpinolene) all had an  $R^2 \leq 0.03$  (Fig. 2, Table S1). Finally,  
278 for *P. flexilis*, tree basal area increment growth, tree age, and elevation were significant factors ( $P$   
279  $< 0.05$ ) which combined to explain a moderate amount of variation ( $R^2 = 0.17$ ) in the second most  
280 abundant monoterpene (limonene); while tree growth rates and elevation were significantly linked  
281 ( $P < 0.05$ ) to models explaining a modest amount of variation in terpinolene ( $R^2 = 0.10$ ) and  
282 myrcene ( $R^2 = 0.21$ ) both of which were moderately abundant in *P. flexilis* (Fig. 2, Table S1)

283         The strong relationship of monoterpene concentration and diversity in *P. contorta* to tree  
284 properties and elevation in generalized regression models led us to propose and test a hypothetical  
285 casual model (i.e., a structural equation model, SEM) of the interactive effects of tree age, size,  
286 growth rate, and elevation on *P. contorta* monoterpene concentration and diversity. Unlike  
287 generalized regressions where collinear predictors can lead to inflated model fit values, SEMs  
288 allow for predictor terms to interact via multiple paths (hence the alternate name ‘path models’).  
289 In our case, both SEMs were considered to be well fit to the observed data with both models having  
290 a Satorra-Bentler  $\chi^2 = 0.043$ , d.f. = 1, and  $P = 0.836$  (a small  $\chi^2$  and a large  $P$ -value indicate an

291 SEM that is well fit to observed data). The SEMs for *P. contorta* explained 75 % ( $R^2 = 0.752$ ) of  
292 the variation in monoterpene concentration (Fig. 4a) and 49% ( $R^2 = 0.493$ ) of the variation in  
293 monoterpene diversity (Fig. 4b). The SEM for monoterpene concentration in *P. contorta* resin  
294 revealed: 1) elevation has the strongest, negative direct-effect on monoterpene concentration, 2)  
295 tree age has a strong, positive direct-effect on total monoterpene concentration, as well as two  
296 paths of indirect influence via tree size and growth rate (basal area increment represented by the  
297 most recent five years of annual growth), and 3) increasing tree growth rates and sizes have  
298 conflicting direct-effects on monoterpene concentrations, with faster growing trees having greater  
299 concentrations and larger trees having lower concentrations (Fig. 4a). The SEM for monoterpene  
300 diversity was similar to that for monoterpene concentrations with regard to the effects of tree age,  
301 but differed in finding no significant links of diversity and elevation. Increasing tree size appeared  
302 to have the strongest, negative direct-effect on monoterpene diversity (Fig. 4b). Taken together,  
303 the SEMs would suggest that smaller, older *P. contorta* trees with larger growth rates have the  
304 greatest levels of constitutive monoterpene defenses. At the same time, trees with these properties  
305 would also be more defended (in terms of total monoterpene concentration) at lower elevations.

306

## 307 Discussion

308 Pests rank among the leading causes of tree mortality and forest disturbance, with their  
309 impacts expected to increase due to global change pressures (Dietze & Matthes 2014; Haynes,  
310 Allstadt & Klimetzek 2014; Kozlov et al. 2014; Anderegg et al. 2015; Jules et al. 2016).  
311 Spatiotemporal patterns in tree defenses are an important feature of the landscape that affects forest  
312 pest dynamics (Ferrenberg 2016). Indeed, range expansion by insects has been linked to greater  
313 susceptibility of host plants within novel versus historical ranges (Desurmont et al. 2011). Also,  
314 studies ranging in focus from herbaceous plants to coniferous trees suggest that a diverse set of  
315 secondary chemicals is important for resisting natural enemies (Keefover-Ring & Linhart 2010;  
316 Iason et al. 2011; Roach et al. 2014; Richards et al. 2015). Previous work has suggested potentially

317 strong influences of tree properties on risk of pest attack and mortality (Iason et al. 2014). Resource  
318 and climatic gradients have also been shown to influence tree defenses in some species (O'Neill  
319 et al. 2002; Moreira et al. 2015; Ferrenberg 2016). At the same time, warming temperatures can  
320 interact with conifer defense chemistry to affect the relative risk of forest insect pest outbreaks  
321 (Kollberg et al. 2015). A greater understanding of the underlying drivers of spatiotemporal patterns  
322 in tree defenses is necessary to improve predictions of forest pest dynamics in relation to global  
323 change pressures (Holdenrieder et al. 2004; Ferrenberg 2016). We employed a study design that  
324 characterized resin defenses of three congeners occurring within shared environments across a  
325 1500 m elevational gradient. The design supported our goal of disentangling potentially interacting  
326 influences of age, growth, size, and climate on defenses of *P. ponderosa*, *P. contorta*, and *P.*  
327 *flexilis*.

