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Physical and chemical characteristics of blue and Engelmann spruce relative to spruce beetle host selection and colonization

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ABSTRACT

Bark beetles are keystone species that can alter the structure and function of forested ecosystems, yet the mechanisms underlying host selection and successful colonization remain poorly understood for most species. Comparison of closely related tree species that vary in their susceptibility to bark beetles could provide insights into such mechanisms. Here, we compare physical and chemical characteristics of blue (*Picea pungens* Engelm.) and Engelmann (*Picea engelmannii* Parry ex Engelm.) spruce, species rarely (blue) and frequently (Engelmann) selected and colonized by the spruce beetle (*Dendroctonus rufipennis* Kirby) in the western U.S. At three sites (Utah, U.S.) where these species co-occur, 15 trees of each species were selected and traits important for bark beetle survival and population dynamics were measured and compared (bark and phloem thickness, resin flow, phloem and volatile chemistry, beetle landing and colonization success). There were significant differences in bark and phloem thickness and resin flow between species. Bark was thicker and phloem was thinner in blue spruce than Engelmann spruce whereas resin flow was highly variable but greater in blue spruce. Concentrations of within-phloem terpenes in blue spruce were more than double those for Engelmann spruce. Engelmann spruce foliage emitted greater concentrations of volatiles than blue spruce. Spruce beetles landed at higher rates on baited Engelmann spruce than baited blue spruce, and Engelmann spruce was more likely to be colonized. Collectively, these results suggest that blue spruce is a less suitable host for spruce beetle than Engelmann spruce due to a combination of factors including: thicker bark, thinner phloem, higher resin flow, lower concentrations of volatile terpenes, and higher concentrations of constitutive terpenes in phloem tissue, several which are known to be toxic to spruce beetles.

1. Introduction

The spruce beetle [(*Dendroctonus rufipennis* (Kirby)] is the primary cause of spruce tree mortality in North America (Massey and Wygant, 1954; Schmid and Frye, 1977; Maroja et al., 2007). Since the 1990s, spruce beetles have killed millions of Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) across entire landscapes in the Rocky Mountains (Holsten et al., 1999; Ross et al., 2001; Maroja et al., 2007; Jenkins et al., 2014). However, spruce beetle colonization of blue spruce (*Picea pungens* Engelm.) in the Rocky Mountains has remained low (Jenkins et al., 2014). In the U.S., blue spruce is found primarily in Colorado and Utah, but its range also extends into parts of Idaho, Wyoming, Arizona, and New Mexico. The high elevational range of blue spruce overlaps the lower elevational range of Engelmann spruce in certain environments. Blue and Engelmann spruce are closely related species (Lockwood et al., 2013) that share many characteristics but differ in cone and needle morphology (Weng and Jackson, 2000) and habitat, with blue spruce more commonly found on mesic sites while Engelmann spruce is more commonly found on drier sites (Massey and Wygant, 1954). Hybridization has only been shown under laboratory conditions where Engelmann spruce is the female (Schaefer and Hanover, 1986; Ernst et al., 1990; Stine and Keathley, 1990; Ledig et al., 2006).

Climate change-induced warming and drying in blue spruce elevational zones and habitats will likely continue (Chmura et al., 2011; Stocker et al., 2013; Intergovernmental Panel on Climate, 2014;

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Anderegg et al., 2015). Therefore, blue spruce may experience increased stress as the climate changes. Rising temperatures are also likely to expand the geographic range of spruce beetle, and to increase outbreak population size in concert with an increase in voltinism (Price, 1997; Bentz et al., 2010; Hansen et al., 2011; Anderegg et al., 2015). Because tree condition and vigor have been shown to affect bark beetle colonization success (Moeck et al., 1981; Hebertson and Jenkins, 2008; Hart et al., 2013), these changes could result in increased spruce beetle pressure on blue spruce, further increasing spruce beetle range expansion and outbreak intensity and size (Bentz et al., 2010).

Tree defense, resistance, tolerance, and resilience to bark beetles have been studied extensively, including studies of spruce beetle in spruce-fir forests across North America. Some studies have linked changes in tree physiology, specifically tree response to drought, to bark beetle susceptibility (Hart et al., 2013; Gaylord et al., 2015; Kolb et al., 2016). Other studies have shown tree physical attributes and chemistry to influence bark beetle host landing (selection) and colonization (Massey and Wygant, 1954; Moeck et al., 1981; Raffa and Berryman, 1983; Byers, 1995; Wallin and Raffa, 1999, 2004; Safranyik and Carroll, 2006; Ott et al., 2011). In conifers, bark and phloem thickness (Amman, 1972), resin flow (Christiansen et al., 1999), and quantity and quality of phloem and volatile terpenes (Wallin and Raffa, 2004; Ott et al., 2011; Gray et al., 2015) have been shown to affect host selection and/or performance of bark beetles. While there are many factors that influence bark beetle host selection and colonization, mechanisms underlying low beetle colonization of blue spruce compared to Engelmann spruce remain unknown. Elucidating mechanisms of resistance to bark beetles is of fundamental interest and could help mitigate future spruce beetle impacts to forest resources.

The overall objective of this research was to identify tree characteristics that influence spruce beetle host selection and colonization of blue and Engelmann spruce. Specifically, the variables measured and compared were: (1) bark and phloem thickness; (2) resin flow following wounding; (3) the quantity and quality of the most abundant constitutive terpenes from volatile and phloem collections; and (4) beetle landing (selection) and colonization in response to synthetic pheromone baits.

