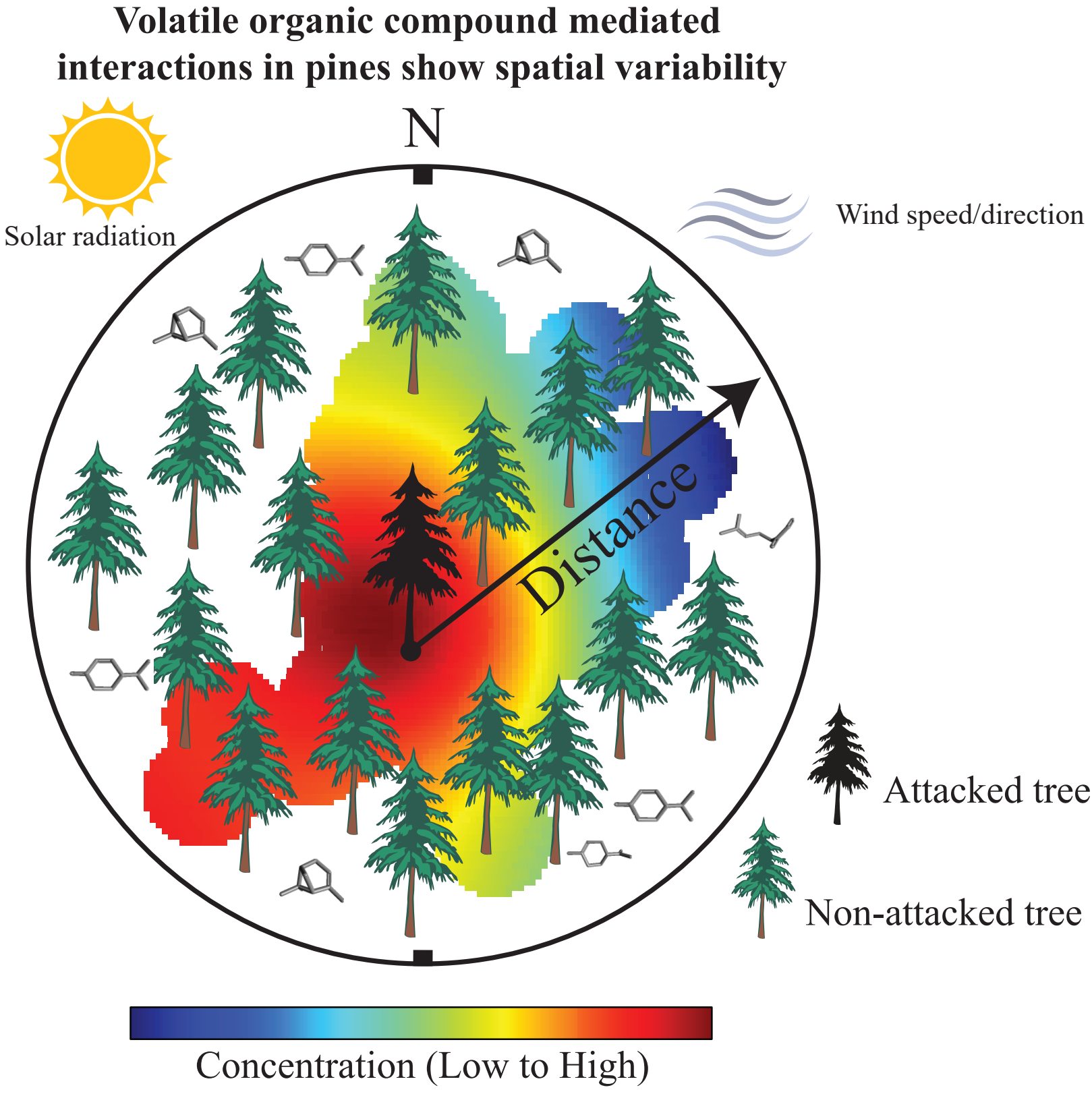
Volatile organic compound mediated interactions in pines show spatial variability
N
Solar radiation
Wind speed/direction
Distance
Attacked tree
Non-attacked tree
Concentration (Low to High)

- Chemotypic expression in pines shows further plasticity with biotic pressure.
- Interactions in pines involving volatile organic compounds depend on distance between interacting pines and site aspects.
- Pines recognize and support kin by using volatile organic compounds.

Spatial characteristics of volatile communication in lodgepole pine trees: evidence of kin recognition and intra-species support

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Abstract

Plant interactions using volatile organic compounds, particularly in the context of kin recognition have received considerable attention in recent years, but several discrepancies and conflicting results have restricted our understanding. We propose that some of these discrepancies in literature are in part due to integral spatial characteristics of sites, and plant attributes. Chemotypic plasticity is commonly used to characterize kin, particularly in conifers. We studied constitutive and induced monoterpene chemotypes of non-attacked lodgepole pine trees within 30 m radii of pine trees attacked by mountain pine beetle. We tested the effects of volatile compounds emitted from the attacked trees on the non-attacked trees by challenge inoculations with a mountain pine beetle associated fungus. We found no relationship between constitutive monoterpene concentrations of the non-attacked trees and distance or direction from the attacked trees or site aspects. In contrast, the effects of volatile compounds were evident after inoculations, depending on distance from the attacked trees and site aspects. However, these interactions only emerged among chemotypically related trees. These results suggest that plants discriminate between chemical cues from kin and strangers, and the emitters likely aid only chemotypically related plants by emitting specific blends of volatiles that can only be deciphered by the receiving kin. These results further demonstrate the importance of incorporating spatial characteristics of sites and plant attributes in studies aimed at investigating intra-species interactions using volatile organic compounds.

Keywords: *Dendroctonus ponderosae*; *Grosmannia clavigera*; kin facilitation; phenotypic plasticity; plant communication; talking trees.

Introduction

Plants mediate aboveground intra- and inter-specific interactions, and respond to environmental stimuli by releasing volatile organic compounds (VOC) (Kessler and Baldwin 2002; Crepy and Casal 2015; Kollist et al. 2018). Most studies have focused on VOC-mediated mutualistic ecological interactions, such as pollination and dispersal (Heil and Karban 2009; Troncoso et al. 2010; Lemaitre et al. 2012), however, little attention has been paid to the role of VOC on antagonistic interactions with herbivores and pathogens (D'Alessandro and Turlings 2006; Biere and Bennett 2013). The VOC-mediated interactions require an 'emitter', a 'receiver' and a 'field' where the exchange of information occurs (Baldwin and Schultz 1983; Kollist et al. 2018). Little is known about the role of heterogeneous field conditions in VOC-mediated interactions despite they affect the dispersal and concentrations of VOC plumes (Thistle et al. 2011; Lowman and Schowalter 2012; Zitouna-Chebbi et al. 2015).

Plant VOC-mediated responses are moderated by gene expression to elicit induced defenses (Kessler and Baldwin 2002), a phenomenon which involves the *de novo* expression of chemical traits at greater concentrations to control tissue damage (Yi et al. 2009; Karban and Maron 2011; Karban et al. 2014a). However, antagonists can spread to the nearby undamaged plant organs, initiating induced responses to be expressed systemically (Heil and Ton 2008; Yi et al. 2009). VOC emitted in response to antagonists may be transmitted either internally or externally by getting airborne as part of the indirect systemic induced defense (Baldwin and Schultz 1983; Dolch and Tscharntke 2000; Heil and Karban 2009; Yi et al. 2009; Karban and Maron 2011; Karban et al. 2014b). Since VOC move spontaneously in the air, they may also influence neighboring non-attacked conspecifics mainly through stomatal uptake (Oikawa and Lerdau 2013). Thus, VOC-mediated communication involves volatile signaling by a plant that causes a response in the same or a different individual that receives the cue (Karban et al. 2014b). Studies focusing on VOC-

46 mediated intra-species plant interactions have mainly compared such communications among
47 strangers and kin (e.g., Karban et al. 2013) or explored the overall existence of kin support among
48 genetically identical plants (e.g., Karban and Shiojiri 2009).