328 We found that the combined influences of tree properties and elevation explained a  
329 substantial amount of the variation in monoterpene concentration and diversity within *P. contorta*  
330 (Tables 1 and S1, Fig. 4), but explained a relatively modest to small amount of the variation for  
331 both *P. ponderosa* and *P. flexilis* (Tables 1 and S1). Specifically, for *P. contorta*, tree properties  
332 and elevation collectively explained roughly 75% of the variation in monoterpene concentrations  
333 and 50% of the variation in monoterpene diversity depending on the modeling approach utilized  
334 (i.e., generalized regression vs. SEM) (Fig. 4, Tables 1 & S1). The same set of factors were  
335 relatively weak predictors of variation in these measures for *P. flexilis* and *P. ponderosa* (Tables  
336 1 & S1) leading us to reject SEMs for these species due to poor model fit. Similarly, monoterpene  
337 dissimilarity was poorly explained by elevation or tree properties for all three species (i.e.,  $R^2 \leq$   
338 0.12 for all). These results suggest that resin defense chemistry is determined by divergent  
339 mechanisms among these pine species, with climate and tree properties varying greatly in their  
340 overall influence at both the individual and species level. This result presents challenges for  
341 predicting spatiotemporal patterns in tree secondary chemistry across coniferous species.

342 In general, support for hypothesized climatic gradients in plant defenses, where plants are

343 predicted to have greater defenses in warm regions, is mixed and a majority of studies refute their  
344 existence (Moles et al. 2011a, 2011b). However, phylogenetically constrained experiments—i.e.,  
345 those performed within the same species or genus—lend stronger support for the LGPD, including  
346 support from studies completed on defenses of coniferous trees (O’Neill et al. 2002; Pearse & Hipp  
347 2012; Moreira et al. 2014; Wang et al. 2016; Zadworny et al. 2016). Support for the EGPD  
348 hypothesis is also mixed, with support available from both community-level studies (Pellissier et  
349 al. 2014; Rasmann et al. 2014b; Descombes et al. 2017) and from phylogenetically constrained  
350 studies suggesting a general decline in plant defenses with elevation (Hengxiao et al. 1999; Smith  
351 2000; O’Neill et al. 2002; Anderson et al. 2015). However, phylogenetically constrained studies  
352 of tree defenses completed across substantial elevational gradients in field settings are exceedingly  
353 rare, possibly limited to our present study and those by Hengxiao et al. (1999) and Smith (2000).  
354 Importantly, these previous studies diverge in their findings as Hengxiao et al. (1999) found  
355 support for the EGPD, while Smith (2000) found no change in the average monoterpene  
356 composition and concentrations in *P. ponderosa* (var. *ponderosa* Lawson) sampled across a 1430  
357 m elevation transect of California’s Sierra Nevada. Additionally, phylogenetically constrained  
358 studies of insect damage on conifers, while also rare, suggest strong influences of soil properties  
359 on pest impacts (Kosunen et al. 2016). Conflicting results in cross-gradient studies, combined with  
360 evidence of alternative drivers of defenses, highlights the need for additional research to justify  
361 broad claims—such as those of Raffa et al. (2014) whose study did not include an elevation  
362 gradient—that climatic conditions have led to trees primarily found at high elevation being poorly  
363 defended against pests compared to low elevation trees.

364 A strong influence of tree age on the number of pests attacking trees and in the amount of  
365 damage they cause has been reported in numerous studies (Boege, Barton & Dirzo 2011). A meta-  
366 analysis by Barton & Koricheva (2010) found that mammalian herbivores consumed more tissue  
367 from older trees than younger ones, but there was no clear preference across studies for  
368 phytophagous insects possibly due to variation in plant defense strategies in relation to diverse



369 insect feeding types. We found that tree age was consistently a significant factor in best-fit models  
370 of monoterpene diversity, dissimilarity, and total concentration for all three species (Table 1), as  
371 well as in models for the concentrations of seven individual monoterpenes within *P. contorta* and  
372 three monoterpenes within *P. flexilis* (Table S1). For *P. contorta*, where models explained a  
373 substantial portion of variation in defenses, we found monoterpene concentration and diversity  
374 significantly increased with tree age—i.e., older trees have higher monoterpene concentrations  
375 than younger trees (Table 1, Fig. 4). Previous studies have also found that chemical defenses—  
376 particularly terpenoid compounds in conifer needles—increased with age in various conifer  
377 species (Barton & Koricheva 2010; Boege et al. 2011). However, Goodman et al. (2013) found  
378 that monoterpene concentrations were greater in younger xylem tissues than in older tissues of  
379 individual *P. contorta* trees—i.e., lower stem material was less defended than upper stem material.  
380 In addition to variation in tree ages and local environmental pressures across study sites, a plausible  
381 explanation for these seemingly contradictory results of our study with that of Goodman et al.  
382 (2013) likely stems from their focus on phloem defenses versus our focus on xylem defenses.  
383 Indeed, dramatic variation in levels and types of defenses across plant structures is a common  
384 feature of nearly all natural plant populations (Moore et al. 2014; Hahn & Maron 2016). At the  
385 same time, we found a significant negative effect of *P. contorta* tree size on monoterpene  
386 concentration and diversity, which initially seems to be in conflict with an age-related increase in  
387 defenses since older trees are generally expected to be larger than younger trees within a given  
388 population. Surprisingly, we found that the relationship among tree age and size was negative for  
389 *P. contorta* trees in our study, while the relationship of tree age and size were positive for *P.*  
390 *ponderosa* and *P. flexilis* (Fig. S1). This negative relationship among tree age and size for *P.*  
391 *contorta* is likely the result of late 19<sup>th</sup> and early 20<sup>th</sup> century mass harvests that targeted *P. contorta*  
392 over other species for building material (Knowles & Grant 1983). This negative relationship  
393 among age and growth in *P. contorta* in our study location might further explain the increase in  
394 monoterpene concentration and diversity with age, since slow growing conifers tend to produce