2. Materials and methods

2.1. Study sites and design

This study was conducted at three sites on the Uinta-Wasatch-Cache National Forest on the Salt Lake and Heber-Kamas Ranger Districts in Utah, U.S.: Shingle Creek (40.61095°N, −111.11794°), Silver Fork (40.63474°, −111.61826°), and Lost Mill (40.93021°, −110.75278°). Blue and Engelmann spruces were present at all sites. Across the three sites, selected study trees were found at elevations from 2300 to 2750 m. Subalpine fir [*Abies lasiocarpa* (Hook.) Nutt.] was the only other tree species present at all sites. Aspen (*Populus tremuloides* Michx.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb,) Franco) were also present in the vicinity of study trees at one or two of the sites. Basal area (m^2/ha) was estimated in July 2015 based on 4 to 20 variable-radius plots (number based on the size of each study site) randomly distributed throughout each site using a Standard Unit 10 BAF prism (Table 1). All study trees were free of any obvious mechanical, insect or disease damage. Individual trees > 25 cm diameter at breast height (DBH, 1.37 m in height) were selected for this study because spruce beetle preferentially colonize larger trees (Schmid and Frye, 1977; DeRose and Long, 2012). Fifteen trees each of blue and Engelmann spruce were selected at each site (N = 90) and their DBH and height measured. All sample trees were located within 500 m of each other at Shingle Creek, 150 m at Silver Fork, and 1750 m at Lost Mill. Spruce beetles were colonizing trees in the vicinity of study trees at Shingle Creek and Lost Mill, but not at Silver Fork. Bark and phloem thickness, resin flow, volatile terpenes, and phloem terpenes were measured on each study tree prior to spruce beetle dispersal in the summers of 2014 or 2015. Finally, a subset of both species of spruce trees were baited in 2014 and 2015 to assess bark beetle landing (selection) and colonization at Lost Mill and Shingle Creek.

Table 1
Mean basal area (m^2/ha) of live and dead dominant tree species at three study sites in Utah, U.S., based on variable-radius plots (10 Standard Unit BAF), July 2015.

	Basal Area		
	Shingle Creek	Silver Fork	Lost Mill
# Variable Radius Plots	20	6	4
Live Engelmann Spruce	4.13	16.84	12.05
Dead Engelmann Spruce	9.41	0	1.72
Live Blue Spruce	2.87	8.42	7.46
Dead Blue Spruce	0	0	0
Live Subalpine Fir	4.48	4.97	5.74
Dead Subalpine Fir	2.18	6.89	3.44
Total Live Trees	21.69	31.76	28.12
Total Dead Trees	13.66	6.89	6.89

2.2. Bark and phloem thickness and resin flow

A 10-mm diameter bark punch and hammer were used to remove a piece of bark and phloem on the north and south sides of each tree at DBH in July 2014. Bark and phloem thickness were measured with a sliding-stage incremental micrometer on each bark section. The two measures of bark and phloem thickness were averaged for each tree. To collect resin flow from each of these bark punch sites, we first attached a metal funnel and pre-weighed 15-ml plastic centrifuge vial to the tree directly below the site of bark and phloem extraction. A piece of duct tape was attached above the bark punch site to keep rain out of the vial. The trees were then mechanically damaged using the 10-mm diameter bark punch by removing bark and phloem in a spiral pattern (Christiansen et al., 1999; Pears and Wallin, 2011). Trees 25–50 cm DBH received five bark punches, trees 50–75 cm DBH received 10 punches, and trees 75–100 cm DBH received 15 punches. Individual punches were 20–50 cm apart depending on DBH. Centrifuge vials were capped and taken to the laboratory after seven days. Vials were weighed, and empty vial weight was subtracted to calculate resin weight.

2.3. Phloem terpenes

The same phloem samples removed for bark and phloem thickness measurements were placed in individually labeled plastic vials that were stored on dry ice in a cooler for transport to the laboratory. Samples were stored at −20 °C at the USDA Forest Service Forest Health Protection Laboratory in Ogden, Utah until they were shipped to the USDA Forest Service Rocky Mountain Research Station Laboratory in Bozeman, Montana, U.S. and stored at −80 °C until they were chemically analyzed.

Terpene extractions were similar to Powell and Raffa, (2011). Samples were removed from individual vials and kept in liquid nitrogen until they were trimmed to ~5-mm cubes and then finely chopped with a razor blade to increase surface area for extraction. Approximately 50 mg of tissue was then placed into 2-ml FastPrep tubes (MP Biomedicals, Solon, Ohio, U.S.) with 1.5 ml of cyclohexane (Page et al., 2014) and sonicated at room temperature for 30 min and left to soak for 24 h at room temperature. Three hundred µl of extract solution was transferred to a gas chromatograph (GC) vial for analysis, and 1 µg of the internal standard *n*-nonyl-acetate was added.

Analyses were performed on an Agilent 7890A GC coupled with a 5975C mass spectrometer (MS) and separated on a HP-1 ms (30 m × 0.25 i.d. 0.25 µm film thickness) column. With helium as a

carrier gas, the GC started at 35 °C for three minutes and incrementally increased by 5 °C/min to 200 °C and then 25 °C/min to 250 °C. The quantities of terpenes were determined by comparison to the internal standard using ChemStation software (Agilent Technologies, Santa Clara, California, U.S.), and compound identification was confirmed by comparison of retention times and mass spectra of commercial standards or by the NIST 08 Mass Spectral Search Program (National Institute of Standards and Technology, Gaithersburg, Maryland, U.S.). When commercial standards were unavailable, compound names are given if match probability was > 50% using NIST Mass Spectral Search Program. Phloem samples were dried at 25 °C for one week, weighed, and terpenes expressed on a dry mass basis (μg/g or ng/g).

2.4. Volatile terpenes

In 2015, volatile terpenes were collected from one randomly-selected lower branch (random bearing), in full sun, on three trees per species at Shingle Creek and Lost Mill, and six trees per species at Silver Fork (Page et al., 2012; Gray et al., 2015; Giunta et al., 2016) for a total of 12 blue and 12 Engelmann spruce. On each tree, the distal ~70 cm of a branch was enclosed in a clear Teflon bag (50 cm wide ×75 cm deep; American Durafilm Co., Holliston, Massachusetts, U.S.) and air was drawn out through a volatile trap (Volatile Assay Systems, Rensselaer, New York, U.S.) containing 30 mg of the adsorbent material (HayeSep-Q, Restek, Bellefonte, Pennsylvania, U.S.) using a portable vacuum pump (Airlite Sampler Model 110–100, SKC Inc., Eighty Four, Pennsylvania). Vacuum pumps sampled air at 0.5 l min^{-1} for 30 min. All needles on each aerated branch were collected in plastic bags, brought to the laboratory, and weighed. Terpene analysis occurred at the USDA Forest Service Rocky Mountain Research Laboratory in Bozeman, Montana.