49 Presently, we lack evidence of VOC-mediated plant-plant interactions in forest trees. A major
50 barrier to assessing such interactions in trees arises from the complex forest conditions (e.g.,
51 density, slope, aspect) as well as tree attributes (e.g., age, size) that prevent us from detecting
52 differences in tree responses. In addition, because airborne VOC are carried by the wind, their
53 dispersal, and thus, their concentrations depend on the distance between the interacting plants, as
54 well as wind direction and speed (Barbosa et al. 2009; Song et al. 2010). The exact concentrations
55 (Baldwin et al. 2006; Kessler et al. 2006) or distance (Dolch and Tscharntke 2000; Karban 2001;
56 Song et al. 2010) at which attacked plants ultimately regulate a non-attacked plant's defensive
57 response remain, largely unknown. However, to verify the ecological relevance of such interactions
58 among trees, it is imperative to validate them in their natural growing conditions (Baldwin et al.
59 2006).

60 Since closely related plant species are more likely to host common antagonists, further
61 research has exposed the complex, yet cooperative nature of chemical interactions among plants
62 (Baldwin and Schultz 1983; Dudley and File 2007; Barbosa et al. 2009; Heil and Karban 2009;
63 Karban and Shiojiri 2009; Crepy and Casal 2015). Nevertheless, these interactions depend on the
64 physiologically active VOC concentrations. However, in a chemotypically diverse community,
65 neighboring plants may respond differentially even if exposed to VOC cues of equal concentrations
66 (Bruin and Dicke 2001; Heil and Karban 2009). Therefore, while assessing population-wide
67 variations in VOC-mediated plant responses, signature patterns may emerge when chemotypic
68 plasticity exhibited by conspecific plants is brought into context, which from an evolutionary

perspective functions to counter adaptations by herbivores and pathogens (Heil and Karban 2009; Karban et al. 2014a; Taft et al. 2015).

The VOC emissions of some plants have been reported to cluster into chemotypes, defined as chemically distinct but morphologically similar individuals of a species within a population (Keefover-Ring et al. 2009; Pieruschka and Schurr 2019). The complex ecological relationship between host chemistry and antagonists suggests the relevance of understanding the phytochemical aspect of multiple chemotypes to interpret VOC-mediated plant communication (Karban and Shiojiri 2009; Keefover-Ring et al. 2009; Karban et al. 2014a; Taft et al. 2015; Pieruschka and Schurr 2019). If kin facilitation occurs, individual plants may respectively increase their survival through improving their defense responses prior to the arrival of the expected antagonists (Axelrod and Hamilton 1981; Waldman 1988; Dudley and File 2007; Karban et al. 2013; Crepy and Casal 2015). However, to our knowledge, no studies have yet tested VOC-mediated communication, kin recognition or support in pines against bark beetles.

The recent unprecedented range expansion by mountain pine beetle (MPB) (*Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae) in western North America (Erbilgin 2019) motivated us to study the roles of VOC in influencing the induced defenses of mature lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) trees. Across its natural range, lodgepole pine monoterpenes are known to persist in different chemotypes at constitutive level, including β-phellandrene, β-pinene and five rare chemotypes (Forrest 1981). Spatial variations in monoterpene concentrations in response to antagonists (i.e., induced defenses) in lodgepole pine are also known to exist (Clark et al. 2014). However, how these chemotypic variations at the constitutive and induced levels and spatial characteristics of attacked and non-attacked trees affect VOC-mediated communication in lodgepole pine, is unknown.

92 Beetles locate and land on suitable hosts, followed by boring through the outer tree bark, and
93 inoculation of the phloem and xylem with symbiotic fungi, including *Grosmannia clavigera* (Six
94 2003). The trees confront the MPB attack with their constitutive defenses in the phloem (Erbilgin
95 2019). However, as the MPB colonization intensifies, the trees respond by producing induced
96 defense compounds, followed by the formation of resin-filled necrotic lesions which comprises of
97 local autolysis of parenchyma cells, and a further increase in the secretion of defense compounds,
98 intended to render the phloem no longer suitable for larval or fungal development (Keeling and
99 Bohlmann 2006). Therefore, comparing monoterpene concentrations is pertinent as they are the
100 most abundant and vastly volatile organic defense compounds in the oleoresins of conifers (Trapp
101 and Croteau 2001; Keeling and Bohlmann 2006) and biologically the most important groups against
102 MPB (Erbilgin 2019). Similarly, lesion length in the attacked trees is considered a good predictor of
103 resistance to a pathogen, and smaller lesions are reported to indicate more efficient defenses
104 (Goodsman et al. 2013; Erbilgin, 2019).

105 We conducted a field survey of trees to retrospectively deduce whether VOC emitted from
106 central trees attacked and killed by MPB have affected the neighboring non-attacked trees across
107 heterogeneous forest conditions by comparing their constitutive and induced chemistry in relation to
108 the distance and direction to the central trees in the same stands. We pursued these research
109 questions. (1) How stable are lodgepole pine chemotypes at constitutive level? (2) Is herbivory
110 informing communication general in lodgepole pine, or only effective in individuals of related
111 chemotypes? (3) Can site aspects and distance between the attacked and non-attacked trees
112 influence such communication? We hypothesized that lodgepole pine trees will exhibit further
113 chemotypic plasticity when challenged and that VOC-mediated interactions will be more
114 pronounced in chemotypically related trees; however, the overall response will depend on the
115 integral spatial characteristics of sites, and tree attributes. As a proxy to MPB and to simulate

induced tree chemical defenses, we inoculated the non-attacked trees with live *G. clavigera*, and as evidence of direct communication in lodgepole pines, we compared lesion lengths formed as a result of subsequent fungal infections (Goodsman et al. 2013).

Material and Methods

Experimental design and sampling

On 13-14 June 2016, we selected non-attacked mature lodgepole pine trees (*N*=201) on five sites within 30 m radii of individual trees (*N*=39) that were attacked by MPB in 2015 in Jasper National Park, Alberta (Table S1). On each site, we established sub-sites by measuring the distances and directions of all non-attacked trees (focal trees hereafter) from their corresponding nearest attacked tree (central tree hereafter). We conducted this field experiment just before MPB emergence from the central trees, allowing the neighboring focal trees to be exposed to the VOC from the central trees for at least a year. We identified MPB attacked trees by the presence of pitch tubes and verified successful beetle attacks on the selected trees (Erbilgin et al. 2017). The non-attacked trees were free of any biotic stress based on external aboveground visual signs and symptoms.

The sites either had an east or west aspect, with slopes roughly 20-25% and elevations ranging from 1,209 m to 1,461 m (Table S1). The mean diameter at 1.3 m of the central and focal trees were 27.12 cm (±0.71 SE) and 24.34 cm (±0.32 SE), respectively. Because VOC-mediated plant interactions likely occur over relatively shorter distances (Dolch and Tscharntke 2000; Karban et al. 2006), we categorized our focal trees in three concentric circles (0-10 m, 10-20 m, and 20-30 m) to detect any spatially distinguishable tree responses. Similarly, VOC plume dispersal is a random process that shows an outward expansion due to factors like wind direction and speed (Song et al. 2010; Thistle et al. 2011), we also categorized the focal trees in four intercardinal directional groups

138 (NE, SE, SW, and NW). This also enabled us to account for the non-uniform and sparse distribution
139 of the focal trees at finer spatial or directional scales.

140 We collected four 1 cm diameter phloem tissue samples (two from north and two from south
141 aspects) from the focal trees at 1.3 m stem heights. We inoculated the focal trees with a single
142 isolate of *G. clavigera* (EL033) on the north aspect by placing 0.9 cm diameter circular fungus-agar
143 plugs in the two bore holes created during tissue collection with the mycelia facing the sapwood
144 (Goodsman et al. 2013). The fungus had been originally isolated from blue-stain sapwood between
145 MPB larval galleries in mature lodgepole pine trees.

