



# Tree response and mountain pine beetle attack preference, reproduction and emergence timing in mixed whitebark and lodgepole pine stands

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- Abstract**
- 1 Mountain pine beetle (*Dendroctonus ponderosae*) is an important disturbance agent in *Pinus* ecosystems of western North America, historically causing significant tree mortality. Most recorded outbreaks have occurred in mid elevation lodgepole pine (*Pinus contorta*). In warm years, tree mortality also occurs at higher elevations in mixed species stands.
  - 2 Mountain pine beetle's relative preference for and performance in *Pinus* species that either commonly or less frequently encounter this insect has received little direct testing. Further, knowledge of the relative proportions of secondary compounds, which can differ among *Pinus* species and play important roles in attack rates and outcomes, is important to understanding host suitability.
  - 3 We monitored mountain pine beetle attacks, adult emergence timing and reproductive capacity in lodgepole and whitebark (*Pinus albicaulis*) pines growing in mixed stands at relatively high elevation. Phloem monoterpene chemistry of trees prior to and during attack was compared within and between species.
  - 4 Although beetles attacked lodgepole pine more frequently, lodgepole pines also resisted attacks more frequently. Overall, there were equal numbers of lethal attacks between species. Brood production and adult emergence timing did not differ between tree species.
  - 5 The relative composition of secondary compounds differed by tree species, although both species contained compounds that affect mountain pine beetle attack and reproductive success.

**Keywords** Bark beetle, *Dendroctonus ponderosae*, monoterpene, *Pinus albicaulis*, *Pinus contorta*, tree defence.

## Introduction

Conifers employ integrated physical, chemical, and histological constitutive and induced defences by which they protect their tissues from a diverse array of insect herbivores (Franceschi *et al.*, 2005; Kane & Kolb, 2010; Zhao *et al.*, 2011; Schiebe *et al.*, 2012). One group of subcortical herbivores, bark beetles, have evolved morphological, behavioural and biochemical specializations that allow them to circumvent or overcome these defences. Bark beetles in the genus *Dendroctonus*, for example, have the

capacity to exploit conifer-produced compounds as pheromone precursors and synergists, and as feeding incitants at low concentrations (Raffa *et al.*, 2005; Blomquist *et al.*, 2010). The ability to tolerate and potentially benefit from low levels of toxic plant-produced secondary compounds is common among insect herbivores that are relative specialists (Ali & Agrawal, 2012). Compared with many other herbivore groups, *Dendroctonus* bark beetles have relatively narrow host preferences because they typically feed on a single species or multiple species within a single tree genus (Wood, 1982). For example, the mountain pine beetle (MPB) *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae) is generally limited to species within the genus *Pinus* that occur in the western U.S.A. and Canada.

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MPB is considered an eruptive species, building rapidly from low to high numbers when environmental and host tree habitat conditions are optimal (Amman & Cole, 1983; Safranyik & Carroll, 2006). Rapid population growth is a by-product of a series of positive feedbacks including host-derived, pheromone-mediated attacks that can overwhelm tree defences when ample conspecifics are available (Raffa *et al.*, 2008). Upon successful attack, the phloem is inoculated with mutualistic fungi, mating occurs, and eggs are laid along a vertical gallery. Larvae mine horizontally feeding on host phloem, thereby severing vessels that transport water and nutrients along the tree bole. After pupation, adult beetles emerge and fly to attack a new tree. Developmental timing that results in synchronous emergence is critical to a successful mass attack and highly temperature dependent (Bentz *et al.*, 1991). A MPB generation is typically univoltine at mid elevations and semivoltine at the highest elevations (Bentz *et al.*, 2014). Because all or part of the host tree is killed during MPB colonization, extensive tree mortality can occur during the high population phase.

As a result of its widespread distribution and silvics-driven habit of commonly growing in stands of relatively homogenous age, size and species composition, Rocky Mountain lodgepole pine *Pinus contorta* var. *latifolia* Dougl. ex. Loud. (hereafter lodgepole pine) is the most common species that supports MPB population outbreaks. Lodgepole pine is ubiquitous, growing in a wide variety of climates in the western U.S.A. and Canada from 480 to 3600 m a.s.l. (Lotan & Critchfield, 1990). Its habitat ranges from relatively single-species stands to mixed stands containing several other MPB hosts, such as whitebark pine *Pinus albicaulis* Engelm., western white pine *Pinus monticola* Dougl., ponderosa pine *Pinus ponderosa* Dougl. and limber pine *Pinus flexilis* James. During the period 1997–2010, the area affected by high MPB populations was 86.8% lodgepole, 6.5% ponderosa, 4.8% whitebark, 1.5% limber and 0.2% each of Rocky Mountain bristlecone *Pinus arista* Engelm. and western white pine (Meddens *et al.*, 2012).

Pine species in the northern Rocky Mountains of the U.S.A. show elevational gradients. Ponderosa pine predominates at lower elevations, lodgepole pine at mid to high elevations, and whitebark pine extends to the highest elevations in the timberline zone. Lodgepole and whitebark pines frequently intermingle, although they can also be separated by a zone of nonhost *Picea* and *Abies* (Arno & Hoff, 1989). Thermal regimes in mid elevation lodgepole pine are often highly suitable for MPB population growth, with summer temperatures that promote univoltinism and relatively mild winter temperatures that enhance survival (Régnière & Bentz, 2007; Safranyik *et al.*, 2010; Bentz *et al.*, 2014). At high elevations where mixed lodgepole and whitebark pine stands transition into pure whitebark pine, however, years with adequate thermal input have been interspersed with thermally unsuitable years over the past century (Arno, 1986; Perkins & Swetnam, 1996; Furniss & Renkin, 2003; Bentz *et al.*, 2011). The result has been pulses of MPB-caused tree mortality at the highest elevations that were less extensive than in pure lodgepole pine stands at lower elevations. MPB fossils found in high elevation lake sediments dominated by whitebark and lodgepole pine pollen and deposited during the Holocene suggest that this pattern of intermittent MPB activity at high elevations has occurred for thousands of years (Brunelle *et al.*, 2008).

A lack of consistent thermal conditions for persistent MPB success could result in reduced levels of evolved defensive responses to MPB attack in species such as whitebark pine that grow at the highest elevations (Raffa *et al.*, 2013). Lack of continual exposure could also influence MPB host preference when whitebark pine grows intermingled with lodgepole pine, although whether one species is preferred remains unclear (Baker *et al.*, 1971; Waring & Six, 2005; Raffa *et al.*, 2013). In recent years, warm temperatures associated with climate change have promoted extensive MPB-caused tree mortality across the western U.S.A. and Canada (Bentz *et al.*, 2010; Safranyik *et al.*, 2010; Meddens *et al.*, 2012), including in mixed and pure whitebark pine stands at high elevations (Gibson *et al.*, 2008; Macfarlane *et al.*, 2013). Understanding MPB host utilization in mixed *Pinus* stands within the current range will improve our ability to predict range expansion into *Pinus* species not currently documented as hosts. For example, many *Pinus* have similar secondary compounds despite high variation in their relative and absolute concentrations (Smith, 2000) and this similarity may allow MPB to be successful in novel host species (Erbilgin *et al.*, 2014).

The present study aimed to investigate MPB host use and reproductive capacity, as well as host tree secondary compounds of whitebark and lodgepole pine growing in mixed stands at relatively high elevations. Specifically we (i) compared the timing of emergence and reproductive capacity between MPB colonizing lodgepole and whitebark pines; (ii) evaluated MPB attack preference and attack success between lodgepole and whitebark pines growing in mixed stands of variable relative compositions; and (iii) evaluated the phloem secondary chemistry of trees prior to attack, and during MPB attack on trees that were ultimately lethally-attacked and trees that ultimately survived.

## Materials and methods

### *Site and stand descriptions*

Two sites, Union Pass and Fish Creek, were selected within the Greater Yellowstone Ecosystem located near Dubois, Wyoming, on the Shoshone National Forest (Table 1). We were interested in evaluating the MPB response to host tree species that were growing in a similar environment, and with varying numbers of each host tree species. Accordingly, at each site, we identified stands that contained (i) predominately whitebark pine (WBP); (ii) predominately lodgepole pine (LPP); and (iii) a mix of lodgepole pine and whitebark pine (MIX). Within a site, the three stand types were within 230 m of one another. Although dispersal remains one of the least understood aspects of MPB biology, dispersal within a stand is on the order of 30 m and long-range movement can be much greater (Safranyik *et al.*, 1992). Both sites were within 1 km of MPB population activity (Gibson *et al.*, 2008; <http://foresthealth.fs.usda.gov/portal>), although few trees in any stands were attacked in spring 2007 prior to the beginning of the study. To estimate tree age, 40 trees (20 whitebark pine and 20 lodgepole pine) at each site were cored. Two cores, one each from the north and south bole aspect, were removed per tree near ground level, and tree age was estimated in the laboratory by counting rings from the pith to outer bark.