395 higher concentrations of constitutive chemical defenses (Moreira et al. 2016). Also, evidence  
396 indicates that conifers exhibiting slower growth rates in early years of their lifespans tend  
397 ultimately to be smaller in size but live longer (Black, Colbert & Pederson 2008; Rötheli, Heiri &  
398 Bigler 2012; Bigler 2016) and suffer less pest damage later in life than trees with rapid early growth  
399 rates (Ruel & Whitham 2002).

400         Similar to results from previous comparisons of monoterpene defenses in these species  
401 (Smith 2000; West et al. 2016), we found that overall, monoterpene ‘communities’ of these pine  
402 species had little overlap (Figs 2 and 3), with interspecific variation exceeding intraspecific  
403 variation regardless of elevational position or tree properties. The large differences among species  
404 was illustrated by the ability of logistic regression (based on monoterpene compositions and  
405 concentrations) to be more than 98% accurate for grouping resin samples within the proper species.  
406 Additionally, we found that monoterpene concentration and diversity were highest on average in  
407 *P. contorta* (Fig. 1). Specifically, monoterpene concentration was significantly lower in *P. flexilis*  
408 than in the other two species, while *P. contorta* had a significantly greater level of monoterpene  
409 diversity than both *P. flexilis* and *P. ponderosa*. Increasing levels of both of these measures have  
410 been linked to greater resistance to natural enemies (Keefover-Ring & Linhart 2010; Raffa 2014).  
411 Thus, our results suggest that *P. contorta* has greater levels of defense against natural enemies  
412 relative to *P. ponderosa* and especially to *P. flexilis*. Our finding that *P. flexilis* (subgenus *Strobus*)  
413 appears to be less defended against natural enemies than co-occurring congeners of the subgenus  
414 *Pinus* joins a result by Bentz et al. (2016) who found *P. flexilis* to be less defended than co-  
415 occurring Great Basin bristlecone pines (*Pinus longaeva*) and foxtail pines (*Pinus balfouriana*)  
416 despite these three species being members of the same subgenus. However, we found that 43% of  
417 *P. contorta*, compared to 11% of *P. ponderosa* and 0% of *P. flexilis*, failed to produce a sufficient  
418 quantity of resin for monoterpene extraction upon wounding in our experiment. Smith (2000)  
419 reported similarly low levels of resin yield in several populations of *P. contorta* suggesting weak  
420 responses to mechanical wounding in the absence of pathogens. Whether the failure to ‘pitch’ resin

421 upon mechanical wounding indicates susceptibility to natural enemies is not entirely clear based  
422 on conflicting outcomes across tree and pest species. For example, while low resin flow could  
423 indicate poor production, trees can also over-produce defensive resins and chemicals in response  
424 to relatively minor pest damage, thereby inducing their own mortality (Hulcr & Dunn 2011).

425         While interspecific variation in monoterpene concentrations and diversity were far greater,  
426 intraspecific variation in monoterpene chemistry was still substantial over the relatively small  
427 spatial scale of our study. Specifically, monoterpene dissimilarity—measured as the mean pairwise  
428 difference in monoterpene ‘communities’ among each tree of a species—was roughly two-fold  
429 greater in *P. contorta* than in *P. ponderosa* and *P. flexilis* (Fig. 1c). In other words, *P. ponderosa*  
430 and *P. flexilis* display less variation in defense from tree to tree than did *P. contorta*. Previous work  
431 has unveiled similar levels of intraspecific variation in resin chemistry of the species studied here,  
432 but the majority of this work was completed over larger spatial scales than present in our study  
433 (Forrest 1980; Smith 2000; Pollack & Dancik 1985; Lindström et al. 1989; Zavarin, Cool &  
434 Snajberk 1993; Pureswaran, Gries & Borden 2004; Clark et al. 2010). However, substantial  
435 variation in secondary resin chemistry in *P. ponderosa* was previously shown to occur in trees near  
436 the lower end of the same elevational gradient used in the current study (Latta et al. 2003).