146 Six weeks later (25-26 July 2016), we exposed the lesions induced by the subsequent fungal
147 infection by removing the outer tree bark, measured their lengths, and collected one (1 cm x 2 cm)
148 sample from each of the two lesions to study the local induced chemical defenses. At the same time,
149 we also collected two samples (1 cm diameter) from non-necrotic phloem tissues adjacent to the
150 edges of fungal inoculation bore marks (about 4 cm away from the lesions). We pooled samples
151 together from each tree by sampling round (constitutive, induced) and tissue type (i.e., phloem or
152 lesion), wrapped them in aluminum foils, and flash froze them in liquid nitrogen before storing
153 them at –40°C in the laboratory.

154 ***Monoterpene analysis***

155 We ground the combined lesion, and phloem samples from each tree in liquid nitrogen with a
156 cryogrinder (SPEX Sample Prep Freezer Mill 6770, Metuchen, NJ, USA), and then stored at −40°C.
157 We extracted monoterpenes from 100 mg (±2) of ground tissue twice with 0.5 ml dichloromethane
158 (Sigma-Aldrich, St Louis, MO, USA) with 0.004% tridecane (Sigma-Aldrich) as surrogate standard
159 at room temperature, as described in (Erbilgin et al. 2014). Briefly, we vortexed samples for 30 s at
160 3,000 rpm, sonicated for 10 min, and centrifuged for 15 min at 0°C and 13,000 rpm, and kept at

161 −40°C for at least 2 h. We transferred the extracts to 2 ml gas chromatograph (GC) vials and stored
162 at −40°C until analysis.

163 For the analysis, we injected 1 µl of extracts with a 10:1 split ratio into a GC fitted with an
164 enantioselective column (HP Chiral 20β; ID 0.25mm, length 30m; Agilent Tech. Santa Clara, CA,
165 USA) and coupled to a Mass Spectrometer (GC-MS; GC: 7890A, MS: 5975C, Agilent Tech.). We
166 used helium as the carrier gas at a flow rate of 1.1 ml min^{-1}, and the temperature program included
167 four ramps, starting at 50°C (held for 5 min), then 75°C min^{-1} to 75°C (held for 3 min), then 1.5°C
168 min^{-1} to 100°C (held for 30 s), then 60°C min^{-1} to 200°C (held for 0 min), and then 25°C min^{-1} to
169 250°C (held for 0 min). We identified the peaks by using the following standards: (−)-α-pinene, (+)-
170 α-pinene, (−)-β-pinene, (−)-camphene, (+)-camphene, myrcene, (S)-(−)-limonene, (R)-(+)-
171 limonene, 3-carene, terpineol (chemical purity >90%), γ-terpinene (>97%), (+)-cymene, sabinene,
172 β-thujone (enantiomeric ratio of 92.5/7.5), pulegone (>97%), terpinolene (>90%), borneol, α-
173 terpinene (>95%) (Sigma-Aldrich), *cis*-ocimene (>90%, SAFC Supply Solutions, St. Louis, MO,
174 USA), and β-phellandrene (>90%, Erbilgin laboratory). We identified compounds by comparing
175 their retention times and mass spectra with those of the standards and quantified their
176 concentrations through calibration curves generated from analyses of a serial of four dilutions of
177 known quantities of standards and calculated as µg of compound per mg of wet weight (WW) of
178 tissue.

179 ***Data analysis***

180 We used R v3.4.4 (R Core Team 2017) for all statistical analyses. We first calculated descriptive
181 statistics, and then checked data for the assumptions of homoscedasticity and normality by using
182 Levene's and Shapiro–Wilk tests, respectively, and where necessary, we transformed data prior to
183 analyses. We performed separate tests for defense compounds at constitutive, induced-phloem, and

induced-lesion levels, and lesion lengths. Our statistical models included Permutational Multivariate Analyses of Variance (PERMANOVA, permutations = 9,999, method = Gower) for multivariate analyses (Oksanen et al. 2017), followed by univariate analyses using either ANOVAs or *t*-tests, and mixed models for lesion lengths.

For the identification of different chemotypes based on the constitutive, induced-phloem, and induced-lesion monoterpene concentrations, we used the *pamk* function of R package *fpc* to determine the optimal number of clusters (Hennig 2018), followed by proportion tests to compare percent representation of each chemotype in each cluster. We also compared the means of monoterpene concentrations between the test groups (chemotypes) using two-sample *t*-tests or one-way ANOVA to confirm differences between chemotypes.

Because of the circular nature of our sampling scheme, we constructed bivariate polar plots in the R package *openair* to visualize statistically different results (Carslaw and Ropkins 2012). We performed PERMANOVAs with the *adonis* function in the R package *vegan* (Oksanen et al. 2017) and used linear mixed models with the *lmer* function in the R package *lme4* (Bates et al. 2015). We constructed separate mixed models for each chemotype identified at constitutive level, and used lesion chemotypes, total monoterpene concentrations, site aspects, and distance and direction of the focal trees from the corresponding central tree as our fixed effects, and sites as a random effect in which the constitutive chemotype for that model was nested. We conducted Tukey's HSD tests to examine pair-wise differences for significant main effects or interactions. We used an alpha level of 0.05 for all statistical tests and constructed all graphs by using raw and non-transformed data.

Results

Chemotypes and spatial characteristics of focal trees before fungal infection

206 Constitutive monoterpene concentrations of the focal trees clustered in Low and High β-
207 phellandrene chemotypes that represented 66.66% and 33.33% of the focal trees, respectively
208 [proportion test, $P<0.001$] (Fig. 1). We found no correlations among monoterpene concentrations,
209 site aspects, direction or distance of the focal trees from their central trees, or any variations among
210 sites for any of the two chemotypes (Table S2).

211 ***Chemotypes and spatial characteristics of focal trees after fungal infection***

212 Induced monoterpene concentrations in the phloem tissues of the focal trees in the High β-
213 phellandrene chemotype at constitutive level further clustered in two distinct myrcene chemotypes
214 (Fig. 2a). The Low and High myrcene chemotype represented 80.60% and 19.40% of the focal
215 trees, respectively [proportion test, $P<0.001$]. Similarly, induced monoterpene concentrations in the
216 phloem tissues of the focal trees in the Low β-phellandrene chemotype at constitutive level further
217 clustered in two distinct 3-carene chemotypes (Fig. 2b). The Low and High 3-carene chemotypes
218 represented 60.45% and 39.55% of the focal trees, respectively [proportion test, $P<0.001$].
219 However, we found no statistical correlations among induced monoterpene concentrations, site
220 aspects, direction or distance of the focal trees from their central trees, or any variations among sites
221 for any of the four induced chemotypes (Table S3).

222 ***Lesion monoterpene chemotypes, spatial characteristics, and lesion lengths***

223 Lesion monoterpene concentrations in the lesion samples of the focal trees in the High β-
224 phellandrene chemotype at constitutive level further clustered in High and Low 3-carene
225 chemotypes (Fig. 3a). The Low and High 3-carene chemotypes represented 70.15% and 29.85% of
226 the focal trees, respectively [proportion test, $P<0.001$]. Similarly, lesion monoterpene
227 concentrations in the lesion samples of the focal trees in the Low β-phellandrene chemotype at
228 constitutive level further clustered in (–)-β-pinene, myrcene, and 3-carene chemotypes. The (–)-β-

229 pinene chemotype represented 45.53%, the myrcene chemotype represented 33.58%, and the 3-
230 carene chemotype represented 20.89% of the focal trees respectively (Fig. 3b).

231 We found no significant correlations between lesion monoterpene concentrations, direction or
232 distance of focal trees from their central trees, or any variations among sites for the Low and High
233 3-carene chemotypes in the High β-phellandrene chemotype, or 3-carene chemotype in the Low β-
234 phellandrene chemotype (Table S4). However, we found significant correlations between lesion
235 monoterpene concentrations and site aspects in the lesion myrcene chemotype, and distance from
236 the central trees and site aspects in the lesion (–)-β-pinene chemotype (Table S4).