**Table 1** Number of trees >10.16 cm diameter at breast height (DBH), mean  $\pm$  SE DBH (cm) and mean  $\pm$  SE tree age at two sites (Fish Creek: 43.5375,  $-109.842$ ; 2749 m; Union Pass: 43.58564,  $-109.958$ , 2716 m) in three stand types (0.25 ha in size): predominately whitebark pine (WBP), predominately lodgepole pine (LPP) and a mix of WBP and LPP (MIX)

Site & Stand type	Number of trees		DBH		Tree age	
	Whitebark	Lodgepole	Whitebark	Lodgepole	Whitebark	Lodgepole
Fish Creek			20.91 $\pm$ 9.9	23.68 $\pm$ 8.47	119 $\pm$ 72	119 $\pm$ 67
WBP	107	6	22.91 $\pm$ 9.74	22.10 $\pm$ 9.38		
LPP	46	117	14.75 $\pm$ 5.03	23.42 $\pm$ 8.53		
MIX	80	75	21.79 $\pm$ 11.01	24.23 $\pm$ 8.38		
Union Pass			21.79 $\pm$ 10.36	23.60 $\pm$ 8.55	181 $\pm$ 70	211 $\pm$ 99
WBP	91	22	26.67 $\pm$ 10.46	23.09 $\pm$ 12.35		
LPP	50	141	16.03 $\pm$ 7.70	22.79 $\pm$ 7.60		
MIX	79	47	19.81 $\pm$ 9.22	26.26 $\pm$ 8.79		

Although 20 trees of each species at each site were cored, only 19 whitebark and 19 lodgepole pines at the Fish Creek site and 20 whitebark and 16 lodgepole pines at the Union Pass site were used to estimate stand age.

At the two sites, a 0.25-ha square plot (50 m per side) was established within the boundary of each stand type (i.e. WBP, LPP, MIX). The boundary of each plot was delineated with flagging and a string line and partitioned into nine blocks (three rows  $\times$  three columns) to facilitate the surveying of trees within each plot. In early June 2007, prior to flight of adult beetles, a 100% survey of all pines in each 0.25 ha plot was conducted. Each live pine was tagged with a number, and diameter at breast height (DBH) (1.37 m) and tree species recorded. DBH of dead pines was also recorded and, if previously MPB-attacked, we estimated whether the attack occurred within 3 years based on characteristics of the foliage. Trees with yellow or red foliage and at least 50% needle retention were recorded as having been attacked within the previous 3 years, and trees with < 50% needles remaining were considered older attacks. Phloem depth (as a measure of MPB food availability; Amman, 1972) of a random sample of live pines in each plot was measured to the nearest millimetre in a 1-cm sample from the north and south bole aspects. Pruning sealant was applied to sample areas to reduce desiccation.

#### MPB population monitoring

Prior to flight of adult beetles, four passive traps were hung in each plot (12 traps total per site). Passive traps, which were not baited with an aggregant pheromone and are designed to measure nondirected beetle flight, were used to monitor background beetle populations within each stand type at each site. Passive traps were constructed of two clear Plexiglass sheets (height 81 cm; width 41 cm) arranged in a cross pattern with a collection funnel to catch flying beetles that intercept the trap. Traps were hung 2–3 m above the ground.

The timing of MPB emergence from the two host species was monitored using cages constructed around portions of infested trees. At each plot, two trees of each species of similar size that were attacked and infested by mountain pine beetle during the previous summer were identified based on pitch tubes on the outer bark and signs of brood and egg galleries within the inner bark. Because phloem temperatures on north and south bole aspects of infested trees can be significantly different (Bentz & Mullins, 1999), two emergence cages were hung on each

infested tree: one on the north and one on the south bole aspect. Emergence cages were hung on trees at the Fish Creek site on 31 May 2007 and at the Union Pass site on 12 June 2007. Emergence cages consisted of a flexible screen stapled over a section (height 60 cm, width 30 cm) of the tree bole, centered at 1.37 m height from the ground. All cages were the same size, and attached to trees such that a similar area of each tree bole was sampled, thereby standardizing the sample size regardless of tree DBH. A tube attached to the bottom of the screen enclosure collected all adults that emerged from the tree bole within the sample space. A total of six whitebark pine (mean  $\pm$  SE: DBH = 37.62  $\pm$  0.29 cm) and six lodgepole pine (DBH = 36.41  $\pm$  0.39 cm) trees were caged at each site. Beginning on 14 June 2007, cage tubes and traps were monitored for adult beetles on a weekly interval. On 27 September 2007, cages and bark beneath each caged area were removed and parent gallery starts were counted for use in estimating adult attack to brood emerged ratios. At this time, we also checked for remaining live brood adults within the sample area, and found none.

Beginning on 25 June 2007, all trees in each plot were monitored weekly for new MPB attacks until 25 September 2007. Each week, MPB attack status was recorded for each tree as either attacked or not attacked. The number of attacks per tree was also monitored on a randomly selected subset of trees. Once attack monitoring for a given tree was initiated, the same tree was followed in subsequent weeks. We subsampled attacks on trees by counting all new attacks in a 20.3-cm band around the circumference of the tree bole at three heights on the tree: 0.76, 1.37, and 1.98 m. Using a permanent marker, new attacks each week were marked within each sample area to avoid counting the same attack during the next week. On 25 September 2007, after MPB flight ended, each tree was assigned an attack type. Trees with attacks on the entire circumference and length of the bole were classified as lethal attacks. Nonlethal attacks included strip-attacked trees, where only a portion of the tree bole was successfully attacked, and pitch-out attacks that had no successful attacks. Although weekly attacks on trees and brood emergence were only monitored in 2007, all trees in each plot were resurveyed after MPB flight in 2008 and 2009. Monitoring each tree for three consecutive years allowed us to confirm the attack type (i.e. lethal or nonlethal) of trees attacked in 2007, in addition to recording new MPB attacked trees in subsequent years.

### Phloem chemistry

Phloem chemistry of unattacked and attacked trees was measured in 2007. To quantify unattacked or constitutive phloem chemistry, phloem samples were collected from a random sample of live, unattacked pines within each plot on 11 July 2007. Although attacks were occurring in some of the stands at this time, the visible bole of each tree, particularly mid-bole where mountain pine beetle initially attacks (Rasmussen, 1974), was checked to ensure there were no attacks. Samples were collected from 10 lodgepole and 10 whitebark pines. Two samples were taken per tree on the north and south bole aspects. A chisel was used to remove a strip of phloem (5 × 2 cm) at DBH from each bole aspect. Strips were placed in labelled vials, sealed with paraffin, placed in dry ice and transported to the laboratory in Logan, UT where all samples were stored at -40 °C.

To quantify phloem chemistry of tree response during MPB attack, phloem strips were removed from a random sample of 41 trees that were within the first 3 days of natural attack. These trees were not always the same as the trees sampled pre-attack as a result of the random timing of natural attacks. During four periods throughout the flight season in 2007 (10–12 July; 27–29 July; 30 July to 1 August; 18–20 August), all trees in each plot were monitored for three consecutive days. Only trees that were attacked within the 3-day period were sampled. One or two samples per tree were collected by removing phloem, as described above, from an area next to a beetle entry site. Samples were placed on dry ice and transported to the laboratory in Logan, Utah, and stored at -40 °C. Because trees were sampled within 3 days of first attack, the fate of each sampled tree was unknown at the time of sampling and the number of attacks per tree at the time of sampling varied.

Volatile compounds were extracted and analyzed by gas chromatography according to a modified method described in Raffa and Smalley (1995). A section (1.5–2.0 cm) of frozen phloem sample was finely chopped, added to a 2.0-mL vial containing 1.0 mL of hexane and shaken vigorously at moderate speed for 24 h at room temperature. The extract was removed from the original vial and filtered through glass wool into second vial. The original sample vial was rinsed twice with 250 µL of hexane, also filtered, and added to the second vial, resulting in a final volume of 1.5 mL. All utensils and equipment were rinsed with methanol between samples. Samples were stored at 5 °C until analysis within 2 days of extraction. The remaining phloem was dried at 25 °C for 1 week and weighed.