Following procedures in Page et al., (2012), 200 μl of dichloromethane were used to elute volatiles from traps and 1 μg of *n*-nonyl-acetate added as an internal standard. An Agilent 7890A GC/5975 MS was used to analyze samples with helium as the carrier gas. Using the same methodology as for phloem, the GC oven temperature program started at 35 °C for three minutes then increased 5 °C per minute to 125 °C, then 25 °C per minute to 250 °C. Volatiles were quantified and identified as described for phloem samples by internal standard comparison using ChemStation software (Agilent Technologies, Santa Clara, California). Rates of volatile emissions were reported based on fresh needle weight. Terpenes were identified using the NIST 08 Mass Spectral Search Program and confirmed by retention time comparison with mass spectra of commercial standards. Volatile terpene amounts are reported on a per gram fresh needle weight basis (ng/g/hr).

2.5. Spruce beetle landing and colonization

The two-component baits for spruce beetle consisted of frontalin and 1-methylcyclohex-2-en-1-ol, releasing at 3 mg/day and 1.25 mg/day at 25 °C, respectively (Product #3123, Synergy Semiochemicals Corp. Burnaby, British Columbia, Canada). During the flight period for spruce beetle, baits were attached to nine study trees of both species that had not been colonized at Lost Mill on June 28, 2014. Baits were attached to the bole of each selected tree at 2.5 m above the ground on the north side. Trees were randomly selected for baiting with the stipulation that a tree containing a bait had to be > 100 m from unbaited study trees to decrease potential "spill over" (i.e., colonization of nearby trees due to placement of baits) (Hansen et al., 2006). At the end of the beetle flight period in September, all study trees were evaluated for spruce beetle colonization by looking for entrance holes with/without boring dust and/or pitch (Schmid and Frye, 1977). During the middle two weeks of June 2015, baiting and assessment of colonization success were repeated at Lost Mill with the same stipulations as 2014. Due to spacing constraints and Engelmann spruce mortality in 2014 only four blue spruce and two Engelmann spruce were selected. Also, in mid-June 2015, baits were attached to eight blue spruce and seven Engelmann spruce trees at Shingle Creek. No baiting was conducted at Silver Fork due to its proximity to a ski resort and concerns from local stakeholders concerning tree mortality. In September 2015, all baited trees were evaluated for spruce beetle colonization as described above.

In mid-June of 2014 and 2015, prior to tree baiting, sticky traps were placed on study trees just above DBH (~1.37 m) to quantify beetle landing or selection. Baited trees, regardless of species, were considered selected when beetles were recovered from sticky traps. Sticky traps were constructed by applying 4 mm of insect adhesive on transparent plastic (216 × 279 mm). Two transparencies were stapled on the north and south sides of each tree. In 2014, Tanglefoot® (The Scotts Company, Maryville, Ohio, U.S.) pest barrier was used and trap catches were low. Therefore, in 2015, transparent plastic traps were coated using Stickum Pro® (TangleTrap, Contech Inc., Victoria, BC, Canada) since this product worked well in a previous study focused on mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (Ott et al., 2011). In September of each year, the presence or absence of spruce beetles was recorded on each sticky trap.

2.6. Statistical analyses

To investigate differences between blue and Engelmann spruce related to spruce beetle colonization, linear mixed-effects models were developed. Species (blue spruce, Engelmann spruce), site (Lost Mill, Shingle Creek, Silver Fork), and the interaction of species and site were used as fixed effects. Sample tree was nested in the models as a random effect. The models assume that random effects from tree and random errors are independent and normally distributed. Response variables were bark thickness, phloem thickness, resin flow, volatile terpene concentrations, and phloem terpene concentrations. Since ratios of compounds are known to be important in host recognition in many herbivore species (Bruce and Pickett, 2011), we compared ratios of some of the most abundant individual terpene compounds. Sites were far enough apart to assume they were independent (> 40 km). Assumptions of constant variance and normality were met using Pearson's standardized residual plots before interpretation of results.

Model adjustments for particular variables are described below: (1) For resin flow and all terpene analyses, the assumption that variances were constant within spruce species was relaxed by adding a weights argument to the models using the varIdent function in the nlme package in R (Pinheiro et al., 2017). This was necessary due to the high proportion of zeros for resin flow and the wide variance in terpene results. This process was used to select a more appropriate model rather than transform the data. (2) For bark thickness estimates of different factors, combinations were made using estimable from the gmodels package in R (Warnes et al., 2015). A 95% Bonferroni correction was used to control for the Type 1 error rate. (3) Chi-squared analyses were used to compare the binary variables of landing, pheromone bait, and colonization between spruce species. Probabilities were used from Fisher's exact test due to its more robust and conservative test for low sample sizes. All statistical analyses were performed using R version 3.5.1 statistical software (R Core Team, 2018).

3. Results

Mean DBH and height were 56.6 cm (SD 15.4) and 23.6 m (SD 3.4) for blue spruce, and 58.4 cm (SD 13.8) and 26.0 m (SD 4.3) for Engelmann spruce, respectively. There were no significant differences in DBH or height between species ($P = 0.42$).

3.1. Bark and phloem thickness and resin flow

Blue spruce bark (mean ± SE, 13.6 ± 0.63 mm) was significantly thicker than Engelmann spruce bark (6.11 ± 0.32 mm), and there was

Table 2
Marginal *F* test for bark thickness (mm), phloem thickness (mm), and resin amount (g) for blue spruce (*Picea pungens*) and Engelmann spruce (*P. engelmannii*) at three study sites in Utah, U.S. Statistically significant results are in bold.