437         Given that congeners occurring in the same environment must deal with similar abiotic and  
438 biotic pressures, large interspecific variation in resin chemistry suggests continued influences of  
439 deep evolutionary pressures alongside contemporary pressures on tree secondary chemistry.  
440 Elucidating the relationship between compositions and concentrations of constitutive and induced  
441 secondary chemicals is challenged by conflicting selection pressures at large and fine scales  
442 (Talluto & Benkman 2014). Local adaptations and low gene-flow have been previously  
443 demonstrated for both *P. ponderosa* and *P. flexilis* populations along the same elevational transect  
444 used in our study (Schuster & Mitton 2000). Such local adaptation is likely driven by numerous  
445 factors. Evidence indicates that pressure by different natural enemies or abiotic factors can select  
446 for diverse monoterpene profiles (Iason et al. 2011) but also lead to divergent plant defense

447 strategies (Carmona & Fornoni 2012; Moreira et al. 2016). Meta-analytical results also indicate  
448 that secondary chemicals can have both detrimental and beneficial effects on insect pests of woody  
449 plants leading to conflicting adaptive influences over spatiotemporal scales (Smilanich, Fincher &  
450 Dyer 2016). Additionally, profiles of monoterpenes and other chemicals in various coniferous tree  
451 species have been associated with a range of interactions with mammalian herbivores and seed  
452 dispersers, insect pollinators, and resistance to fungal pathogens (Snyder 1993; Langeheim 1994;  
453 Latta & Mitton 1997; Litvak & Monson 1998; Witzell & Martín 2008). Similarly, the presence of  
454 ‘poorly defended’ pine species (e.g., *P. flexilis*) at higher elevations could be linked to  
455 physiological responses in response to low atmospheric pressure, high UV irradiance, and other  
456 factors unrelated to pest-selection pressures (Bentz et al. 2016). Finally, a focus on temperature in  
457 the absence of consideration for precipitation regime is potentially at the heart of incongruence  
458 results among studies testing the LGPD and EGPD hypotheses in coniferous trees. Work by  
459 O’Neill et al. (2002) found both constitutive and inducible defenses of spruce trees (*Picea spp.*)  
460 decreased with elevation and latitude in western Canada, but increased with aridity. Understanding  
461 the drivers of interspecific and intraspecific variation in defense chemistry of the species studied  
462 here and others will require additional experimental and observational efforts to disentangle  
463 influences of multiple, interacting pressures on conifer defense expression.

464

#### 465 **Authors’ Contributions**

466 SF conceived and designed the study; SF, JL, and JM implemented the study and collected samples  
467 and data; SF, JL, SL and LR implemented laboratory procedures to quantify monoterpene  
468 concentrations; SF analyzed the data and led the writing of the manuscript; JL and JM edited drafts.  
469 All authors gave final approval for publication.

470

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475

#### 476 **Data Accessibility**

477 Data from this study will be made publicly available from FigShare pending acceptance.

478

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**Table 1.** Generalized regression models\* of monoterpene concentration (mg monoterpenes / mg resin), diversity (Shannon H'), and dissimilarity (Bray-Curtis dissimilarity) relationships to tree age, size (DBH), growth rate, and elevation in *Pinus contorta* (lodgepole pine), *Pinus ponderosa* (ponderosa pine), and *Pinus flexilis* (limber pine)

Monoterpene measure	Factor	<i>P. contorta</i>			<i>P. ponderosa</i>			<i>P. flexilis</i>		
		Wald $\chi^2$	P	$R^2$	Wald $\chi^2$	P	$R^2$	Wald $\chi^2$	P	$R^2$
concentration	age	30	<0.001	0.76	removed	0.08	0.5	> 0.1	0.17	
	size	18.8	<0.001		removed		1.0	> 0.1		
	basal growth		removed		removed		2.1	> 0.1		
	radial growth	6.2	0.013		removed		2.4	> 0.1		
	elevation	27.2	< 0.001		4.1	0.044	4.0	0.044		
diversity	age	30.0	<0.001	0.50	removed	0.00	removed	0.11		
	size	15.8	<0.001		removed		removed			
	basal growth		removed		0.08	> 0.1	1.2	> 0.1		
	radial growth		removed		removed		removed			
	elevation		removed		removed		6.7	0.010		
dissimilarity	age	0.01	> 0.1	0.01	5.0	0.026	0.12	2.8	0.095	0.10
	size		removed		2.6	> 0.1		removed		
	basal growth		removed		3.4	0.065		removed		
	radial growth		removed		3.3	0.069		removed		
	elevation		removed		removed		4.7	0.030		

\* $R^2$  are for full generalized regression models including all factors not listed as "removed". Factors retained in each model were determined with the adaptive lasso estimation method and best-fit models were validated by AICc scores.

728

729 **Figure Legends**

730 **Figure 1:** Monoterpene measures from constitutive stem resin of *P. contorta* (PICO), *P.*  
731 *ponderosa* (PIPO), and *P. flexilis* (PIFL): (a) Monoterpene concentration (mg of monoterpenes  
732 per mg of resin), (b) monoterpene diversity ( $H'$ ), and (c) monoterpene dissimilarity (pairwise  
733 Bray-Curtis distance among each tree; i.e., higher dissimilarity values indicates larger differences  
734 in chemistry among trees). Box plots show the median (center line) and the 1<sup>st</sup> and 3<sup>rd</sup> quartiles,  
735 and whiskers represent 1.5 inter-quartile range (IQR). The *P*-value is from a Kruskal-Wallis test;  
736 boxes with different lettering are significantly different ( $P < 0.05$ ) via Steel-Dwass  
737 nonparametric pairwise comparison.