Samples were analyzed with a Shimadzu 17 gas chromatograph (Shimadzu Corp., Japan) with a DB Wax column (Agilent Technologies Inc., Santa Clara, California) (inner diameter 0.25 mm, length 30.0 m, 0.25-µm film) with Helium carrier gas. The oven was programmed with an initial temperature of 50.0 °C held for 10 min, then increased at a rate of 5 °C/min up to a final temperature of 240 °C and held for 10 min. Flow rate was 45 mL He/min and total run time was 58 min. Individual compounds were identified by matching retention times of compounds to those of pure standards (Sigma-Aldrich, St Louis, Missouri) run with the same oven programme as previously above. The quantities of individual compounds were calculated by comparing the area under the curve of each compound to an external standard, and the proportions of individual

compounds relative to the total monoterpene content were calculated.

### Statistical analysis

Generalized linear mixed models were used to analyze all statistical relationships (GLIMMIX, SAS, version 9.4; SAS Institute, Inc., Cary, North Carolina). Site and block within site were considered as random effects in tests for differences in trap catch among stand types. In tests aiming to evaluate differences in counts of adults emerging into cages, site and tree within site were considered as random effects, tree species was a fixed effect, and a Poisson error distribution was used. This same random effects structure and error distribution was used in tests to evaluate counts of attacks on trees, and stand type, DBH, attack type and tree species were considered fixed effects. Site and tree within site were considered random effects in tests to analyze differences in phloem thickness between the species. Post-hoc pairwise comparisons were conducted using Tukey's honestly significant difference test. Differences in the proportion of each tree species attacked were also evaluated with GLIMMIX using the variance-stabilizing lognormal error distribution and the random effect site. We also used contingency analyses with Pearson's chi-square test to evaluate the likelihood that more whitebark pine were attacked than lodgepole pine.

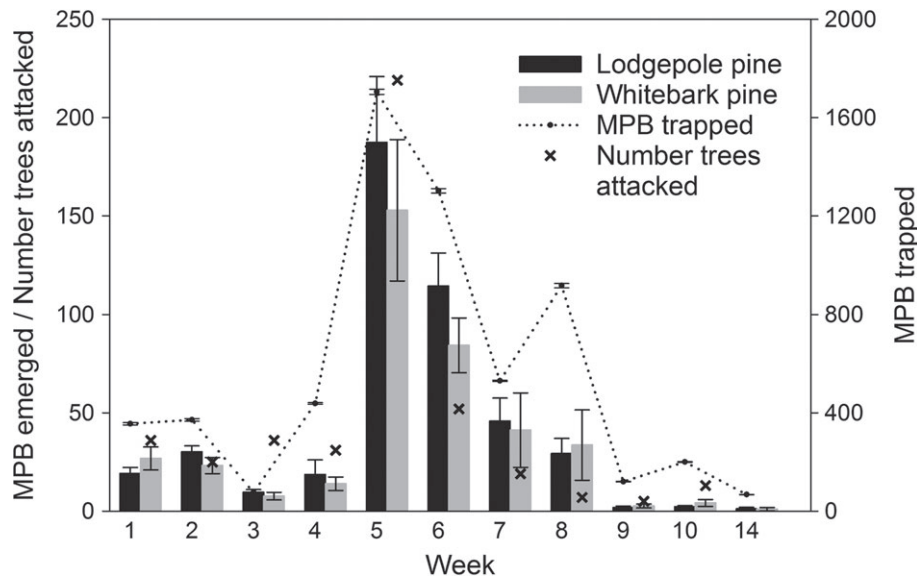
Phloem chemistry of unattacked and attacked trees was also analyzed using GLIMMIX, with random effects site and tree within site. Because the trees sampled for phloem chemistry prior to attack were not the same trees as those sampled during attack, analyses were conducted separately for unattacked and attacked trees. We compared the relative amount of individual compounds between tree species and, in the case of attacked trees, also between lethal and nonlethal attacks. All compounds, except 4-allylanisole, were calculated as a proportion of the total monoterpenes for a tree. Prior to mixed model analyses, transformed proportions using either arcsine or  $\log_{10}$  were tested for normality and the appropriate transformed value was used in the analyses.

## Results

### Site and stand conditions

Approximately 4% of both lodgepole pine and whitebark pine in our stands had been attacked and killed by MPB in the 3 years prior to the start of the study in 2007, and a few trees of both species had also been killed by MPB >3 years ago. There were no significant differences in age between the two tree species ( $F_{1,69} = 1.86$ ,  $P = 0.1766$ ) or among stand types ( $F_{2,69} = 1.64$ ,  $P = 0.2006$ ), although trees at the Union Pass site were older than trees at the Fish Creek site (Table 1). Lodgepole pines were larger than whitebark pines in the LPP ( $t_{854,2} = -7.07$ , adjusted  $P < 0.0001$ ) and MIX ( $t_{853,3} = -3.82$ , adjusted  $P = 0.0020$ ) stand types, although the mean DBH values of lodgepole and whitebark pine were not different in the WBP stand type ( $t_{1,820,1} = 0.99$ , adjusted  $P = 0.9198$ ) (Table 1). Phloem thickness was measured on two bole aspects of 106 lodgepole ( $0.0988 \pm 0.0022$  mm) and 109 whitebark ( $0.1153 \pm 0.023$  mm)





**Figure 1** Mean  $\pm$  SE number of mountain pine beetle (MPB) that emerged into tree cages per week by tree species; MPB trapped in 24 passive traps (12 per site); and trees attacked at the Fish Creek and Union Pass study sites. Week 1 is 25 June 2007 and week 14 is 27 September 2007. Emergence counts between species did not differ for a given week (week 1:  $t_{29.82} = 1.24$ ,  $P = 0.9998$ ; week 2:  $t_{28.04} = -1.15$ ,  $P = 0.9999$ ; week 3:  $t_{45.39} = -1.31$ ,  $P = 0.9996$ ; week 4:  $t_{32.96} = -1.82$ ,  $P = 0.9714$ ; week 5:  $t_{22.23} = -1.83$ ,  $P = 0.9686$ ; week 6:  $t_{22.97} = -1.88$ ,  $P = 0.9589$ ; week 7:  $t_{25.23} = -1.16$ ,  $P = 0.9999$ ; week 9:  $t_{26.87} = 0.21$ ,  $P = 1.0000$ ; week 10:  $t_{116.2} = 1.50$ ,  $P = 0.9972$ , week 14:  $t_{456.1} = -0.55$ ,  $P = 1.000$ ).

pinus, and did not differ by species ( $F_{1,208.5} = 0.87$ ,  $P = 0.3515$ ) or attack type ( $F_{2,203.6} = 1.47$ ,  $P = 0.2325$ ), although the north bole aspects had thicker phloem in both species ( $F_{1,211.6} = 6.83$ ,  $P = 0.0096$ ). Larger trees (DBH:  $F_{1,203.4} = 8.96$ ,  $P = 0.0031$ ) of both species (DBH  $\times$  species:  $F_{1,204.2} = 4.00$ ,  $P = 0.0469$ ) also had thicker phloem.

#### Background populations and within-tree MPB development and reproduction

On the first collection date, 14 June 2007, one MPB was found in a single passive trap and all other traps and cages contained 0 MPB. Beginning the week of 25 June, the number caught increased for 2 weeks, then declined until the week of 24 July when the peak number of MPB was caught in both traps and cages. Numbers of beetles collected from both traps and cages declined thereafter until the week of 25 September 2007 when few adults were found (Fig. 1).

The number of MPB caught in passive traps was greater at Fish Creek than Union Pass, although trap catch did not differ among the stand types ( $F_{2,1} = 32.97$ ,  $P = 0.1222$ ), suggesting an equal beetle pressure among stand types within a site. Within a given week, no differences were found between the number of adults emerged from lodgepole and whitebark pines (Fig. 1), suggesting that emergence timing did not differ between the species. Passive trap catches followed the trend in emergence from caged trees indicating that trap catch was a good representation of background population level within a stand type. The total number of adult MPB that emerged into cages did not differ by species ( $F_{1,20.7} = 1.12$ ,  $P = 0.3024$ ) or DBH ( $F_{1,20.6} = 0.15$ ,  $P = 0.7054$ ), although bole aspect (i.e. north or south) ( $F_{1,43} = 75.41$ ,  $P < 0.001$ ) and the interaction of aspect

and species ( $F_{1,43} = 251.30$ ,  $P < 0.001$ ) were significant. Emergence from lodgepole pine was greater from the north bole aspect ( $t_{44} = 17.81$ , adjusted  $P < 0.0001$ ), and emergence was greater from the south bole aspect in whitebark pine ( $t_{44} = -4.94$ , adjusted  $P < 0.0001$ ) (Table 2). In the area of each tree bole that was caged, there were more gallery starts on whitebark pine than lodgepole pine ( $F_{1,21} = 6.18$ ,  $P = 0.0214$ ). However, brood to attack ratio (adults emerged/gallery starts) adjusted for tree size was greater on lodgepole pine than whitebark pine ( $F_{1,22} = 3.72$ ,  $P = 0.0668$ ) (Table 2).