237 For the lesion myrcene chemotype, we found significantly higher concentrations of myrcene,
238 (–)-α-pinene, (–)-camphene, (+)-camphene, and total monoterpenes in the lesion samples collected
239 from the focal trees on the west-facing sites (Fig. 4, Table 1). For the lesion (–)-β-pinene
240 chemotype, the concentrations of β-phellandrene, myrcene, (+)-limonene, (–)-α-pinene, (–)-
241 camphene, and total monoterpenes decreased with an increase in the distance from the central trees,
242 whereas the concentration of (–)-β-pinene increased (Fig. 5, Table 2). We did not find any
243 differences in the lesion lengths for any of the lesion chemotypes, or their correlation with total
244 lesion monoterpene concentration, site aspects, direction or distance of the focal trees from their
245 green attack trees, or variations among sites (Table S5). Chemotypic expression and their percent
246 representation in constitutive phloem, induced phloem, and lesion tissue samples have been
247 summarized in (Fig. S1).

248 **Discussion**

249 We identified two distinct monoterpene chemotypes in lodgepole pine at constitutive level,
250 characterized by low or high concentrations of β-phellandrene, in agreement with Forrest (1981).
251 We anticipated such variations because an environmental change within the range of a plant species

252 decreases the likelihood of a single chemotype to persist and show equal resilience under an
253 environment of predictable challenges (Via et al. 1995; Pieruschka and Schurr 2019). The
254 coexistence of chemically heterogeneous forests is also a strong indication of genetic influence on
255 chemotypic expression in lodgepole pine, supporting earlier conclusions that pine monoterpenes are
256 in part genetically controlled (Hanover 1971; Forrest 1981; Clark et al. 2014; Taft et al. 2015).

257 We found an intensification of chemotypic plasticity in our focal trees in response to the
258 fungal infection at induced levels. These results highlight the co-evolutionary roles of biotic
259 pressures across the heterogeneous environments, driving intraspecific differentiation of the
260 defensive metabolome in time and space (Via et al. 1995; Keefover-Ring et al. 2009; Karban et al.
261 2014a; Pieruschka and Schurr 2019). Therefore, as the defense compounds of plants can
262 differentially influence the attacks by diverse antagonists, plants may have evolved to fine-tune
263 their defenses against specific threats by further optimizing their chemotypes (Heil and Karban
264 2009; Hansen et al. 2012; Karban et al. 2014a; Taft et al. 2015; Erbilgin 2019; Zhao et al. 2019).
265 The observed refined differentiation of chemotypic expression with the severity of fungal threat also
266 suggests that pines potentially evolved by facing an array of selective pressures, enabling them to
267 favor one chemotype over the other under specific conditions in their dynamic life-long
268 environments. Such a chemical polymorphism in plants is critical for reciprocal organismal natural
269 selection (Via et al. 1995, 1998; Agrawal 2011; Mithöfer and Boland 2012; Taft et al. 2015; Bamba
270 et al. 2019).

271 Interestingly, we also found significant correlations of monoterpene concentrations with
272 distance from the central trees and site aspects in two chemotypes identified in induced-lesion
273 monoterpenes. In the (–)-β-pinene chemotype, concentrations of total and some individual
274 monoterpenes, such as β-phellandrene, (–)-α-pinene, myrcene, and (+)-limonene decreased, whereas

the concentration of (–)-β-pinene increased with distance from the central trees. These results suggest that VOC-mediated communications in pines can occur, but the mechanisms are likely spatially constrained, and therefore, may be very fine-grained. Our results are consistent with the findings of Dolch and Tscharntke (2000) who found alder (*Alnus glutinosa*) defoliation induced defenses only in the nearby plants, but the response was greatly concentrated within a few meters of the damaged tree.

Although the roles of site aspects or distance from central trees in the VOC-mediated communication are not fully understood, VOC plume dispersal in plants is known to be multidimensional and a complex process which heavily depends on the ambient environment (Baldwin et al. 2006; Thistle et al. 2011; Lowman and Schowalter 2012). Therefore, factors such as wind speed or direction, and the intricate mosaic of solar insolation due to topographic and surface aerodynamic properties may potentially influence VOC plume dispersal (Barbosa et al. 2009; Thistle et al. 2011; Zitouna-Chebbi et al. 2015). In fact, these landscape features can be linked to the diurnal and nocturnal variations observed in VOC concentrations in angiosperms (e.g., De Moraes et al. 2001; Loughrin et al. 2006); the downwind enhanced induced resistance in neighboring plants (Karban 2001) or greater VOC dispersal in tall plants (Lowman and Schowalter 2012). In the current study, we only observed these interactions in chemotypically identical focal lodgepole pine trees, suggesting kin facilitated VOC communication.

As chemotypes are heritable, they are reasonably a reliable way to predict relatedness in plants (Hanover 1971; Axelrod and Hamilton 1981; Karban et al. 2014a). Therefore, the patterns observed in the responses of chemotypically related trees in our study may highlight an important mechanism in pines, i.e., to recognize and support kin by keeping the VOC-mediated communication very discrete within the family (Baldwin and Schultz 1983; Waldman 1988; Dudley

298 and File 2007; Barbosa et al. 2009; Heil and Karban 2009; Karban and Shiojiri 2009; Karban et al.
299 2014a).

300 The ability of kin recognition in order to cooperate is prevalent across all taxa (Lizé et al.
301 2006; Waldman 1988; Karban et al. 2013; Crepy and Casal 2015). Surprisingly, most studies
302 focusing on kin recognition and support in plants have looked at belowground responses in
303 environments of competitive interactions and niche partitioning. For example low competition for
304 resources in *Cakile edentula* when planted with siblings (Dudley and File 2007); interspecific
305 genetic material exchange in plants via mycorrhizal connections (Giovannetti et al. 2004); greater
306 mycorrhiza-mediated carbon sharing in roots of Douglas-fir siblings (Pickles et al. 2017), and
307 conspecific facilitation of younger trees by older trees (Beiler et al. 2010). However, our results
308 show that pines may also have the ability of kin recognition and cooperation by using the
309 aboveground VOC cues.

310 Since we did not sample the central trees prior to MPB colonization, we cannot speculate that
311 the central and focal trees were chemotypically similar or hence within the same kinship, this may
312 limit our interpretation of our results. Nevertheless, consistent results across sites suggest the
313 importance of incorporating spatial characteristics of sites and tree attributes in studies aimed at
314 investigating intra-species interactions using volatile organic compounds.

315 **Conclusions**

316 Whether to term the differences observed in our study 'communication' or 'kin recognition and
317 support' is currently lacking consensus in the literature (Dudley and File 2007; Scott-Phillips 2008;
318 Crepy and Casal 2015). Nevertheless, an interactive neighborhood could reduce potential losses due
319 to antagonists (Baldwin and Schultz 1983; Barbosa et al. 2009; Heil and Karban 2009; Karban and
320 Shiojiri 2009). Although it is not clear which VOC elicit induced responses, it is commonly thought

that the interacting kin have similar VOC profiles, thereby the high chemical incompatibility exhibited by strangers makes it difficult for them to decipher the critical airborne information (Karban et al. 2013). These abilities in interacting individuals have been linked to evolution and speciation potential through natural selection (Platt and Bever 2009; Gardner and West 2010). Additional studies with spatially-explicit models and genetic markers are needed to further substantiate our findings.

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Author contributions

N.E. and A.H. designed the study. A.H. carried out the field and laboratory work, analyzed the data and wrote the manuscript. J.C.R.R helped with the fieldwork.

Conflict of interest

The authors declare no conflict of interest.