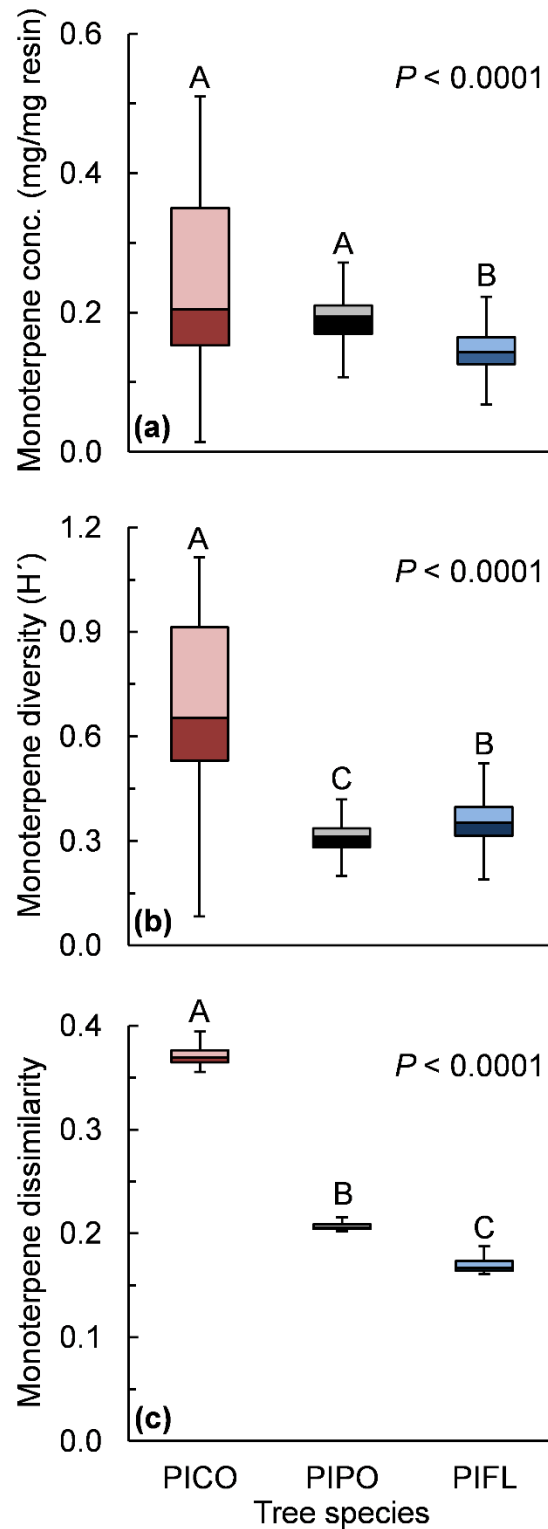
738  
739 **Figure 2:** Mean relative abundance of 10 identified monoterpenes and unidentified (other)  
740 monoterpenes in the constitutive resin of lodgepole pines (*P. contorta*; *P. CONTORTA*),  
741 ponderosa pines (*P. ponderosa*, PIPO), and limber pines (*P. flexilis*, PIFL). Monoterpene relative  
742 abundances significantly differ among all three species ( $\chi^2=203.7$ ,  $P < 0.0001$ ).

743  
744 **Figure 4:** Canonical correlation plot illustrating the differences in monoterpene chemistry  
745 among lodgepole pine (*P. contorta*; red triangles), ponderosa pine (*P. ponderosa*; grey circles),  
746 and limber pine (*P. flexilis*; blue squares) trees. For each species, the larger open concentric  
747 circle shows the 50% contour (the region in ordination space that contains ~50% of  
748 observations), while the smaller open concentric circle indicates the 95% confidence ellipses.  
749 Vectors indicate the strength and influence of specific monoterpenes on point locations relative  
750 to canonical axis 1 and axis 2.

751  
752 **Figure 5:** Structural equation models relating resin monoterpene concentration (a) and diversity  
753 (b) to tree properties and elevation. Values are standardized coefficient estimates, lines widths  
754 are scaled to represent effects sizes with solid lines indicating significant paths ( $P < 0.05$ ) and  
755 dashed lines nonsignificant paths. Measures of tree age, tree growth increment (basal area

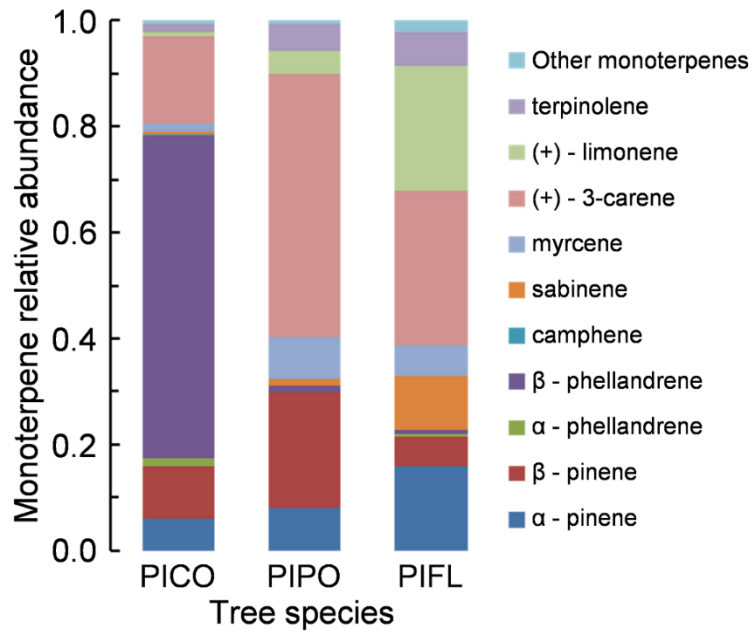
756 increment, BAI, represented by the most recent five years of annual growth), tree size (diameter  
757 at breast height, DBH), and monoterpene content of constitutive resin (mg monoterpenes/mg  
758 resins) were determined from samples (increment cores and liquid resin) collected 1.4 meters  
759 above the ground surface.