		Variables			
		numDF	denDF	*F*-value	*p*-value
Bark Thickness	Intercept	1	82	867.71	< 0.0001
	Species	1	82	126.53	< **0.0001**
	Site	2	82	1.19	0.31
	Species: Site	2	82	5.46	< **0.01**
Phloem Thickness	Intercept	1	58	1469.20	< 0.0001
	Species	1	58	42.37	< **0.0001**
	Site	1	58	0.72	0.40
	Species: Site	1	58	0.46	0.50
Resin	Intercept	1	82	4.36	< 0.05
	Species	1	82	3.50	0.07
	Site	2	82	1.11	0.33
	Species: Site	2	82	1.61	0.21

a significant species by site interaction (Table 2). At all sites, confidence intervals did not cross zero and had significant *P*-values, indicating that blue spruce had thicker bark than Engelmann spruce (Fig. 1).

Blue spruce phloem (2.97 ± 0.12 mm) was significantly thinner than Engelmann spruce phloem (4.19 ± 0.13 mm), and there were no significant effects of site or species by site interaction (Table 2). Resin flow tended to be greater in blue spruce than Engelmann spruce ($P = 0.07$). Mean blue spruce resin flow was 0.37 ± 0.16 g (n = 43) and Engelmann spruce was 0.07 ± 0.05 g (n = 45). The smaller sample size for blue spruce was the result of two vials being knocked off the trees apparently by wildlife. Each species had 30 trees with no resin flow. There was no significant effect of site or the interaction of species and site on resin flow (Table 2).

3.2. Terpenes in phloem

A total of 22 compounds were identified in the phloem of both blue and Engelmann spruce using GC–MS analysis (Table S1). Compounds were composed entirely of terpenoids, mostly monoterpenoids (18 compounds) but also sesquiterpenoids (4 compounds). The most abundant compound in both species was α-pinene followed by 3-carene, β-pinene, β-phellandrene, myrcene, limonene, sabinene, and terpinolene. Blue spruce phloem (3,040,762 ± 1,518,941 ng/g) contained more than twice the concentration of terpenes than Engelmann spruce phloem (1,460,454 ± 708,810 ng/g) ($P < 0.0001$). Among individual compounds, α-pinene, sabinene, myrcene, β-phellandrene, limonene, terpinolene, and linalool varied significantly by species. All of the terpenes were present in greater quantities in blue spruce compared to Engelmann spruce except for β-phellandrene and myrcene, which were greater in Engelmann spruce (Table 3 & Fig. 2A). Concentrations of 3-carene also tended to be greater in blue spruce than Engelmann spruce ($P = 0.09$). Concentrations of sabinene, myrcene, β-phellandrene, limonene, terpinolene, and α-phellandrene varied significantly by site (Table 3). There was a species by site interaction for some compounds (α-pinene, sabinene, and limonene).

There were also differences in the ratios of some major compounds in the phloem of blue and Engelmann spruce. Of note, ratios of β-phellandrene: limonene, α-pinene: 3-carene, and myrcene: terpinolene varied significantly by spruce species (Fig. 3A). The ratio of α-pinene: 3-carene was higher in blue spruce while the ratios of β-phellandrene: limonene and myrcene: terpinolene were higher in Engelmann spruce. The only ratio that did not vary by species, 3-carene: limonene, varied significantly by site. Only β-phellandrene: limonene varied significantly by the interaction of species and site ($F_{2,74} = 3.97$, $P < 0.05$).

3.3. Volatile compounds emitted from foliage

A total of 41 volatile compounds were identified from the headspace of foliage in blue and Engelmann spruce using GC–MS analysis (Table S1). Compounds were composed of mostly monoterpenoids (33 compounds) but also sesquiterpenoids (7 compounds), and benzenoids (2 compounds). Volatiles emitted by foliage of blue and Engelmann spruce were qualitatively similar, both contained the same 41 compounds. Engelmann spruce foliage (507 ± 539 ng/g/h) tended to emit almost twice the total amount of volatiles than blue spruce (262 ± 289 ng/g/h; $P = 0.09$). Emissions of β-pinene and α-pinene tended to be greater in Engelmann spruce than blue spruce (Fig. 3B; $P < 0.07$). No other significant differences in the eight volatile terpenes examined were observed. For selected terpene ratios, no significant differences were observed by species, site, or the interaction of species and site.

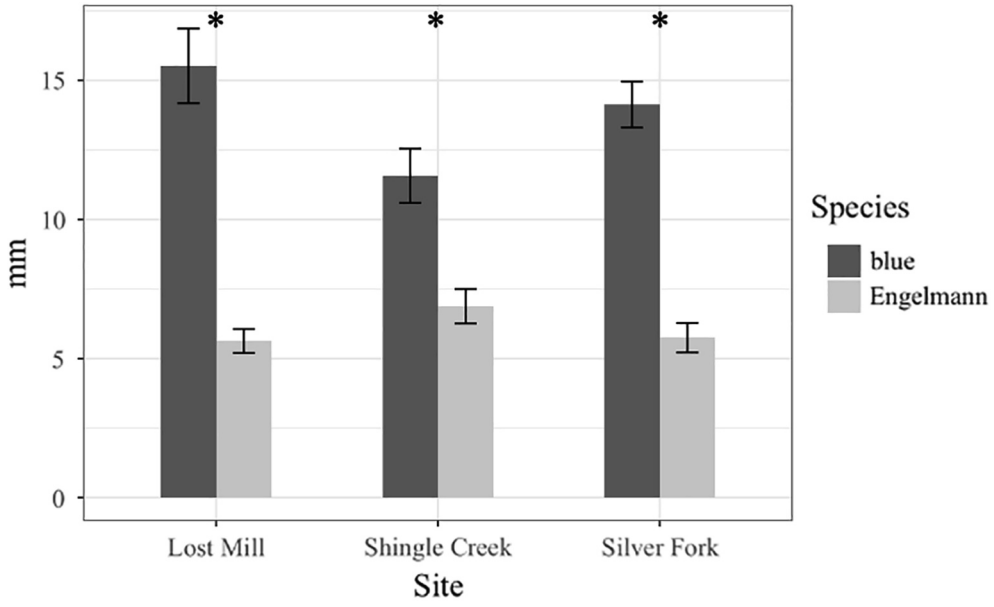

Fig 1. Mean bark thickness of blue spruce (*Picea pungens*) and Engelmann spruce (*P. engelmannii*) at three study sites in Utah, U.S. Error bars (± SE). * denotes significant differences between species within sites ($P < 0.05$).