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Table 1 Mean concentrations and enantiomeric ratios of defense compounds in lesion samples of myrcene chemotype (*N*=45) characterized in the Low β-phellandrene chemotype (at constitutive level) of *Pinus contorta* trees sampled at five sites in Jasper National Park. Non-attacked trees were categorized in East (*n*=10) or West (*n*=35) facing aspects around their corresponding *Dendroctonus ponderosae* attacked central trees (*N*=22). *P*-values significant at α=0.05.

Compounds	Mean (SE) concentration (μg mg^{-1} FW)			
	East-facing	West-facing	$t_{(df)}$ †	*P-value*
β-phellandrene	37.68 (3.72)	42.77 (1.60)	1.40 $_{(43)}$	0.165
Myrcene	9.09 (0.93) b	12.42 (0.42) a	3.56 $_{(43)}$	<0.001
3-Carene	8.91 (2.77)	14.29 (1.82)	1.31 $_{(43)}$	0.194
(–)-limonene	1.53 (0.13)	2.97 (0.61)	1.26 $_{(43)}$	0.213
(+)-limonene	0.34 (0.04)	0.71 (0.03)	1.77 $_{(43)}$	0.083
(–)-β-pinene	9.67 (1.44)	14.05 (1.74)	0.88 $_{(43)}$	0.383
(–)-α-pinene	2.50 (0.27) b	3.47 (0.22) a	2.57 $_{(43)}$	0.013
(+)-α-pinene	1.44 (0.19)	3.11 (0.53)	1.58 $_{(43)}$	0.121
4-allylanisole	0.56 (0.27)	0.50 (0.06)	0.64 $_{(43)}$	0.524
Terpinolene	0.68 (0.18)	1.04 (0.12)	1.49 $_{(43)}$	0.142
(–)-camphene	0.24 (0.02) b	0.33 (0.01) a	2.89 $_{(43)}$	0.005
(+)-camphene	0.09 (0.02) b	0.16 (0.01) a	2.84 $_{(43)}$	0.006
γ-terpinene	0.10 (0.02)	0.13 (0.01)	1.02 $_{(43)}$	0.310
p-cymene	0.12 (0.01)	0.14 (0.01)	1.81$_{(27.99)}$ †	0.081
Total monoterpenes	72.97 (6.70) b	95.80 (3.14) a	3.32 $_{(43)}$	0.001
(–):(+)-α-pinene ratio	81.02 (11.19)	90.01 (19.80)	0.04 $_{(36.03)}$ †	0.963
(–):(+)-limonene ratio	368.25 (37.01)	638.96 (152.43)	0.92 $_{(28.62)}$ †	0.363

†Welch's *t*-test (when homogeneity of variance was not equal).

Table 2 Mean concentrations and enantiomeric ratios of defense compounds in lesion samples of the (−)-β-pinene chemotype of *Pinus contorta* trees (*N*=61) sampled at five sites in Jasper National Park. Non-attacked focal trees were categorized in 0-10 m, 10-20 m, 20-30 m distances from their corresponding *Dendroctonus ponderosae* attacked central trees (*N*=26). *P*-values significant at α=0.05. df=2.

Compounds	Mean (SE) concentration (μg mg^{-1} FW)			ANOVA	
	0-10 m	10-20 m	20-30 m	*F*	*P-value*
β- phellandrene	79.85 (3.02) a	69.09 (1.63) b	69.03 (2.57) b	6.178	0.003
Myrcene	18.91 (0.55) a	15.97 (0.35) b	17.92 (0.59) ab	10.95	<0.001
3-carene	8.22 (1.19)	7.14 (0.87)	8.77 (2.08)	0.11	0.895
(−)-limonene	4.93 (1.03)	4.42 (0.76)	6.49 (1.98)	0.80	0.453
(+)-limonene	0.80 (0.03) a	0.63 (0.02) b	0.68 (0.04) ab	12.12	<0.001
(−)-β-pinene	19.89 (1.85) ab	14.78 (1.85) b	24.38 (4.52) a	3.42	0.039
(−)-α-pinene	5.59(0.26) a	4.49 (0.20) b	6.03 (0.63) a	7.15	0.001
(+)-α-pinene	3.53 (0.33)	2.55 (0.21)	2.88 (0.44)	2.51	0.089
4-allylanisole	1.07 (0.17)	0.62 (0.08)	0.95 (0.29)	1.65	0.201
Terpinolene	0.79 (0.09)	0.60 (0.05)	0.87 (0.16)	1.49	0.234
(−)-camphene	0.57 (0.02) a	0.47 (0.01) b	0.53 (0.03) ab	11.81	<0.001
(+)-camphene	0.23 (0.01)	0.19 (0.01)	0.23 (0.04)	2.04	0.139
γ-terpinene	0.12 (0.01)	0.09 (0.01)	0.11 (0.02)	1.55	0.221
p-cymene	0.19 (0.01)	0.19 (0.01)	0.16 (0.02)	0.64	0.527
Total monoterpenes	144.75 (4.01) a	121.25 (2.12) b	139.03 (4.26) a	14.73	<0.001
(−):(+)-α-pinene	89.28 (16.56)	97.30 (13.89)	144.90 (39.02)	1.46	0.239

(–):(+)-limonene	537.74 (134.16)	691.29 (179.24)	838.32 (266.78)	1.05	0.355

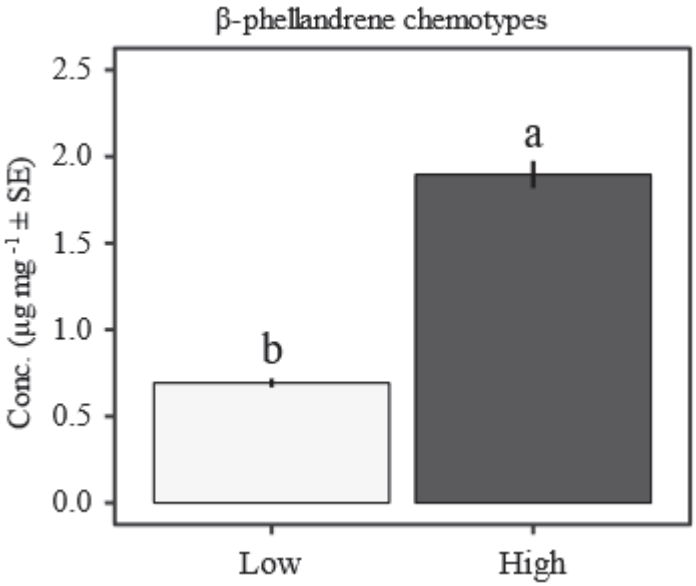


Fig. 1. Concentrations of β-phellandrene in the Low (*N*=134) and High (*N*=67) β-phellandrene chemotypes characterized in *Pinus contorta* trees on five field sites around central trees (*N*=39) in 30 m radii in Jasper National Park. Different letters indicate significant differences at α=0.05 in two-sample *t*-test, *P*<0.001.