#### Attack preference and success

Attacks on trees were first recorded during the week of 25 June, coinciding with catches in traps and cages (Fig. 1). When all attack types are considered (i.e. lethal and non-lethal), the proportion of each species attacked in 2007 varied by week ( $F_{11,45} = 5.23$ ,  $P < 0.0001$ ), although tree species ( $F_{1,45} = 0.01$ ,  $P = 0.9401$ ) and the week  $\times$  species interaction ( $F_{10,45} = 1.57$ ,  $P = 0.1472$ ) were not significant. Also, when only the first 4 weeks of attacks were included in the analyses, week ( $F_{3,14.5} = 0.68$ ,  $P = 0.5783$ ) and species ( $F_{1,14.7} = 0.58$ ,  $P = 0.4600$ ), as well as their interaction ( $F_{3,14.1} = 1.64$ ,  $P = 0.2249$ ), were not significant, suggesting that one tree species was not attacked before the other.

Over the 3 years of the study, lodgepole pines were more frequently attacked than whitebark pines ( $\chi^2 = 12.2813$ ,  $P = 0.0005$ ) (Fig. 2A). When lethal attacks, the product of entry and success, are considered alone, the species did not differ ( $\chi^2 = 1.0748$ ,  $P = 0.3264$ ) (Fig. 2B). A lower percentage of attacks on lodgepole pine resulted in lethal attacks (59.9%) than on whitebark pine (82.57%) (Fig. 2C and Table 3; see also

**Table 2** Mean  $\pm$  SE mountain pine beetle emergence from the north and south bole aspect of 12 whitebark and 12 lodgepole pine

Species	Number of adults emerged	Gallery starts	Brood : attack
Whitebark pine			
North aspect	181.42 $\pm$ 22.30	33.75 $\pm$ 3.59	6.46 $\pm$ 1.12
South aspect	209.67 $\pm$ 46.73	37.00 $\pm$ 3.86	5.75 $\pm$ 1.13
Mean per tree	195.54 $\pm$ 25.49	35.38 $\pm$ 2.60	6.10 $\pm$ 0.78
Lodgepole pine			
North aspect	284.59 $\pm$ 30.94	30.33 $\pm$ 1.70	9.70 $\pm$ 1.23
South aspect	173.42 $\pm$ 32.29	25.08 $\pm$ 3.69	7.41 $\pm$ 1.17
Mean per tree	229.00 $\pm$ 24.78	27.71 $\pm$ 2.06	8.56 $\pm$ 0.87

Mean  $\pm$  SE diameter at breast height (DBH) of caged whitebark pine = 36.4  $\pm$  1.31 cm and caged lodgepole pine = 37.6  $\pm$  0.99 cm. Cages on each bole aspect covered a section (height 60 cm, 30 cm) of the tree bole. Gallery starts are the number of attacks within each caged area. The brood : attack ratio was calculated as the number emerged/gallery starts per tree.

Supporting information, Table S1). Among all trees, 24.3% of lodgepole pines and 8.4% of whitebark pines showed nonlethal attacks. Linear mixed models also indicated that, although the overall percentage of each species with lethal attacks did not differ significantly, a greater percentage of lodgepole than whitebark pine had lethal attacks in the LPP stand type ( $t_5 = -5.03$ , adjusted  $P = 0.0260$ ), resulting in a significant effect of stand type (Table 3). No differences were found in the overall percentage of each species with lethal attacks in the MIX ( $t_5 = 0.43$ , adjusted  $P = 0.9969$ ) or WBP stand types ( $t_5 = 1.93$ , adjusted  $P = 0.4790$ ). A similar relationship was seen when we tested the effect of the percentage of each species in a stand on the percentage of that species being lethally-attacked. The percentage of whitebark pine with lethal attacks was lower in stands that contained a low percentage of whitebark pine ( $F_{1,4} = 12.02$ ,  $P = 0.0256$ ), although whitebark pine was also smaller than lodgepole pine in these stands (Table 1). Conversely, the percentage of lodgepole pine in a stand did not influence the percentage of lodgepole pine with lethal attacks ( $F_{1,4} = 0.40$ ,  $P = 0.5594$ ).

Trees with nonlethal attacks in 2007 were monitored for new attacks in 2008 and 2009 after mountain pine beetle flight each year. In 2008, 57% of trees that had been strip-attacked in 2007 ( $n = 30$ ) were strip-attacked again and remained alive, and 30% had lethal attacks. One of the trees strip-attacked in both 2007 and 2008 was attacked again in 2009 and remained alive. Of the 122 trees recorded as a pitch-out in 2007, 8% were attacked again and remained alive, and 8% were attacked again and died.

Trees of both species with lethal attacks were larger than trees that were not attacked (lodgepole pine:  $t_{855} = 17.26$ ,  $P < 0.0001$ ; whitebark pine:  $t_{854} = 28.26$ ,  $P < 0.0001$ ) and trees with nonlethal attacks (lodgepole pine:  $t_{854} = 10.14$ ,  $P < 0.0001$ ; whitebark pine  $t_{854,3} = 8.85$ ,  $P < 0.0001$ ) (see Supporting information, Fig. S1). Among all stand types, we found no significant differences in DBH or phloem thickness between lodgepole pine with lethal attacks and whitebark pine with lethal attacks, although lodgepole pine that survived attacks were larger than whitebark pine that survived attacks ( $F_{1,134,2} = 9.55$ ,  $P = 0.0024$ ). When all attack types were combined, the size of the attacked trees did not differ between the species ( $F_{1,460,5} = 2.90$ ,  $P = 0.0892$ ).

As expected, the number of attacks per tree (subsampling at three heights on a tree bole), when adjusted for DBH, was greater on trees with lethal attacks (1.60  $\pm$  0.06) than nonlethal attacks (0.40  $\pm$  0.09) (Table 4). Also, there were more

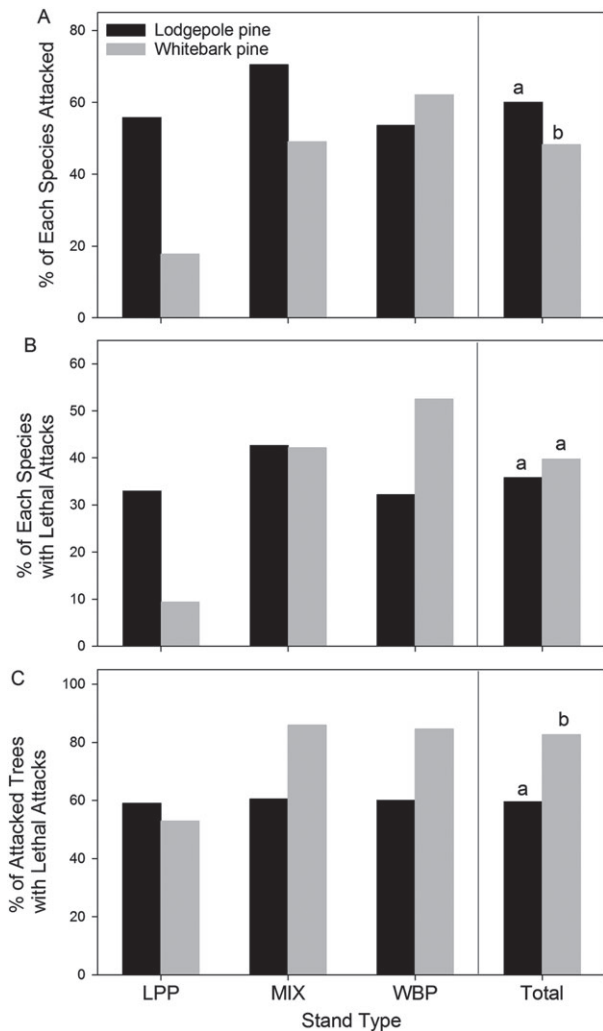
attacks on whitebark pine with lethal attacks than lodgepole pine with lethal attacks ( $t_{131,1} = -2.77$ ,  $P = 0.0318$ ). The number of attacks on both tree species was greater at the low than high sample height ( $t_{256,3} = -3.96$ ,  $P = 0.0003$ ), although no differences were observed between the middle and low heights ( $t_{254,7} = 1.89$ ,  $P = 0.14341$ ) or middle and high heights ( $t_{253,1} = -2.13$ ,  $P = 0.0866$ ).