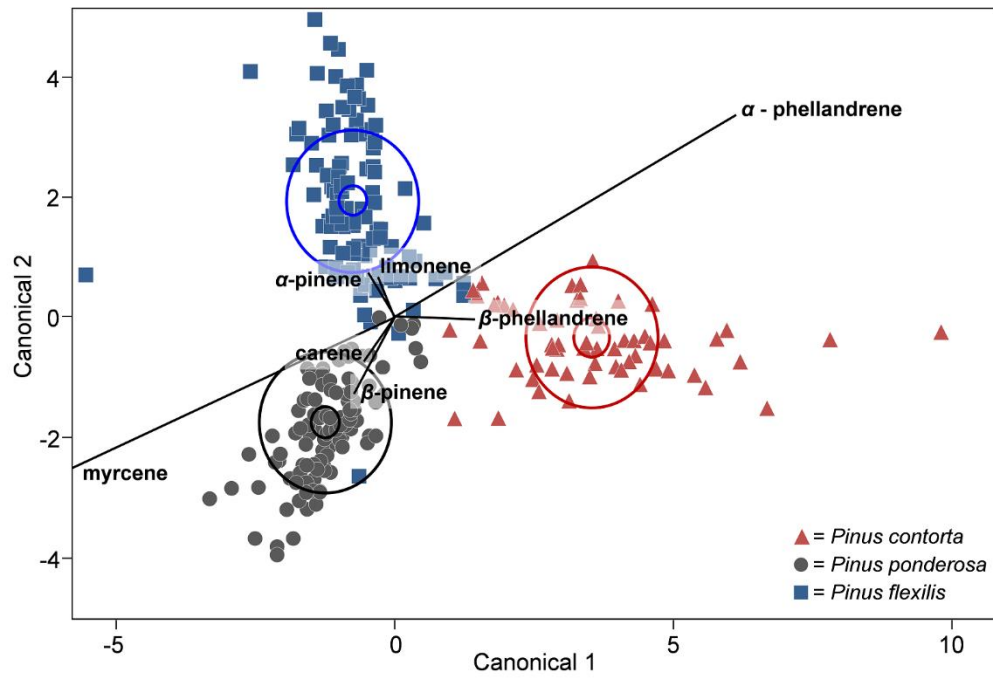
760

761 **Figure 1**



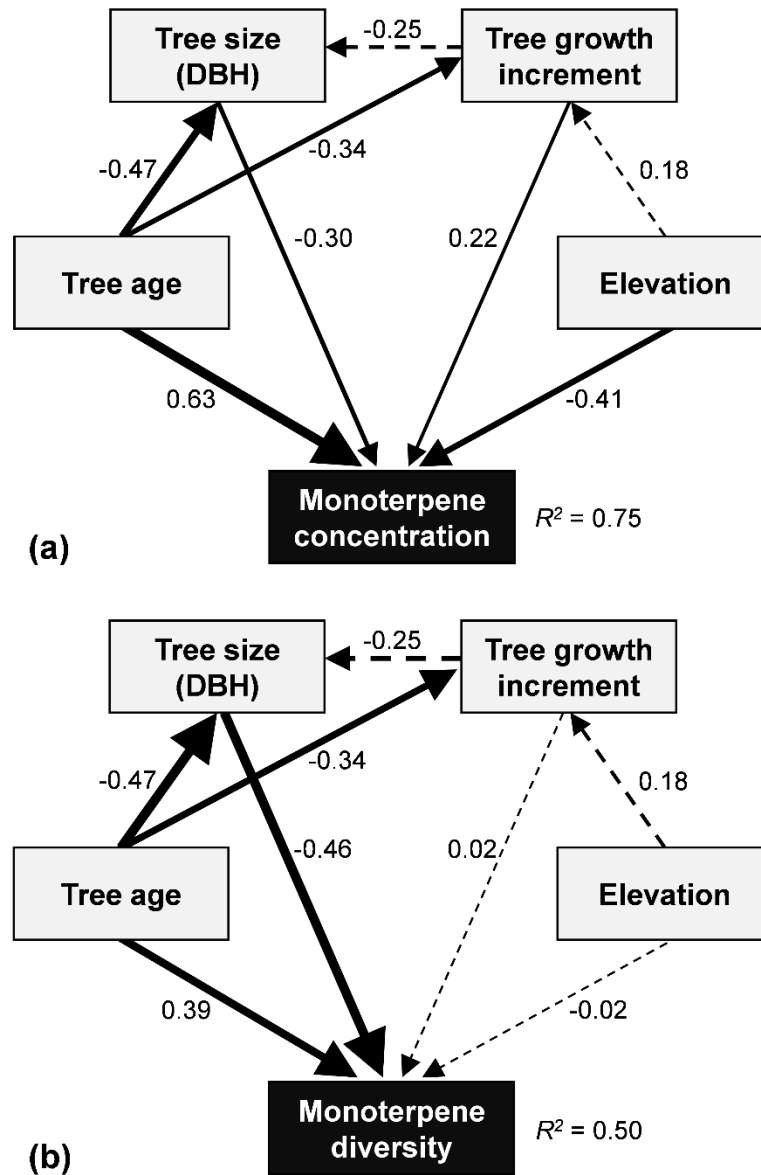
762

763 **Figure 2**



764  
765

**Figure 3**



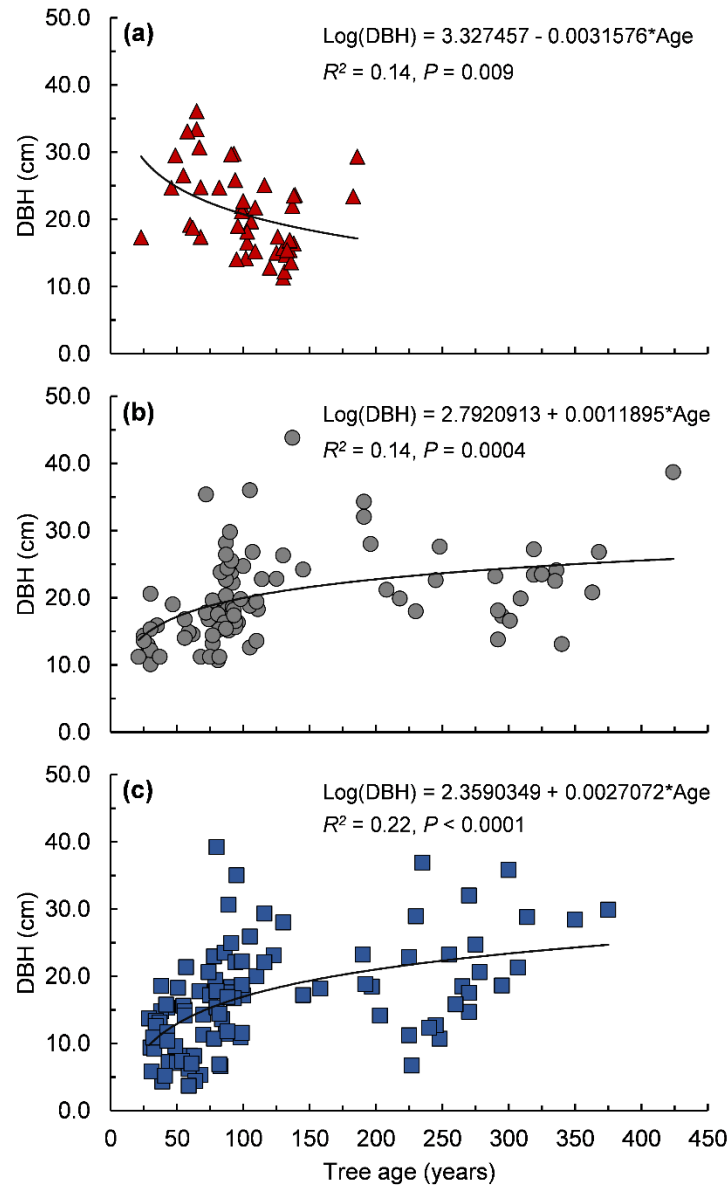
766  
767 **Figure 4**

768 **Supporting Materials**  
769

**Table S1.** Generalized regression models\* of monoterpene relationships to tree age, size (DBH), growth rate, and elevation across *Pinus contorta* (lodgepole pine), *Pinus ponderosa* (ponderosa pine), and *Pinus flexilis* (limber pine)