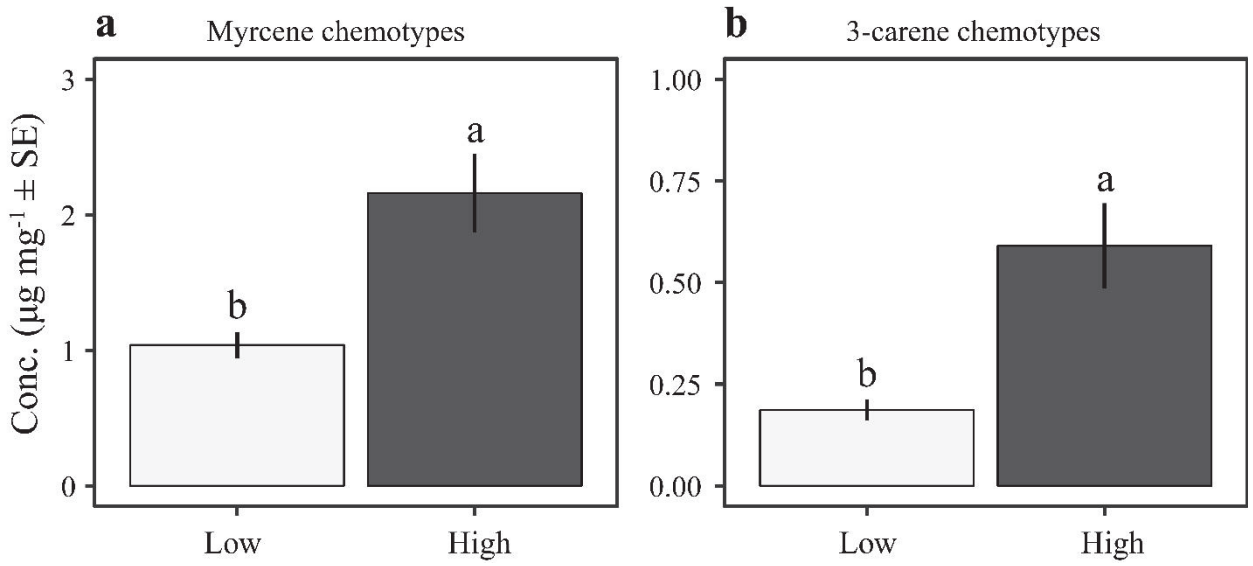


Fig. 2. Concentrations of induced myrcene and 3-carene chemotypes characterized in *Pinus contorta* trees on field sites around central trees in 30 m radii in Jasper National Park. a) The myrcene chemotype characterized in the High β-phellandrene chemotype (at constitutive level) had Low and High myrcene chemotypes (n=54 and 13 respectively). b) The 3-carene chemotype characterized in the Low β-phellandrene chemotype (at constitutive level) had Low and High 3-carene chemotypes (n=81 and 53 respectively). Different letters indicate significant differences at α=0.05 in two-sample t-tests, P<0.001 in both instances.

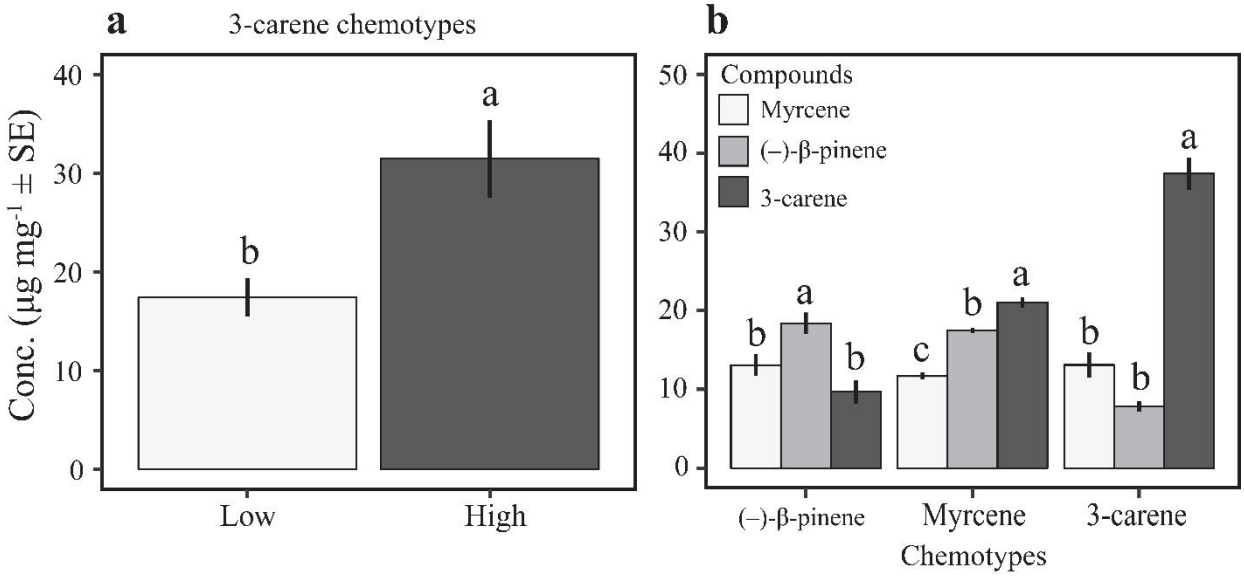


Fig. 3. Concentrations of 3-carene, (–)-β-pinene, and myrcene characterized in lesion samples of *Pinus contorta* in their respective chemotypes on field sites around central trees in 30 m radii in Jasper National Park. a) The 3-carene chemotype characterized in the High β-phellandrene chemotype (at constitutive level) had Low and High 3-carene chemotypes (n=47 and 20 respectively). b) The (–)-β-pinene chemotype (n=61), myrcene chemotype (n=45) and 3-carene chemotype (n=28) characterized in the Low β-phellandrene chemotype (at constitutive level). Different letters indicate significant differences at α=0.05, P<0.001 in all instances.