Table 3
Marginal *F* Test for volatile and phloem-based terpenes and ratios of specific terpenes by species, site, and the interaction of species by site for blue spruce (*Picea pungens*) and Engelmann spruce (*P. engelmannii*) at three study sites in Utah, U.S. Phloem terpene compounds are the same as volatile compounds from the foliage unless indicated otherwise. Statistically significant results are in bold.

		Volatile Terpenes					Phloem Terpenes			
		numDF	denDF	*F*-value	*p*-value		numDF	denDF	*F*-value	*p*-value
α-Pinene	Intercept	1	18	23.30	< 0.0001		1	74	204.96	< 0.0001
	Species	1	18	3.59	0.07		1	74	48.21	< **0.0001**
	Site	2	18	6.45	< **0.01**		2	74	2.57	0.08
	Species:Site	2	18	2.48	0.11		2	74	3.98	< **0.05**
Sabinene	Intercept	1	18	175.62	< 0.0001		1	74	60.76	< 0.0001
	Species	1	18	1.34	0.26		1	74	21.71	< **0.0001**
	Site	2	18	17.82	< **0.0001**		2	74	4.87	< **0.01**
	Species:Site	2	18	0.49	0.62		2	74	3.30	< **0.05**
β-Pinene	Intercept	1	18	17.79	< 0.0001		1	74	88.58	< 0.0001
	Species	1	18	4.40	< **0.05**		1	74	2.56	0.11
	Site	2	18	6.18	< **0.001**		2	74	0.59	0.56
	Species:Site	2	18	1.24	0.31		2	74	2.29	0.11
Myrcene	Intercept	1	18	12.51	< 0.001		1	74	117.20	< 0.0001
	Species	1	18	1.96	0.18		1	74	3.80	< **0.05**
	Site	2	18	2.19	0.14		2	74	4.88	< **0.01**
	Species:Site	2	18	0.13	0.88		2	74	0.64	0.53
3-Carene	Intercept	1	18	17.20	< 0.001		1	74	49.90	< 0.0001
	Species	1	18	0.03	0.86		1	74	2.89	0.09
	Site	2	18	4.67	< **0.05**		2	74	0.89	0.42
	Species:Site	2	18	0.45	0.64		2	74	0.90	0.41
β-Phellandrene	Intercept	1	18	28.53	< 0.0001		1	74	283.08	< 0.0001
	Species	1	18	1.39	0.25		1	74	16.35	< **0.001**
	Site	2	18	1.87	0.18		2	74	3.78	< **0.05**
	Species:Site	2	18	0.13	0.88		2	74	1.87	0.16
Limonene	Intercept	1	18	16.77	< 0.001		1	74	143.43	< 0.0001
	Species	1	18	0.10	0.76		1	74	26.31	< **0.0001**
	Site	2	18	5.31	< **0.05**		2	74	6.18	< **0.01**
	Species:Site	2	18	0.42	0.66		2	74	3.30	< **0.05**
Terpinolene	Intercept	1	18	39.37	< 0.0001		1	74	81.24	< 0.0001
	Species	1	18	1.64	0.22		1	74	17.77	< **0.0001**
	Site	2	18	11.36	< **0.001**		2	74	3.08	< **0.05**
	Species:Site	2	18	0.12	0.88		2	74	1.77	0.18
Total Terpenoids	Intercept	1	18	29.19	< 0.0001		1	74	330.86	< 0.0001
	Species	1	18	3.12	0.09		1	74	42.47	< **0.0001**
	Site	2	18	6.64	< **0.01**		2	74	5.08	< **0.01**
	Species:Site	2	18	1.67	0.22		2	74	2.14	0.12
Verbenone	Intercept	1	18	36.53	< 0.0001	Linalool	1	74	169.65	< 0.0001
	Species	1	18	1.86	0.19		1	74	5.41	< **0.05**
	Site	2	18	0.11	0.90		2	74	3.07	0.05
	Species:Site	2	18	1.08	0.36		2	74	0.81	0.45
β-Phellandrene/Limonene	Intercept	1	18	13.32	< 0.01		1	74	66.43	< 0.0001
	Species	1	18	0.95	0.34		1	74	27.18	< **0.0001**
	Site	2	18	2.08	0.15		2	74	1.77	0.18
	Species:Site	2	18	0.45	0.65		2	74	3.97	< **0.05**
3-Carene/Limonene	Intercept	1	18	17.63	< 0.001		1	74	60.91	< 0.0001
	Species	1	18	0.91	0.35		1	74	1.77	0.19
	Site	2	18	1.44	0.26		2	74	4.45	< **0.05**
	Species:Site	2	18	0.45	0.64		2	74	2.37	0.10
α-Pinene/3-Carene	Intercept	1	18	21.44	< 0.001		1	74	42.93	< 0.0001
	Species	1	18	1.83	0.19		1	74	9.92	< **0.01**
	Site	2	18	0.81	0.46		2	74	0.54	0.59
	Species:Site	2	18	2.41	0.12		2	74	2.78	0.07
Myrcene/Terpinolene	Intercept	1	18	49.37	< 0.0001		1	74	43.88	< 0.0001
	Species	1	18	0.60	0.45		1	74	9.10	< **0.01**
	Site	2	18	1.46	0.26		2	74	0.67	0.52
	Species:Site	2	18	0.85	0.44		2	74	0.26	0.77

However, our data suggest there are perhaps fundamental differences in ratios of some compounds between tree species (e.g., those with limonene). All volatile terpenes, except for myrcene and β-phellandrene, varied significantly by site (Table 3). There was no interaction of species and site for any compound.