#### Phloem chemistry

We were interested in comparing the relative concentrations between the tree species of secondary compounds known to influence MPB. In unattacked tissue  $\alpha$ -pinene was 3.8-fold greater in whitebark pine, myrcene was 2.9-fold greater in whitebark pine, 3-carene was 1.5-fold greater in whitebark pine, an unknown monoterpene was 12.9-fold greater in whitebark pine, and  $\beta$ -phellandrene was 2.9-fold greater in lodgepole pine (Table 5). During MPB attack, the relative concentrations of these same compounds differed between the species, although in greater amounts.  $\alpha$ -Pinene was 4.2-fold greater, myrcene was 4.7-fold greater, and an unknown monoterpene with the same retention time as above was 10.7-fold greater in whitebark than lodgepole pine. However, attacked whitebark pine also had greater percentage of limonene (7.2-fold) than attacked lodgepole pine. Similar to differences between unattacked trees, attacked lodgepole pine had greater proportions of  $\beta$ -phellandrene (3.7-fold) than whitebark pine, and  $\beta$ -pinene (2.8-fold) was also induced in lodgepole pine (Fig. 3; see also Supporting information, Table S2). Within a species, the only compound significantly different between lethal and nonlethal attacked trees was myrcene in whitebark pine (see Supporting information, Table S3).

#### Discussion

Over all stands and years, MPB showed a preference for lodgepole over whitebark pines growing in the same stands. The greater proportion of lodgepole pine attacked could not be explained by tree size. However, this relationship was influenced by forest composition. Specifically, the proportion of whitebark pines within a stand that were attacked tended to increase as the availability of lodgepole pine decreased, a trend also observed



**Figure 2** (A) Percentage of each tree species attacked by mountain pine beetle by stand type, predominately whitebark pine (WBP), lodgepole pine (LPP), a mix of both species (MIX) and all trees (Total). (B) Percentage of each species with lethal attacks. (C) Percentage of attacked trees with lethal attacks.

by (Raffa *et al.*, 2013). A lack of preference for whitebark pine was further supported by our findings that neither species was consistently attacked first. Two previous studies conducted in the same general area also found that, in mixed stands, MPB were more likely to attack lodgepole pine (Baker *et al.*, 1971; Raffa *et al.*, 2013). Furthermore, in no-choice assays, MPB entered both species equally (Raffa *et al.*, 2013).

Among those trees that were attacked in 2007, a greater proportion of lodgepole pines survived. When MPB entered whitebark pines, they were more likely to generate lethal attacks. The combination of behavioural preference for lodgepole pine with an increased likelihood of beetle attack success in whitebark pine yielded equivalent rates of lethal attacks between these species among all trees. The size of lethal-attacked trees did not differ by species, and they were most commonly the largest trees of both species, which is consistent with previous observations (Amman & Cole, 1983; Safranyik & Carroll, 2006).

**Table 3** Mixed model results testing for differences in the percentage of each tree species (*i.e.* lodgepole and whitebark pine) attacked by mountain pine beetle among three stand types: predominately whitebark pine, predominately lodgepole pine and a mix of both species

Effect	F <sup>a</sup>	P
All attacks		
Species	35.85 <sub>1,5</sub>	0.0019
Stand type	28.91 <sub>2,5</sub>	0.0018
Species × stand type	21.57 <sub>2,5</sub>	0.0035
Lethal attacks		
Species	2.39 <sub>1,5</sub>	0.1828
Stand type	15.08 <sub>2,5</sub>	0.0076
Species × stand type	13.42 <sub>2,5</sub>	0.0098
Percentage attacked trees with lethal attacks		
Species	6.42 <sub>1,6</sub>	0.0444
Stand type	2.15 <sub>2,6</sub>	0.1972
Species × stand type	3.28 <sub>2,6</sub>	0.1091

<sup>a</sup>Degrees of freedom, numerator, denominator.

**Table 4** Mixed model results testing for differences in the number of attacks on trees, adjusted for tree diameter at breast height (DBH), as a function of tree species (*i.e.* lodgepole and whitebark pine), attack type (*i.e.* lethal and nonlethal attack), and attack height on tree

Effect	F <sup>a</sup>	P
Species	7.22 <sub>1,148.5</sub>	0.0080
Attack type	117.02 <sub>1,150.6</sub>	< 0.0001
Attack height	7.87 <sub>2,254.6</sub>	< 0.0001
Species × attack type	0.24 <sub>1,148.9</sub>	0.6256

<sup>a</sup>Degrees of freedom, numerator, denominator.

Number of attacks per tree was subsampled using three (*i.e.* 0.76, 1.37 and 1.98 m above ground level) 20.3-cm wide bands along each tree bole.

We observed no differences in MPB brood production in whitebark and lodgepole pines. This result agrees with previous laboratory (Amman, 1982) and field (Bentz *et al.*, 2014; Dooley *et al.*, 2014) studies, and suggests that phloem thickness (Amman, 1972), rather than host tree species, controls the number of brood produced in successfully attacked trees. At our sites, phloem thickness did not differ between lodgepole and whitebark pine and, as expected (Amman & Cole, 1983), phloem was thicker in larger trees of both species. Results from previous studies have been mixed, with some showing greater phloem thickness in whitebark than in comparatively sized lodgepole pine (Dooley *et al.*, 2014; Lahr & Sala, 2014) and another showing no difference (Baker *et al.*, 1971). High within-species variability both among and within sites makes interspecific generalizations about phloem thickness difficult. Phloem is the major food source for MPB and its symbionts, and so trees with thick phloem, which are often the largest, typically support the most brood (Amman & Cole, 1983; Safranyik & Carroll, 2006; Graf *et al.*, 2012). We also found no differences between these species in the timing of brood adult emergence, as also previously observed by Amman (1982) and Bentz *et al.* (2014). Collectively, these results suggest that emergence timing and reproductive output were not affected by tree species. However, without measures of the size of adults emerging from each tree species, we cannot fully evaluate the

**Table 5** Mean  $\pm$  SE of individual compounds in unattacked lodgepole and whitebark pine phloem tissue

Species	$\alpha$ -Pinene	$\beta$ -Pinene	Myrcene	Limonene	$\beta$ -Phellandrene	3-Carene	Unknown	4-Allylanisol
Lodgepole	3.26 $\pm$ 0.43	7.94 $\pm$ 0.96	1.71 $\pm$ 0.24	4.96 $\pm$ 1.21	31.8 $\pm$ 3.83	6.58 $\pm$ 2.19	0.28 $\pm$ 0.06	0.039 $\pm$ 0.007
Whitebark	12.59 $\pm$ 2.05	6.47 $\pm$ 1.70	4.09 $\pm$ 0.75	8.12 $\pm$ 1.23	12.63 $\pm$ 3.11	13.86 $\pm$ 3.47	3.62 $\pm$ 0.58	0.047 $\pm$ 0.015
<i>F</i> <sub>d.f. num,den</sub>	22.40 <sub>1,17.08</sub>	2.99 <sub>1,17.01</sub>	4.21 <sub>1,18</sub>	2.87 <sub>1,18.02</sub>	9.90 <sub>1,17.05</sub>	8.08 <sub>1,16.24</sub>	15.53 <sub>1,15.92</sub>	0.03 <sub>1,15.92</sub>
<i>P</i>	0.0002	0.1020	0.0550	0.1076	0.0059	0.0116	0.0012	0.8588

4-Allylanisol is shown as an absolute concentration and all other compounds as a mean percent. Trace compounds are not shown. Also shown are the *F* statistic and *P*-value testing for differences in individual compounds between tree species. Two samples per tree from nine whitebark pine and one sample from one whitebark pine and two samples per tree from 10 lodgepole pine were used in the analyses.

influence of host species on overall beetle fitness. MPB emerging from similar sized whitebark and western white pine were larger than beetles emerging from lodgepole (Bentz *et al.*, 2014); stored resources that influence beetle size can be higher in whitebark than lodgepole pine (Lahr & Sala, 2014); and larger beetles likely have greater flight and reproductive potential (Graf *et al.*, 2012). The higher attack densities that we observed on whitebark pine, however, could negate the positive influence of tree species on adult size (Amman & Pace, 1976).

As a result of their historical association with tree-killing bark beetles, conifers have evolved numerous physical and physiological traits that confer defence against attacks (Franceschi *et al.*, 2005; Kane & Kolb, 2010). Although terpenoids can be costly to synthesize and store (Keeling & Bohlmann, 2006), they are key components of tree defence against attacking beetles, and can also be toxic to adults, eggs and symbionts upon entry (Smith, 1965; Raffa & Berryman, 1983; Manning & Reid, 2013; Bohlmann & Gershenzon, 2009). In addition to being constitutively produced in pines, monoterpenes can be induced upon bark beetle attack or inoculation with bark beetle-associated fungi (Zhao *et al.*, 2011; Schiebe *et al.*, 2012). The composition and inducibility of monoterpenes likely reflects the evolutionary histories between particular tree and bark beetle species (Cates & Alexander, 1982; Huber *et al.*, 2004). In the present study, there were several ecologically relevant differences in the proportions of individual compounds (i.e. chemotype) between the species in both unattacked and attacked phloem tissue.

