



Ethanol and primary attraction of red turpentine beetle in fire stressed ponderosa pine



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ABSTRACT

Trees injured and weakened by wild or prescribed fires are susceptible to bark beetle attacks. Nonaggressive *Dendroctonus valens* in North American forests is often the first bark beetle to colonize fire injured pine. Although not an important contributor to post-fire mortality it does vector pathogens, and surviving diseased trees may serve as pathogen redistribution centers. Traps baited with both ethanol and monoterpene lures are known to attract *D. valens*, and fire injured pine are known to accumulate ethanol in tissues containing monoterpenes. The primary objective of this study was to quantify ethanol concentrations in fire injured ponderosa pine tissue near pioneering *D. valens* gallery entrances and compare them with levels from the unattacked side of the same tree, or their unattacked neighbors. The secondary objective was to quantify α -pinene in the same samples. Two separate but related studies were conducted, one in a June wildfire, the other in an October prescribed fire, near Bend, Oregon, USA where phloem and sapwood cores were collected for analysis by headspace gas chromatography. Wildfire damaged trees attacked by *D. valens* were sampled above beetle gallery entrance holes and on the opposite bole side without an entrance hole. An adjacent unattacked tree of similar size and injury was sampled at the same aspect and height. Prescribed fire damaged trees were sampled in groups of three characterized by: (1) one or more *D. valens* attacks with 100% crown scorch; (2) unattacked with 100% crown scorch; and (3) unattacked with $\leq 95\%$ crown scorch. They were sampled above a beetle gallery entrance or equivalent positions on unattacked trees. Ethanol and α -pinene concentrations at both fires were higher in tissues above beetle entrance holes than in corresponding tissues from unattacked sides of the same tree, or adjacent trees. Ethanol concentrations in wildfire damaged trees were three or more orders of magnitude greater than in prescribed fire trees, likely a consequence of wildfire trees experiencing higher temperatures and greater heat stress indicated by injury measurements. Ethanol concentrations in stem bark char, analyzed only in the prescribed fire were two to four orders of magnitude greater than in the underlying phloem, a result of it being adsorbed and concentrated during outward diffusion. Ethanol synthesis, accumulation, and atmospheric release in combination with host monoterpenes, is proposed as the critical physiological process contributing to initial *D. valens* host tree and bole position selection of fire stressed ponderosa pine.

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1. Introduction

Wildfire has been a key disturbance agent in western US forests for millennia with a slight long-term decline in burning until the late 1800s when a more abrupt decline led to a “fire deficit” (Marlon et al., 2012). In the mid-1980s, in response to rising temperatures and drought enhancing fuel aridity there has been an increase in numbers of large wildfires, their severity, and annual area burned (Abatzoglou and Williams, 2016; Dennison et al.,

2014; Littell et al., 2009, 2016; Peterson and Marcinkowski, 2014). Temperatures, drought, and annual area burned are all projected to increase in western US ecoregions by mid-21st century (Miniat and Peterson, 2014; Peterson and Littell, 2014). Since the mid- to late 1800s western forest ecosystems have undergone structural, compositional, and functional changes with undesirable ecological and economic consequences in response to various forest management practices, including fire exclusion, as illustrated for Inland Pacific forests (Hessburg et al., 2015). Prescribed fire is a treatment tool (Tappeiner et al., 2015) used by managers to help reset conditions in fire-prone forests, reduce fuels to mitigate wildfire severity, and improve ecosystem resilience to insects, pathogens, and drought.

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Regardless of fire type, severely burned trees will die from their injuries, while many others with lesser damage survive, but are left weakened and more susceptible to other stress agents, particularly bark beetles (Breece et al., 2008; Davis et al., 2012; Fettig and McKelvey, 2014; Parker et al., 2006). Red turpentine beetle, *Dendroctonus valens* LeConte, is frequently one of the first bark beetles to attack fire injured pines (Bradley and Tueller, 2001; Fettig and McKelvey, 2014; Fettig et al., 2008, 2010; Hood et al., 2007; McHugh et al., 2003; Perrakis and Agee, 2006; Schwilke et al., 2006; Six and Skov, 2009; Youngblood et al., 2009), sometimes within 24 h (Ganz et al., 2003). Initial attacks by pioneering beetles are made by females that bore into the inner bark and produce a dual function aggregation, sex pheromone (Liu et al., 2013; Owen et al., 2010). But, unlike more aggressive beetle species, its presence does not contribute substantially to post-fire mortality (Fettig and McKelvey, 2014; Fettig et al., 2008; Owen et al., 2010). Other woody materials colonized by *D. valens* in North America include diseased trees or otherwise weakened, dying, or recently dead trees, plus stumps and fresh logs (Owen et al., 2010). Offspring emerging from diseased hosts can vector pathogen spores (Lu et al., 2009, 2010; Owen et al., 1987; Schweigkofler et al., 2005; Taerum et al., 2013), spreading disease among surviving fire injured trees, or other susceptible hosts in the surrounding unburned forest. Management of fire effects can benefit from a more thorough understanding of the physiological changes in fire injured trees, and improved insight of how fire treatments change the vulnerability of surviving trees to bark beetle attacks and vectored pathogens.