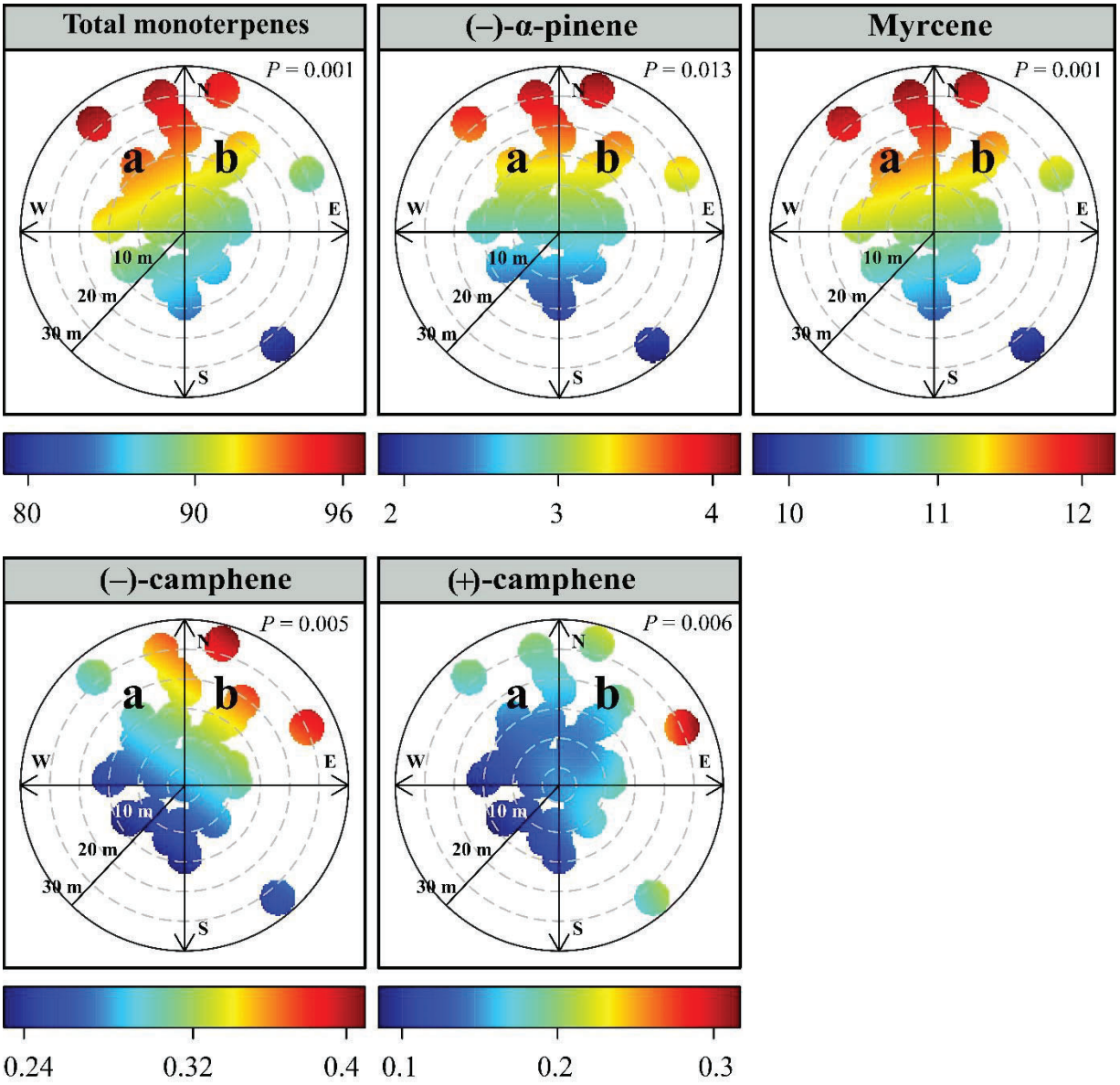
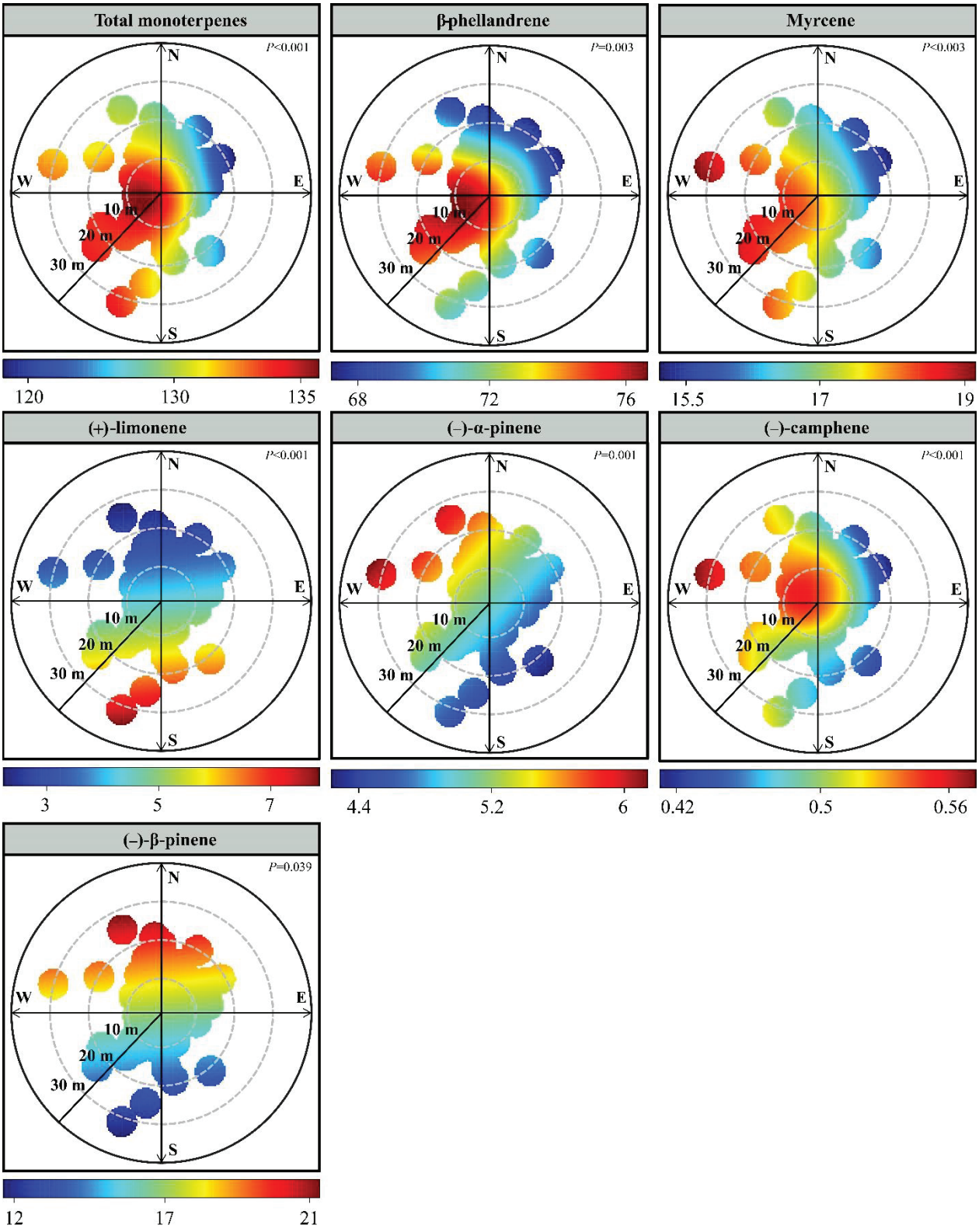


Fig. 4. Mean monoterpene concentrations (µg mg^{-1} WW) of focal *Pinus contorta* trees on east (*n*=10) and west (*n*=35) site aspects in the myrcene chemotype characterized in the Low β-phellandrene chemotype (at constitutive level) in Jasper National Park. Different letters indicate significant differences at α=0.05 in two sample *t*-tests, *P*<0.05 in all instances (Table 1).



Mean concentration (µg mg^{-1} WW)

Fig. 5. Variations in mean lesion monoterpene concentrations (μg mg^{-1} WW) of focal *Pinus contorta* trees (*N*=45) and their correlation with distance from their corresponding central trees (*N*=22) and site aspects on east (*n*=10) and west (*n*=35) in the (–)-β-pinene chemotype characterized in the Low β-phellandrene chemotype (at constitutive level) at field sites in Jasper National Park. Different letters indicate significant differences at α=0.05 in one-way ANOVAs, *P*<0.05 in all instances (Table 2).

Table S1. Details of site characteristics of lodgepole pine (*Pinus contorta* var. *latifolia*) forests selected for the study.

Site	Aspect	Elevation (m)	Central (ID)	Focal (*n*)	Location
1	East	1280	A	8	52.766533, -118.025617
			B	2	
			C	7	
			D	9	
			E	5	
			F	2	
			G	3	
			H	4	
			I	2	
2	East	1209	A	2	52.774233, -118.029467
			B	2	
			C	4	
			D	2	
			E	2	
			F	4	
			G	7	
			H	6	
3	West	1461	A	7	52.838967, -117.718467
			B	9	
			C	2	
			D	8	
			E	8	
			F	3	
			G	2	
			H	7	
			I	6	
4	West	1261	A	2	52.918918, -118.090646
			B	2	
			C	4	
			D	8	
5	West	1246	A	7	52.916625, -118.084935
			B	4	
			C	9	
			D	9	
			E	7	
			F	6	
			G	7	
			H	7	
			I	6	

Table S2. Results of the four-way PERMANOVAs comparing differences in the concentrations of constitutive monoterpenes in the Low (*N*=134) and High (*N*=67) β-phellandrene chemotypes of *Pinus contorta* trees on field sites (*N*=5). *P*-values are significant at α=0.05. Subscript numbers indicate the numbers of residuals of each *F* test.

Chemotypes	Factors	df	SS	MS	R^2	*F-value*	*P- value*
Low β-phellandrene	$Aspects_{124}$	1	0.01	0.01	0.01	1.42	0.197
	$Direction_{124}$	3	0.01	0.00	0.01	0.56	0.891
	$Distance_{124}$	2	0.01	0.00	0.00	0.58	0.812
	$Sites_{124}$	3	0.05	0.01	0.03	1.70	0.071
High β-phellandrene	$Aspects_{57}$	1	0.00	0.00	0.00	0.37	0.897
	$Direction_{57}$	3	0.07	0.02	0.05	1.23	0.231
	$Distance_{57}$	2	0.02	0.01	0.02	0.68	0.749
	$Sites_{57}$	3	0.06	0.02	0.04	1.08	0.358

Table S3. Results of the PERMANOVAs comparing differences in the concentrations of induced monoterpenes and site aspects, direction and distance of the non-attacked *Pinus contorta* trees from their corresponding mountain pine beetle (*Dendroctonus ponderosae*) attacked trees, and variations among sites. *P*-values are significant at $\alpha=0.05$. Subscript numbers indicate the numbers of residuals of each *F* test.