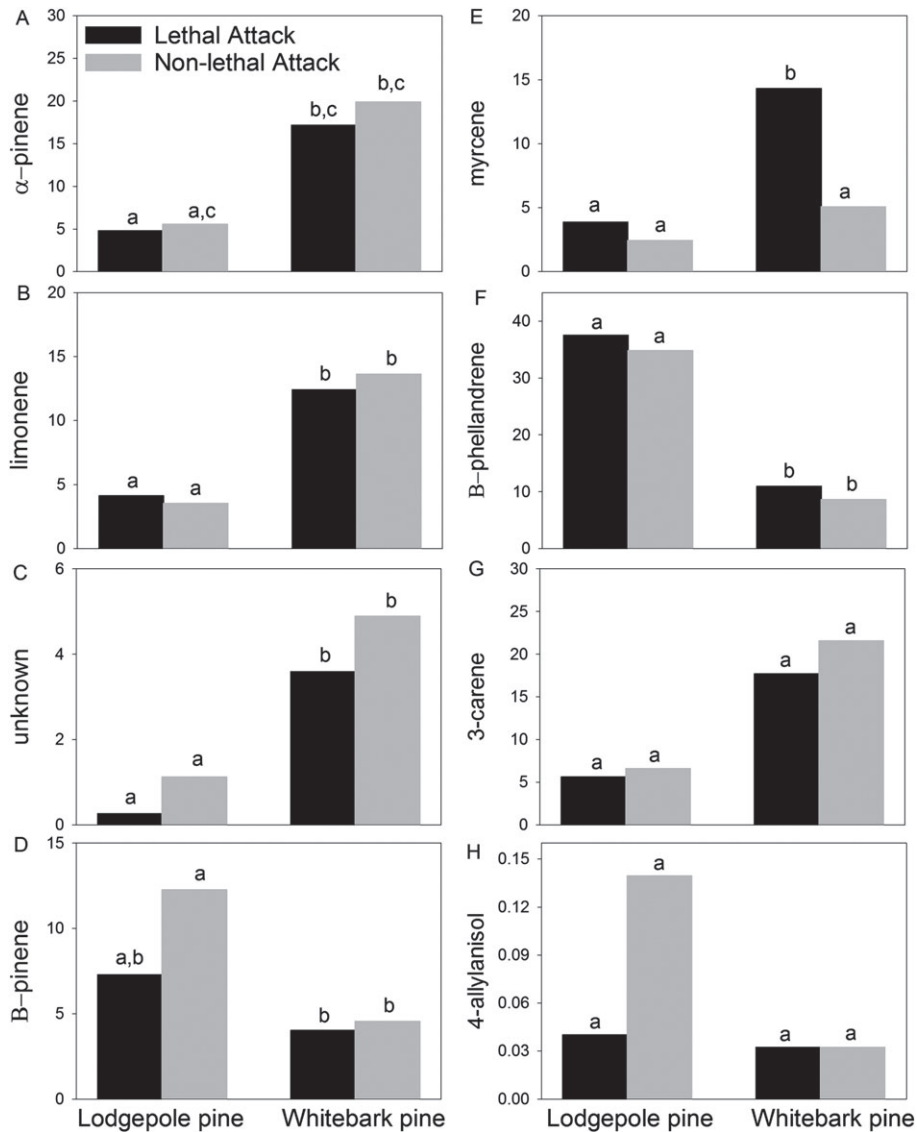
Unattacked and attacked whitebark pine tissue contained 3.8–4.2-fold more  $\alpha$ -pinene and 2.9–4.7-fold more myrcene, each of which are exploited by MPB for pheromonal communication, than lodgepole pine. In addition to whitebark pine (Zavarin *et al.*, 1991; Raffa *et al.*, 2013),  $\alpha$ -pinene is a main component of multiple MPB hosts, including ponderosa pine, Rocky Mountain bristlecone pine and limber pine (Zavarin *et al.*, 1993; Latta *et al.*, 2000; Smith, 2000; Davis & Hofstetter, 2012). In comparison, concentrations of another compound known to positively influence MPB host selection,  $\beta$ -phellandrene, were 2.9–3.7-fold greater in unattacked and attacked lodgepole pine.  $\beta$ -Phellandrene elicits antennal responses in MPB (Huber *et al.*, 2000) and is considered to function as a host-detection kairomone (Miller & Borden, 1990; Jost *et al.*, 2008), potentially resulting in the greater number of lodgepole pine attacked at our study sites. Myrcene and  $\alpha$ -pinene synergize attraction to and serve as biosynthetic precursors for MPB aggregation pheromones (Miller & Borden, 2000; Borden *et al.*, 2008; Blomquist *et al.*, 2010). Greater amounts of these compounds would serve to increase the number of beetles arriving during

colonization and, indeed, we found a greater attack density on whitebark than lodgepole pine with lethal attacks.

In addition to facilitating attraction to host trees, specific compounds found in pine tissue are known to inhibit attraction to MPB and can be toxic to attacking beetles and their eggs. For example, in addition to being a biosynthetic precursor to MPB aggregation pheromones,  $\alpha$ -pinene is also auto-oxidized to and biosynthetically converted via verbenol to verbenone, a ketone that repels beetles and thus prevents overcrowding by terminating mass attacks (Borden *et al.*, 1987; Hunt *et al.*, 1989; Flechtmann *et al.*, 1999; Lindgren & Miller, 2002; Blomquist *et al.*, 2010). We found that whitebark pine had a more than four-fold greater proportion of  $\alpha$ -pinene than lodgepole pine. Attacked whitebark pine also had a more than seven-fold greater proportion of limonene than attacked lodgepole pine. Limonene is highly toxic to bark beetles (Werner, 1995; Raffa *et al.*, 2005) and can negatively influence MPB reproduction (Manning & Reid, 2013). Norway spruce that survived *Ips typographus* L. attacks also had higher limonene concentrations than those that were killed (Schiebe *et al.*, 2012). Compared with whitebark pine, attacked lodgepole pine had greater concentrations of 4-allylanisol, a phenylpropanoid found to inhibit beetle attraction to its aggregation pheromones (Hayes & Strom, 1994; Emerick *et al.*, 2008), although the difference was not statistically significant. Similarly, in response to fungi vectored by MPB, Raffa *et al.* (2013) found that lodgepole pine had greater concentrations of 4-allylanisol than whitebark pine.

A history of predation is predicted to result in directional selection for compounds that confer greater defence (Rhoades & Cates, 1976). The presence and relative composition of multiple compounds in both species that influence attraction to and defence against MPB suggests that both tree species have a history of infestation by MPB at our study sites. For example,  $\alpha$ -pinene and myrcene are strong attractants and limonene is a strong toxin to MPB, and whitebark pine contained greater proportions of these compounds. Other studies have suggested that these relationships indicate a long history between tree and herbivore (Sturgeon, 1979; Borden *et al.*, 2008). On the other hand, lodgepole pine contained greater proportions of  $\beta$ -phellandrene, known to serve in host recognition, and higher concentrations of 4-allylanisol, a pheromone inhibitor. These results highlight the complex evolutionary interactions between MPB, its symbiotic fungi and its hosts, and suggest multiple pathways of evolution among multiple host trees in secondary defence traits. We currently lack information on how defensive capacities are distributed among populations of lodgepole, whitebark and other





**Figure 3** Mean of individual compounds in lodgepole pine and whitebark pine phloem tissue within the first 3 days of natural mountain pine beetle (MPB) attack. At the end of MPB flight, sampled trees were categorized as either a lethal or nonlethal attack. (A)  $\alpha$ -Pinene, (B) limonene, (C) unknown monoterpene, (D)  $\beta$ -pinene, (E) myrcene, (F)  $\beta$ -phellandrene and (G) 3-carene are reported as a mean percentage of total monoterpenes. (H) 4-allylanisol is shown as an absolute concentration in mg/g phloem tissue. Trace compounds are not shown. Different letters within individual compounds denote significant differences based on Tukey's honestly significant difference for multiple comparisons after GLIMMIX analyses (see Supporting information, Tables S2 and S3). Note the difference in y-axis scale among compounds.

pinus across their geographical ranges; on the relative concentrations of other important secondary compounds such as diterpenes and phenolics (Faccoli & Schlyter, 2007; Zhao *et al.*, 2011); and on how the costs of biosynthesizing secondary compounds affect other physiological processes, such as growth, cold tolerance and seed production.