3.4. Spruce beetle landing and colonization

In 2014 at the Lost Mill site, spruce beetles selected and colonized baited Engelmann spruce at higher frequencies than baited blue spruce (Table 4). Eight of the nine baited Engelmann spruce trees had spruce beetles select and colonize them. Only two of nine baited blue spruce trees were selected (i.e., spruce beetle were recovered from sticky traps), and only one of those trees was colonized. A single entrance hole was observed on this blue spruce, but colonization was unsuccessful due to resin flow that encapsulated and killed the beetle. In 2015, all baited Engelmann spruce at both sites were successfully colonized based on the presence of boring dust in bark crevices and at the root collar. In 2015, two of the four baited blue spruce trees at Lost Mill were selected and colonized. At Shingle Creek, none of the baited blue spruce

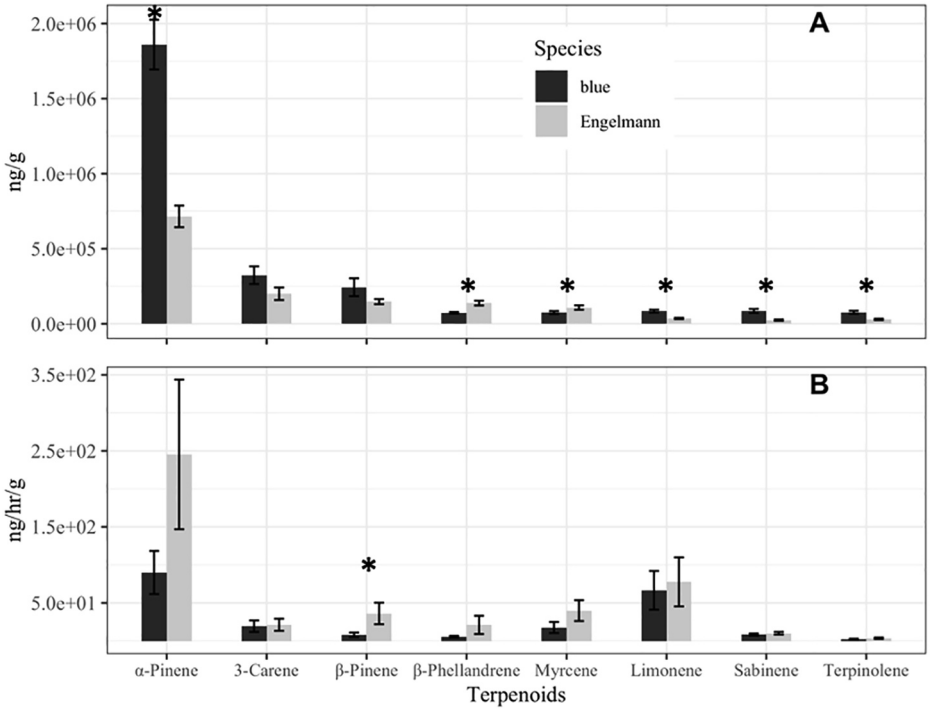

Fig 2. Means of the most abundant phloem and volatile (foliage) terpenes in blue spruce (*Picea pungens*) and Engelmann spruce (*P. engelmannii*) at three study sites in Utah, U.S. A. Phloem terpenes (n = 41 blue spruce, n = 39 Engelmann spruce). B. Volatile terpenes (n = 12, both species). Error bars (± SE). * denotes significant differences between species ($P < 0.05$).

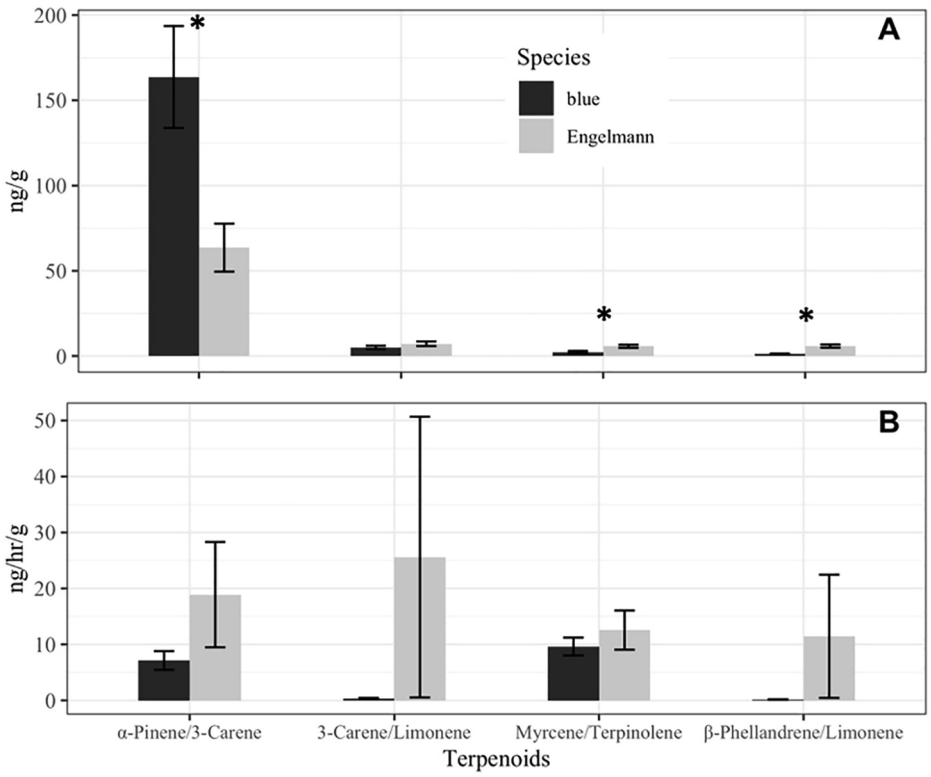

Fig 3. Selected ratios of mean amounts of some major phloem and volatile (foliage) terpenes in blue spruce (*Picea pungens*) and Engelmann spruce (*P. engelmannii*) spruce at three study sites in Utah, U.S. A. Ratios of phloem terpenes (n = 41 blue, n = 39 Engelmann). B. Ratios of volatile terpenes (n = 12). Error bars (± SE). * denotes significant differences between species ($P < 0.05$).

were selected or colonized.