Chemotype (Constitutive)	Chemotype (Induced)	Factors	df	SS	MS	R^2	*F*	*P*
High β-phellandrene (*N=67*)	Low Myrcene	$Aspects_{44}$	1	0.02	0.02	0.02	1.06	0.369
		$Direction_{44}$	3	0.06	0.02	0.06	1.06	0.378
		$Distance_{44}$	2	0.02	0.01	0.02	0.61	0.798
		$Sites_{44}$	3	0.06	0.01	1.01	0.05	0.432
	High Myrcene	$Aspects_{3}$	1	0.02	0.02	0.03	0.90	0.480
		$Direction_{3}$	3	0.37	0.12	0.56	2.16	0.061
		$Distance_{3}$	2	0.08	0.04	0.12	1.47	0.267
		$Sites_{3}$	3	0.09	0.03	0.14	1.20	0.384
Low β-phellandrene (*N=134*)	Low 3-Carene	$Aspects_{71}$	1	0.02	0.02	0.01	1.25	0.255
		$Direction_{71}$	3	0.06	0.02	0.03	1.06	0.371
		$Distance_{71}$	2	0.02	0.01	0.01	0.71	0.729
		$Sites_{71}$	3	0.06	0.02	0.03	1.03	0.405
	High 3-Carene	$Aspects_{43}$	1	0.02	0.02	0.02	1.30	0.245
		$Direction_{43}$	3	0.05	0.01	0.06	1.10	0.319
		$Distance_{43}$	2	0.01	0.00	0.02	0.51	0.810
		$Sites_{43}$	3	0.04	0.01	0.04	0.84	0.460

Table S4. Results of the PERMANOVAs comparing differences in the monoterpene concentrations in lesions and site aspects, direction and distance of the non-attacked *Pinus contorta* trees from their corresponding central trees, and variations among sites. *P*-values are significant at α=0.05. Subscript numbers indicate the numbers of residuals of each *F* test.

Chemotypes (Constitutive)	Chemotypes (Lesion)	Factors	df	SS	MS	R^2	*F*	*P*
High β-phellandrene (*N=67*)	Low 3-carene	$Aspects_{37}$	1	0.04	0.04	0.03	1.90	0.109
		$Direction_{37}$	3	0.11	0.03	0.09	1.48	0.130
		$Distance_{37}$	2	0.02	0.01	0.02	0.51	0.855
		$Sites_{37}$	3	0.11	0.03	0.08	1.43	0.159
	High 3-carene	$Aspects_{13}$	1	0.02	0.02	0.02	0.45	0.745
		$Direction_{13}$	3	0.12	0.04	0.12	0.66	0.726
		$Distance_{13}$	1	0.04	0.04	0.04	0.70	0.561
		$Sites_{13}$	1	0.04	0.00	0.00	0.07	0.985
Low β-phellandrene (*N=134*)	Myrcene	$Aspects_{35}$	1	0.12	0.12	0.08	4.22	0.003
		$Direction_{35}$	3	0.06	0.02	0.04	0.75	0.725
		$Distance_{35}$	2	0.10	0.05	0.06	1.60	0.089
		$Sites_{35}$	3	0.11	0.03	0.08	1.32	0.196
	3-carene	$Aspects_{18}$	1	0.09	0.09	0.10	2.71	0.061
		$Direction_{18}$	3	0.11	0.03	0.11	1.02	0.408
		$Distance_{18}$	2	0.06	0.03	0.06	0.89	0.485
		$Sites_{18}$	3	0.03	0.01	0.03	0.27	0.989
	(−)-β-pinene	$Aspects_{51}$	1	0.05	0.05	0.03	2.27	0.048
		$Direction_{51}$	3	0.11	0.03	0.06	1.51	0.104
		$Distance_{51}$	2	0.22	0.11	0.12	4.41	<0.001
		$Sites_{51}$	3	0.07	0.02	0.04	1.03	0.443

Table S5. Results of the linear mixed model analyses comparing mean lesion lengths (cm) and their correlation with the concentration of total monoterpenes (µg mg^{-1} WW), site aspects (East or West), direction (NE, SE, SW and NW) and distance (0-10, 10-20 and 20-30 m) of the non-attacked focal trees (*N*=201) from their corresponding mountain pine beetle attacked central trees (*N*=39) in two lesion chemotypes originating from the High (*N=67*), and three originating from the Low (*N=134*) β-phellandrene chemotypes (at constitutive level) in *Pinus contorta* trees. *P*-values are significant at α=0.05.

Chemotype (constitutive)	Fixed effect	Wald χ^2 (*2*)	*P*
High β-phellandrene	Total monoterpenes	0.49	0.480
	Lesion chemotypes	2.67	0.101
	Aspects	1.91	0.166
	Direction	4.20	0.239
	Distance	3.39	0.182
Low β-phellandrene	Total monoterpenes	0.50	0.477
	Lesion chemotypes	2.89	0.234
	Aspects	0.30	0.582
	Direction	4.68	0.196
	Distance	1.85	0.395

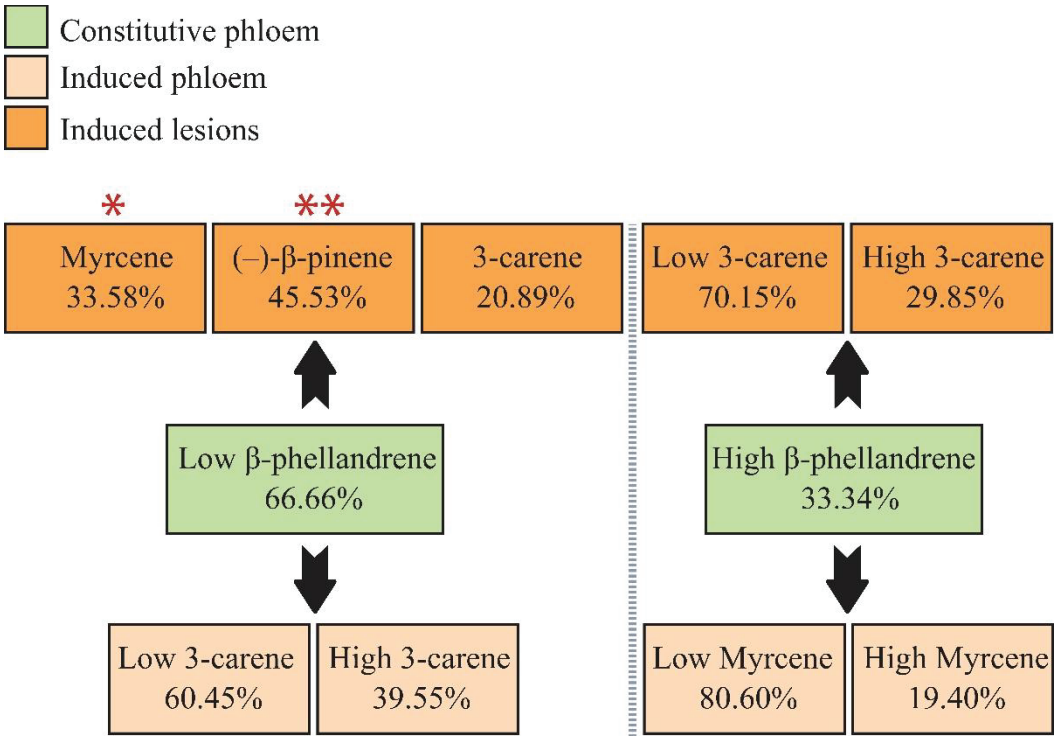


Fig. S1. Chemotypic expression of lodgepole pine trees and their percent representation in constitutive and induced phloem, and induced lesion samples. Chemotypes indicated with '*' and '**' correlated with site aspects, and distance from the central trees and site aspects, respectively. None of the other chemotypes correlated with any of these two variables.