## Conclusions

We compared MPB attack preference, attack success, brood production and monoterpene chemistry of unattacked and attacked lodgepole and whitebark pine. In contrast to studies evaluating

induced responses in a random sample of the host population by simulating attack using mechanical wounds combined with beetle-vectored fungi, we allowed MPB to choose trees and then measured the tree response in those trees that elicited beetle entry. We found that beetles attacked lodgepole pine more frequently, although whitebark pine less commonly resisted attacks, with the combined result of an overall equivalent incidence of lethal attacks between these two species. Brood production and adult emergence timing from trees of similar DBH did not differ among species, implying a greater role for phloem thickness than host tree species in reproductive capacity. Chemotypes of both species suggest an evolutionary history with MPB. For

example, whitebark pines had higher proportions of  $\alpha$ -pinene and myrcene, which MPB exploits to facilitate mass attacks, and lodgepole pines had higher proportions of  $\beta$ -phellandrene, which MPB exploits for host recognition. To further evaluate how historical associations with MPB influence allocation to defence versus other functions in host trees, additional comparative data between *Pinus* species growing at the same site, as well as data on species at their distributional margins (i.e. elevation and latitude), where we would expect reduced defences, are needed. Our ability to predict MPB range expansion and host tree defensive response to ongoing and future changing abiotic conditions will greatly benefit from an understanding of *Pinus* chemotype evolution.

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## Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference: 10.1111/afe.12124

**Fig. S1.** Mean  $\pm$  SE diameter at breast height (DBH) (cm) of lodgepole and whitebark pine attacked by mountain pine beetle (MPB) by attack type. Different letters indicate significant differences in DBH among attack types within and between species.

**Table S1.** Percentage of each tree species attacked by mountain pine beetle (number of trees) by attack type at two sites (Fish Creek, Union Pass) and within three stand types at each site: predominately whitebark pine (WBP), predominately lodgepole pine (LPP) and a mix of both species (MIX).

**Table S2.** Mean  $\pm$  SE of individual compounds in lodgepole and whitebark pine phloem tissue within the first 3 days of natural mountain pine beetle (MPB) attacks.

**Table S3.** Tukey's honestly significant difference results testing for differences between tree species (lodgepole, whitebark pine) and attack type (lethal, nonlethal) in individual compounds.

## References

- Ali, J.G. & Agrawal, A.A. (2012) Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, **17**, 293–302.
- Amman, G.D. (1972) Mountain pine beetle brood production in relation to thickness of lodgepole pine phloem. *Journal of Economic Entomology*, **65**, 138–140.
- Amman, G.D. (1982) Characteristics of mountain pine beetles reared in four pine hosts. *Environmental Entomology*, **11**, 590–593.
- Amman, G.D. & Cole, W.E. (1983) *Mountain Pine Beetle Dynamics in Lodgepole Pine Forests. Part II: Population Dynamics*. General Technical Report No INT-145. Intermountain Forest and Range Experiment Station, USDA Forest Service, Ogden, Utah.
- Amman, G.D. & Pace, V.E. (1976) *Optimum Egg Gallery Densities for the Mountain Pine Beetle in Relation to Lodgepole Pine Phloem Thickness*. General Technical Report No INT-209. Intermountain Forest and Range Experiment Station, USDA Forest Service, Ogden, Utah.
- Arno, S.F. (1986) Whitebark pine cone crops a diminishing source of wildlife food. *Western Journal of Applied Forestry*, **9**, 92–94.
- Arno, S.F. & Hoff, R.J. (1989) *Silvics of Whitebark Pine* (*Pinus albicaulis*). General Technical Report No INT-253. USDA Forest Service, Ogden, Utah.
- Baker, B.H., Amman, G.D. & Trostle, G.C. (1971) *Does the Mountain Pine Beetle Change Hosts in Mixed Lodgepole and White Bark Pine Stands?* General Technical Report No INT-151. Intermountain Forest and Range Experiment Station, USDA Forest Service, Ogden, Utah.
- Bentz, B.J. & Mullins, D.E. (1999) Ecology of mountain pine beetle (Coleoptera: Scolytidae) cold hardening in the Intermountain West. *Environmental Entomology*, **28**, 577–587.
- Bentz, B.J., Logan, J.A. & Amman, G.D. (1991) Temperature-dependent development of the mountain pine beetle (Coleoptera: Scolytidae) and simulation of its phenology. *Canadian Entomologist*, **123**, 1083–1094.
- Bentz, B.J., Régnière, J., Fettig, C.J. *et al.* (2010) Climate change and bark beetles of the western United States and Canada: direct and indirect effects. *Bioscience*, **60**, 602–613.
- Bentz, B.J., Campbell, E., Gibson, K., Kegley, S., Logan, J. & Six, D. (2011) Mountain pine beetle in high-elevation five-needle white pine ecosystems. *The Future of High-Elevation, Five-Needle White Pines in Western North America: Proceedings of the High Five Symposium* (ed. by R. E. Keane, D. F. Tomback, M. P. Murray and C. M. Smith), pp. 78–84. USDA Forest Service, RMRS-P-63, Ft. Collins, Colorado. [WWW document]. URL [http://www.fs.fed.us/rm/pubs/rmrs\\_p063.html](http://www.fs.fed.us/rm/pubs/rmrs_p063.html) [accessed on 30 November 2014].
- Bentz, B., Vandygriff, J., Jensen, C. *et al.* (2014) Mountain pine beetle voltinism and life history characteristics across latitudinal and elevational gradients in the western United States. *Forest Science*, **60**, 434–449.
- Blomquist, G.J., Figueroa-Teran, R., Aw, M. *et al.* (2010) Pheromone production in bark beetles. *Insect Biochemistry and Molecular Biology*, **40**, 699–712.
- Bohlmann, J. & Gershenzon, J. (2009) Old substrates for new enzymes of terpenoid biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 10402–10403.
- Borden, J., Ryker, L., Chong, L., Pierce, H. Jr., Johnston, B. & Oehlschlager, A.C. (1987) Response of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), to five semiochemicals in British Columbia lodgepole pine forests. *Canadian Journal of Forest Research*, **17**, 118–128.
- Borden, J.H., Pureswaran, D.S. & Lafontaine, J.P. (2008) Synergistic blends of monoterpenes for aggregation pheromones of the mountain pine beetle (Coleoptera: Curculionidae). *Journal of Economic Entomology*, **101**, 1266–1275.
- Brunelle, A., Rehfeldt, J., Bentz, B. & Munson, S. (2008) Holocene records of mountain pine beetle infestation in the US Northern Rocky Mountains. *Forest Ecology and Management*, **255**, 836–846.
- Cates, R.G. and Alexander, H. (1982) Host resistance and susceptibility. *Bark Beetles in North American Conifers* (ed. by Mitton, J. B. and K. B. Sturgeon), pp. 212–263. University of Texas Press, Austin, Texas.
- Davis, T.S. & Hofstetter, R.W. (2012) Plant secondary chemistry mediates the performance of a nutritional symbiont associated with a tree-killing herbivore. *Ecology*, **93**, 421–429.