4. Discussion

In this study, several tree traits related to host selection and colonization success of spruce beetle were compared in blue and Engelmann spruce. In areas where blue and Engelmann spruce are sympatric, Engelmann spruce experiences much higher rates of tree mortality attributed to spruce beetle than blue spruce (Schmid and Frye, 1977). Factors such as bark and phloem thickness may serve as a physical barrier to colonizing spruce beetle adults or available food resources for developing spruce beetle larvae. Tree characteristics such as resin flow and constitutive terpene concentration are important indicators of tree defensive capacity while volatile terpenes may play a role in primary host selection and synergizing aggregation pheromones during secondary host selection.

Table 4
Species frequency tests (chi squared, Fisher's exact) of binary variables for baited blue spruce (*Picea pungens*) and Engelmann spruce (*P. engelmannii*) in Utah, U.S. in 2014 and 2015 (i.e., the likelihood if a bait was placed on a tree then it was landed upon by spruce beetle (selected)). Site specific data is also included (Lost Mill and Shingle Creek). Overall selection and colonization in 2014 includes Shingle Creek where spruce beetle was active. No statistical test could be completed for blue spruce at Shingle Creek in 2015 because there was no variation (represented by "."). Statistically significant results are in bold.

Interaction + Year	Blue				Engelmann			
Variable A × B	*N*	χ^2	Fisher's $P > \chi^2$	Bait #	*N*	χ^2	Fisher's $P > \chi^2$	Bait #
Lost Mill Bait × Selection 2014	16	1.78	0.3	9	18	14.4	< **0.001**	9
Lost Mill Bait × Colonization 2014	16	0.83	0.56	9	18	14.4	< **0.001**	9
Lost Mill Selection × Colonization 2014	16	7.47	0.13	9	18	18	< **0.0001**	9
Bait × Selection 2014	32	5.45	0.07	9	34	29.06	< **0.0001**	9
Bait × Colonization 2014	32	2.64	0.28	9	34	29.06	< **0.0001**	9
Selection × Colonization 2014	32	15.48	0.06	9	34	34	< **0.0001**	9
Lost Mill Bait × Selection 2015	16	6.86	**0.05**	4	18	0.45	0.69	2
Lost Mill Bait × Colonization 2015	16	6.86	**0.05**	4	18	0.45	0.69	2
Lost Mill Selection × Colonization 2015	16	16	< **0.001**	4	18	18	< **0.0001**	2
Shingle Creek Bait × Selection 2015	16	.	.	8	16	12.44	< **0.001**	7
Shingle Creek Bait × Colonization 2015	16	.	.	8	16	12.44	< **0.001**	7
Shingle Creek Selection × Colonization 2015	16	.	.	8	16	16	< **0.0001**	7
Bait × Selection 2015	32	3.56	0.13	12	34	5.85	< **0.05**	9
Bait × Colonization 2015	32	3.56	0.13	12	34	5.85	< **0.05**	9
Selection × Colonization 2015	32	32	< **0.001**	12	34	34	< **0.0001**	9

4.1. Bark and phloem thickness and resin flow

Blue spruce bark was thicker than Engelmann spruce bark (Fig. 1), meaning adult spruce beetles must tunnel through more bark (a physical barrier) when attempting to colonize blue spruce thus using more energy to reach the phloem where gallery formation, mating and egg laying occurs (Safranyik and Carroll, 2006; Graf et al., 2012; Raffa et al., 2015). Blue spruce also has thinner phloem than Engelmann spruce, which limits the amount of food available for growth and development of spruce beetle larvae (Cole and Amman, 1969; Amman, 1972). Further, blue spruce has less available phloem substrate for spruce beetle symbiotic fungus [*Leptographium abietinum* [(Peck) M.J. Wingf.] to propagate in while concentrating nitrogen, phosphorus and protein near spruce beetle galleries as a food source (Ayres et al., 2000; Davis et al., 2019). Spruce beetle symbiotic fungi have also been shown to decrease spruce beetle antagonistic microbes and concentrations of toxic terpenes like 3-carene (Davis et al., 2019).

Numerous studies have shown that preformed resin is an important conifer defense against bark beetles (Raffa and Berryman, 1982, 1983; Wainhouse et al., 1990; Ruel et al., 1998; Ayres and Lombardero, 2000; Lombardero et al., 2000; Wallin and Raffa, 2001; Safranyik and Carroll, 2006). In our study, the difference in resin flow between blue and Engelmann spruces was marginally significant (Table 1) where blue spruce had higher resin flow following wounding. Many resin flow studies have been conducted in loblolly pine (*Pinus taeda* L.), Norway spruce [*Picea abies* (L.) H. Karst.], and lodgepole pine (Cook and Hain, 1986; Ruel et al., 1998; Lombardero et al., 2000; Rocchini et al., 2000; Roberds et al., 2003) and most report higher mean resin flow rates than observed in our study for either blue or Engelmann spruce. The absence of resin flow on many blue and Engelmann spruce in our study influenced our results. However, some blue spruce in our study had resin flows triple the means reported for loblolly pine in approximately half the time (Klepzig et al., 2005). Whether resin flow in blue and Engelmann spruce is inherently more variable than other conifer species, and identification of factors influencing this variation, warrants further study.