Monoterpene measure	Factor	<i>P. contorta</i>			<i>P. ponderosa</i>			<i>P. flexilis</i>		
		Wald $\chi^2$	<i>P</i>	<i>R</i> <sup>2</sup>	Wald $\chi^2$	<i>P</i>	<i>R</i> <sup>2</sup>	Wald $\chi^2$	<i>P</i>	<i>R</i> <sup>2</sup>
$\alpha$ -pinene	age	36.5	< 0.0001	0.58		removed	0.03	1.7	0.1960	0.03
	size	10.8	0.0010			removed			removed	
	basal growth		removed		3.4	0.0668		2.2	0.1355	
	radial growth		removed			removed			removed	
	elevation		removed			removed			removed	
$\beta$ -pinene	age	6.0	0.0147	0.14		removed	0.03		removed	0.07
	size		removed			removed			removed	
	basal growth		removed		2.4	0.1245		5.6	0.0178	
	radial growth		removed			removed			removed	
	elevation		removed			removed		1.3	0.2630	
$\alpha$ -phellendrene	age	40.0	<0.0001	0.69		removed	0.00		removed	0.00
	size	11.6	<0.0001			removed			removed	
	basal growth		removed			removed			removed	
	radial growth	17.9	<0.0001			removed			removed	
	elevation	22.8	<0.0001			removed			removed	
$\beta$ -phellendrene	age	27.5	<0.0001	0.71		removed	0.07	5.1	0.0235	0.04
	size	11.3	0.0008			removed			removed	
	basal growth	4.5	0.0342			removed			removed	
	radial growth	5.2	0.0224			removed		1.7	0.1930	
	elevation	16.5	<0.0001		4.1	0.0439			removed	
camphene	age		removed	0.17		removed	0.08		removed	0.04
	size	12.8	0.0003		2.4	0.1239			removed	
	basal growth	5.0	0.0260			removed		0.3	0.6038	
	radial growth		removed			removed		0.6	0.4330	
	elevation		removed		3.3	0.0677			removed	
$\delta^3$ -carene	age	3.6	0.0587	0.23		removed	0.00		removed	0.03
	size	2.5	0.1125			removed			removed	
	basal growth		removed		0.11	0.7446			removed	
	radial growth		removed			removed			removed	
	elevation	3.8	0.0512			removed		1.7	0.1915	
limonene	age	3.8	0.0522	0.22		removed	0.04	9.5	0.0021	0.17
	size		removed		1.9	0.1721			removed	
	basal growth	3.7	0.0547		2.7	0.1020		10.18	0.0014	
	radial growth	3.3	0.0682			removed			removed	
	elevation	2.1	0.1432			removed		5.4	0.0201	
myrcene	age		removed	0.10	5.1	0.0234	0.02		removed	0.21
	size	9.6	0.0020			removed			removed	
	basal growth	3.3	0.0676			removed		13.0	0.0003	
	radial growth		removed			removed		9.2	0.0024	
	elevation		removed			removed		19.9	<0.0001	
sabinene	age		removed	0.17		removed	0.06		removed	0.04
	size	9.1	0.0025			removed			removed	
	basal growth		removed			removed		3.4	0.0672	
	radial growth	1.4	0.2353		1.6	0.2041			removed	
	elevation		removed			removed			removed	
terpinolene	age	1.8	0.1749	0.12		removed	0.00		removed	0.1
	size	0.5	0.4610			removed			removed	
	basal growth		removed			removed		8.2	0.0041	
	radial growth		removed		0.09	0.7694		6.4	0.0117	
	elevation	3.4	0.0662			removed		4.6	0.0327	

770 \**R*<sup>2</sup> are for full generalized regression models with all factors not listed as "removed". Best-fit models were validated by AICc scores.



771

772 **Figure S1**

773 Relationship of tree size (diameter at breast height, DBH) to tree age (# annual xylem rings at  
774 DBH) in *Pinus contorta* (a), *Pinus ponderosa* (b), and *Pinus flexilis* (c). DBH was log-  
775 transformed for all three species to meet linear model assumptions of normality.

```
776 R script for SEM
777
778 ## Structural Equation Model(SEM) code for pine resin monoterpene concentration and
779 diversity in relation to tree age, size, growth rate, and elevation.
780 ## scott.ferrenberg@colorado.edu
781
782 #####
783 # Set your working directory (in this example a folder named SEM on the C drive)
784 setwd("C:/SEM")
785
786 #Load library
787 library(lavaan)
788
789 #Load data
790 ResChem <- read.csv("ResinChemistryPICO.csv")
791
792 #Examine contents of data file
793 names(ResChem)
794 head(ResChem)
795
796 ##MODEL 1
797 ##Monoterpene Concentration
798
799 mod1<-'Mt_conc ~ Age + DBH + BAI5 + Elev
800     DBH ~ Age + BAI5
801     BAI5 ~ Age + Elev'
802
803 #Note that this model was fit with Fml, but, due to multivariate non-normality
804 #we scaled the test of fit and the standard errors
805 #monoterpene concentrations were normally distributed
806
807 mod1Fit <- sem(mod1, data=ResChem, estimator="MLM")
808 summary(mod1Fit)
809
810 varTable(ResChem)
811
812 #standardized coefficients are in Fig. 5 of the model
813
814 standardizedSolution(mod1Fit)
815 modindices(mod1Fit)
816
817 #variation explained
818 inspect(mod1Fit, "r2")
819
820 #model fit BIC
821 fitMeasures(mod1Fit, "bic")
822
823 #####
824
825 ##Model 2
826 ##Monoterpene Diversity
```

```
827
828 mod2<-'Mt_div ~ Age + DBH + BAI5 + Elev
829     DBH ~ Age + BAI5
830     BAI5 ~ Age + Elev'
831
832 #Note that this model was fit with Fml, but, due to multivariate non-normality
833 #we scaled the test of fit and the standard errors
834 #monoterpene diversity was cube-root transformed to better meet the assumption of normality
835
836 mod2Fit <- sem(mod2, data=ResChem, estimator="MLM")
837 summary(mod2Fit)
838
839 varTable(ResChem)
840
841 #standardized coefficients are in Fig. 5 of the model
842
843 standardizedSolution(mod2Fit)
844 modindices(mod2Fit)
845
846 #variation explained
847 inspect(mod2Fit, "r2")
848
849 #model fit BIC
850 fitMeasures(mod2Fit, "bic")
```