- Dooley, E.M., Six, D.L. & Powell, J.A. (2014) A comparison of mountain pine beetle (Coleoptera: Curculionidae, Scolytinae) productivity and survival in lodgepole and whitebark pine after a region-wide cold weather event. *Forest Science*, **60**, 235–246.
- Emerick, J.J., Snyder, A.I., Bower, N.W. & Snyder, M.A. (2008) Mountain pine beetle attack associated with low levels of 4-allylanisole in ponderosa pine. *Environmental Entomology*, **37**, 871–875.
- Erbilgin, N., Ma, C., Whitehouse, C., Shan, B., Najar, A. & Evenden, M. (2014) Chemical similarity between historical and novel host plants promotes range and host expansion of the mountain pine beetle in a naïve host ecosystem. *New Phytologist*, **201**, 940–950.
- Faccoli, M. & Schlyter, F. (2007) Conifer phenolic resistance markers are bark beetle antifeedant semiochemicals. *Agricultural and Forest Entomology*, **9**, 237–245.
- Flechtmann, C., Dalusky, M. & Berisford, C. (1999) Bark and ambrosia beetle (Coleoptera: Scolytidae) responses to volatiles from aging loblolly pine billets. *Environmental Entomology*, **28**, 638–648.
- Franceschi, V.R., Krokene, P., Christiansen, E. & Krekling, T. (2005) Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist*, **167**, 353–376.
- Furniss, M.M. & Renkin, R. (2003) Forest entomology in Yellowstone National Park, 1923–1957: a time of discovery and learning to live. *American Entomologist*, **49**, 198–209.
- Gibson, K., Skov, K., Kegley, S., Jorgensen, C., Smith, S. & Witcosky, J. (2008) *Mountain Pine Beetle Impacts in High-Elevation Five-Needle Pines: Current Trends and Challenges*. R1-08-020. US Department of Agriculture, Forest Service, Northern Region Forest Health Protection, Missoula, Montana.
- Graf, M., Reid, M., Aukema, B. & Lindgren, B. (2012) Association of tree diameter with body size and lipid content of mountain pine beetles. *Canadian Entomologist*, **144**, 467–477.
- Hayes, J.L. & Strom, B.L. (1994) 4-Allylanisole as an inhibitor of bark beetle (Coleoptera: Scolytidae) aggregation. *Journal of Economic Entomology*, **87**, 1586–1594.
- Huber, D.P., Gries, R., Borden, J.H. & Pierce, Jr. H.D. (2000) A survey of antennal responses by five species of coniferophagous bark beetles (Coleoptera: Scolytidae) to bark volatiles of six species of angiosperm trees. *Chemoecology*, **10**, 103–113.
- Huber, D.P., Ralph, S. & Bohlmann, J. (2004) Genomic hardwiring and phenotypic plasticity of terpenoid-based defenses in conifers. *Journal of Chemical Ecology*, **30**, 2399–2418.
- Hunt, D., Borden, J., Lindgren, B. & Gries, G. (1989) The role of autoxidation of  $\alpha$ -pinene in the production of pheromones of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *Canadian Journal of Forest Research*, **19**, 1275–1282.
- Jost, R.W., Rice, A.V., Langor, D.W. & Boluk, Y. (2008) Monoterpene emissions from lodgepole and jack pine bark inoculated with mountain pine beetle-associated fungi. *Journal of Wood Chemistry and Technology*, **28**, 37–46.
- Kane, J.M. & Kolb, T.E. (2010) Importance of resin ducts in reducing ponderosa pine mortality from bark beetle attack. *Oecologia*, **164**, 601–609.
- Keeling, C.I. & Bohlmann, J. (2006) Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytologist*, **170**, 657–675.
- Lahr, E.C. & Sala, A. (2014) Species, elevation, and diameter affect whitebark pine and lodgepole pine stored resources in the sapwood and phloem: implications for bark beetle outbreaks. *Canadian Journal of Forest Research*, **44**, 1312–1319.
- Latta, R.G., Linhart, Y.B., Lundquist, L. & Snyder, M.A. (2000) Patterns of monoterpene variation within individual trees in ponderosa pine. *Journal of Chemical Ecology*, **26**, 1341–1357.
- Lindgren, B.S. & Miller, D.R. (2002) Effect of verbenone on five species of bark beetles (Coleoptera: Scolytidae) in lodgepole pine forests. *Environmental Entomology*, **31**, 759–765.
- Lotan, J. & Critchfield, W. (1990) Lodgepole pine (*Pinus contorta* Dougl. ex. Loud.). *Silvics of North America*, **1**, 302–314.
- Macfarlane, W.W., Logan, J.A. & Kern, W.R. (2013) An innovative aerial assessment of Greater Yellowstone Ecosystem mountain pine beetle-caused whitebark pine mortality. *Ecological Applications*, **23**, 421–437.
- Manning, C.G. & Reid, M.L. (2013) Sub-lethal effects of monoterpenes on reproduction by mountain pine beetles. *Agricultural and Forest Entomology*, **15**, 262–271.
- Meddens, A.J., Hicke, J.A. & Ferguson, C.A. (2012) Spatiotemporal patterns of observed bark beetle-caused tree mortality in British Columbia and the western United States. *Ecological Applications*, **22**, 1876–1891.
- Miller, D.R. & Borden, J.H. (1990)  $\beta$ -Phellandrene: kairomone for pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). *Journal of Chemical Ecology*, **16**, 2519–2531.
- Miller, D.R. & Borden, J.H. (2000) Dose-dependent and species-specific responses of pine bark beetles (Coleoptera: Scolytidae) to monoterpenes in association with pheromones. *Canadian Entomologist*, **132**, 183–195.
- Perkins, D.L. & Swetnam, T.W. (1996) A dendroecological assessment of whitebark pine in the Sawtooth-Salmon River region, Idaho. *Canadian Journal of Forest Research*, **26**, 2123–2133.
- Raffa, K.F. & Berryman, A.A. (1983) The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). *Ecological Monographs*, **53**, 27–49.
- Raffa, K.F. & Smalley, E.B. (1995) Interaction of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle-fungal complexes. *Oecologia*, **102**, 285–295.
- Raffa, K.F., Aukema, B.H., Erbilgin, N., Klepzig, K.D. & Wallin, K.F. (2005) Chapter four-interactions among conifer terpenoids and bark beetles across multiple levels of scale: an attempt to understand links between population patterns and physiological processes. *Recent Advances in Phytochemistry*, **39**, 79–118.
- Raffa, K.F., Aukema, B.H., Bentz, B.J., Carroll, A.L., Hicke, J.A., Turner, M.G. & Romme, W.H. (2008) Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. *Bioscience*, **58**, 501–517.
- Raffa, K.F., Powell, E.N. & Townsend, P.A. (2013) Temperature-driven range expansion of an irruptive insect heightened by weakly coevolved plant defense. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 2193–2198.
- Rasmussen, L.A. (1974) *Flight and Attack Behavior of Mountain Pine Beetles in Lodgepole Pine of Northern Utah and Southern Idaho*. INT-180. USDA Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah.
- Régnière, J. & Bentz, B. (2007) Modeling cold tolerance in the mountain pine beetle, *Dendroctonus ponderosae*. *Journal of Insect Physiology*, **53**, 559–572.
- Rhoades, D.F. & Cates, R.G. (1976) Toward a general theory of plant antiherbivore chemistry. *Biochemical Interaction between Plants and Insects, Recent Advances in Phytochemistry*, **10**, pp. 168–213. Plenum Press, New York, New York.
- Safranyik, L. & Carroll, A.L. (2006) The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. *The Mountain Pine Beetle: A Synthesis of Biology, Management and Impacts on Lodgepole Pine* (ed. by L. Safranyik and B. Wilson), pp. 3–66. Pacific Forestry Centre, Victoria, Canada.
- Safranyik, L., Linton, D.D., Silversides, R. & McMullen, L.H. (1992) Dispersal of released mountain pine beetle under the canopy of a mature lodgepole pine stand. *Journal of Applied Entomology*, **113**, 441–450.
- Safranyik, L., Carroll, A., Régnière, J. *et al.* (2010) Potential for range expansion of mountain pine beetle into the boreal forest of North America. *Canadian Entomologist*, **142**, 415–442.

- Schiebe, C., Hammerbacher, A., Birgersson, G. *et al.* (2012) Inducibility of chemical defenses in Norway spruce bark is correlated with unsuccessful mass attacks by the spruce bark beetle. *Oecologia*, **170**, 183–198.
- Smith, R.H. (1965) Effect of monoterpene vapors on the western pine beetle. *Journal of Economic Entomology*, **58**, 509–510.
- Smith, R.H. (2000) *Xylem Monoterpenes of Pines: Distribution, Variation, Genetics, Function*. PSW-GTR-177. US Department of Agriculture, Forest Service, Albany, California.
- Sturgeon, K.B. (1979) Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. *Evolution*, **33**, 803–814.
- Waring, K.M. & Six, D.L. (2005) Distribution of bark beetle attacks after whitebark pine restoration treatments: a case study. *Western Journal of Applied Forestry*, **20**, 110–116.
- Werner, R.A. (1995) Toxicity and repellency of 4-allylanisole and monoterpenes from white spruce and tamarack to the spruce beetle and eastern larch beetle (Coleoptera, Scolytidae). *Environmental Entomology*, **24**, 372–379.
- Wood, S.L. (1982) The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *The Great Basin Naturalist Memoirs*, **6**, 1–1359.
- Zavarin, E., Rafii, Z., Cool, L.G. & Snajberk, K. (1991) Geographic monoterpene variability of *Pinus albicaulis*. *Biochemical Systematics and Ecology*, **19**, 147–156.
- Zavarin, E., Cool, L.G. & Snajberk, K. (1993) Geographic variability of *Pinus flexilis* xylem monoterpenes. *Biochemical Systematics and Ecology*, **21**, 381–387.
- Zhao, T., Krokene, P., Hu, J. *et al.* (2011) Induced terpene accumulation in Norway spruce inhibits bark beetle colonization in a dose-dependent manner. *PLoS ONE*, **6**, e26649.

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