4.2. Phloem terpenes

Blue and Engelmann spruce phloem were qualitatively similar, but phloem of Engelmann spruce contained more than twice the concentration of terpenoids. Terpenes in phloem are well-known to function as a defense against bark beetles and can be directly toxic to beetles (Chiu et al., 2017), interfere with insect digestion, and inhibit germination and growth of beetle-associated fungi (Klepzig et al., 1996; Klepzig and Six, 2004; Davis et al., 2018). Moreover, recent work suggests that the high levels of constitutive terpenes in the phloem of Great Basin bristlecone pine (*Pinus longaeva* Bailey) and foxtail pine (*P. balfouriana* Grev. and Balf.) could explain poor performance and avoidance of these species by mountain pine beetle (Bentz et al., 2017; Eidson et al., 2018). The more abundant compounds in phloem, including α-pinene, limonene, and 3-carene, were all present in greater amounts in blue spruce than in Engelmann spruce (Fig. 2A). In particular, limonene and 3-carene are known to be highly toxic at high concentrations to phloem feeding insects, including spruce beetle (Smith, 1965; Raffa and Smalley, 1995; Werner, 1995; Lindgren et al., 1996; Rocchini et al., 2000; Ott et al., 2011). Davis et al., (2018) found that just the presence of linalool and low concentrations of terpinolene suppressed the growth of spruce beetle associated fungi *Leptographium abietinum*. In our study, linalool levels were significantly greater in Engelmann spruce while terpinolene levels were significantly higher in blue spruce. Some phloem terpenes are potentially beneficial to spruce beetle: myrcene and terpinolene are synergistic with bark beetle aggregation pheromones that regulate colonization (Borden, 1982; Raffa and Berryman, 1983; Safranyik and Carroll, 2006) and myrcene concentrations were greater in Engelmann spruce. α-Pinene was more abundant in blue spruce than Engelmann spruce and has been shown to be important in the production of both aggregation pheromones (Borden, 1982; Seybold et al., 1995; Wallin and Raffa, 2001), and anti-aggregation pheromones (Pureswaran and Borden, 2005) for mountain pine beetle. Byers and Birgersson (1990), found that many species of phloem feeding insects use myrcene and α-pinene for pheromone production. The specific precursors for spruce beetle pheromones are not known. In combination, these results suggest that differences in concentrations of constitutive phloem terpenes could, at least in part, explain differences in selection and colonization of blue and Engelmann spruce by spruce beetle.

4.3. Volatiles

As with phloem terpenes, compounds emitted by foliage of blue and Engelmann spruce were qualitatively similar, but total volatile emissions by foliage of Engelmann spruce tended to be greater than blue spruce. While the greater amount of odor emanating from Engelmann spruce might render this species more attractive to spruce beetles, it is the relative proportion of compounds in volatile blends emitted by

plants that is usually most important in host recognition by insect herbivores (Bruce and Pickett, 2011). Since blue and Engelmann spruce emitted the same compounds, if olfaction is used in host selection by spruce beetles, then they must rely on species specific differences in the ratios of compounds in the odor blends. Gray et al., (2015) showed that pioneering mountain pine beetles were repelled by foliage volatiles of non-host Great Basin bristlecone pine, but strongly attracted to volatiles from a preferred host, limber pine. Both species emitted the same volatile compounds and evidence suggests that multiple compounds were involved in mountain pine beetle avoidance and attraction (Gray et al., 2015). Indeed, the ratios of blue and Engelmann spruce foliage volatiles appears to differ substantially for some major compounds (Fig. 3B), and also for some less abundant compounds (Table S1), providing a plausible mechanism allowing host choice by foraging spruce beetles. It is possible that volatiles themselves are not toxic, but can be used as cues by beetles to locate suitable hosts (e.g., Engelmann spruce) and avoid less suitable hosts (e.g., blue spruce).

Still, differences in several individual volatile compounds might affect spruce beetle behavior. For example, the rate of α-pinene emission from Engelmann spruce was approximately double that of blue spruce. Dyer and Chapman (1971) suggested that α-pinene (and frontalin) played a role in spruce beetle attraction and commercially available lures for spruce beetles typically include α-pinene (Seybold et al., 2018). Moreover, Wallin and Raffa, (2004) found that high levels of the most abundant terpene, α-pinene, on a host medium (i.e., consisting of spruce phloem, agar, and water) repelled spruce beetle while intermediate levels were attractive to spruce beetle. It is possible that levels of α-pinene emitted by Engelmann spruce fall in this attractive range, whereas lower levels emitted from blue spruce do not reach the threshold of attraction. There were no significant differences between tree species in emission of other terpene compounds thought to affect spruce beetle attraction.

4.4. Spruce beetle landing and colonization

Even with baits present, selection and colonization of blue spruce was rare, and of the two blue spruce colonized (indicated by a pitch tube and frass) only one died (Table 4). This is consistent with previous observations for blue spruce in which levels of tree mortality attributed to spruce beetle were reported to be low (Massey and Wygant, 1954; Schmid and Frye, 1977; Colorado State Forest, 2017). Due to a spruce beetle outbreak, most Engelmann spruce at Lost Mill and Shingle Creek were successfully colonized and killed by spruce beetle by the end of the study, regardless of whether or not they were baited.

4.5. Summary

The overall objective of this study was to identify tree characteristics that might explain differences in selection and colonization of blue and Engelmann spruces by spruce beetle. Blue spruce phloem had greater concentrations of defensive terpenes than Engelmann spruce, suggesting beetles might avoid this species because it is relatively well defended. Volatile terpenoids emanating from foliage of blue and Engelmann spruce were consistently quantitatively (but not qualitatively) different, suggesting that spruce beetle might be preferentially attracted to Engelmann spruce by the higher concentrations of volatiles. Laboratory studies are needed to determine if the phloem of blue spruce is indeed toxic to spruce beetle or reduces offspring survival, and laboratory and field studies are needed to determine how volatiles from each spruce species affect behavior of host-searching spruce beetles. Several additional characteristics were identified that may help explain higher levels of colonization in Engelmann spruce compared to blue spruce. These include Engelmann spruce having thinner bark and thicker phloem. Future research should focus on differences in bark and phloem nitrogen (for host suitability), beetle vision and foliage reflectance (for host selection), and other physical characteristics such as antifeedant structures (e.g., calcium oxalate crystals, stone cells, phenolics and lignin). Other gaps in research related to spruce beetle host defenses include speed and extent of induced resin duct formation and phloem terpenes, which can determine beetle success (Lombardero et al., 2000; Martin et al., 2002; Faldt et al., 2003).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2020.118577.

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