Host tree oleoresin, or individual monoterpenes, function as *D. valens* primary attractants to baited traps, but (+)-3-carene lures typically attract more beetles than (–)-β-pinene, (+) or (–)-α-pinene, or 1:1:1 (+)-α-pinene:(–)-β-pinene:(+)-3-carene mixed lures when tested across North America and in China (Erbilgin et al., 2007; Sun et al., 2004; Vité and Gara, 1962). But (–)-β-pinene did attract more beetles than (+)-3-carene, or a mixture of α-pinene enantiomers in a California study (Hobson et al., 1993). This beetle's preference for (+)-3-carene lures aligns with many North American pine species producing it as a major component in their xylem resin (Smith, 2000). However, host *P. tabularis* trees in China contain α-pinene as the major component with minimal amounts of (+)-3-carene, and in laboratory choice tests *D. valens* was attracted to its oleoresin, or mimic synthetic blends (Liu et al., 2011; Xu et al., 2014). These responses illustrate a broad plasticity in host monoterpene attraction for *D. valens*.

Ethanol is synthesized and accumulates in tree tissues stressed by mechanical injury to the crowns (Sjödin et al., 1989), pathogens (Kelsey et al., 2013), severe water deficits (Kelsey et al., 2014), and in stumps and logs after severing the stem (Kelsey, 1994a, 1994b; Kelsey and Joseph, 1999; von Sydow and Birgersson, 1997). Many of these woody materials function as hosts for *D. valens* (Owen et al., 2010). Ethanol baited traps typically capture low numbers of *D. valens*, but when ethanol lures are combined with host oleoresin or monoterpenes lures the number of responding beetles increase (Gandhi et al., 2010; Vité and Gara, 1962). Changing trap lures from turpentine to 1:1 turpentine:ethanol raised the captured beetle numbers by 60 times (Klepzig et al., 1991). Traps using ethanol lures paired with the mixed 1:1:1 (+)-α-pinene:(–)-β-pinene:(+)-3-carene lure caught 1.2 times more *D. valens* than traps with just the monoterpene mixture, but it was not statistically greater (Fettig et al., 2004). Ethanol release rates influence *D. valens*, with greater numbers attracted to high release lures than those with low rates, when each was paired with the same monoterpene lure (Joseph et al., 2001). But their response to different ethanol release rates may be mitigated by the monoterpenes chiral form. More beetles were attracted to traps with (–)-α-pinene:high release ethanol (1:5) lures than (–)-α-pinene:low

release ethanol (1:1) lures, whereas the opposite response was observed for the same comparison using (+)-α-pinene lures (Erbilgin et al., 2001).

Ethanol concentrations in ponderosa pine, *Pinus ponderosa* Douglas ex P. Lawson & C. Lawson, stem tissues injured by a fall wildfire were higher than in corresponding tissues from undamaged trees, and the quantities increased with severity of injury to the bole and crown (Kelsey and Joseph, 2003). The following year *D. valens* landed in greater abundance on burned trees than unburned controls, with a weak relationship between numbers landing per tree and sapwood ethanol concentrations the previous September. The probability of *D. valens* attacks on wildfire injured ponderosa pine in the Intermountain Region can be estimated with logistic regression models using bole or crown scorch height (Negrón et al., 2016). This beetle's preference for stressed hosts known to produce ethanol and their attraction to traps with ethanol and host monoterpene lures together strongly suggest that elevated ethanol concentrations in fire-injured trees may function in their primary attraction and initial host selection of burned trees.

The primary objective for this study was to select fire stressed trees attacked by pioneering *D. valens* and analyze ethanol concentrations in tissues above a gallery entrance hole and compare them with concentrations in tissues from the unattacked side of the same tree, and with tissues from an adjacent, unattacked tree. The secondary objective was to quantify α-pinene concentrations in these same samples. Trees were sampled in two fires, one was a late spring wildfire where intense heat caused severe tree injury and stress, and the other was a fall prescribed fire of low intensity and less tree injury and stress.

2. Methods and materials

2.1. Study sites and tree selection

Fires for this study occurred in forests just east of the Cascade Mountains in Oregon, USA where ponderosa pine is a dominant tree. Previous observations of other fires in this area indicated early pioneering *D. valens* typically attack trees with 100% crown scorch, but occasionally those with 95% or lower crown scorch, during the first week or two after ignition. There also were adjacent unattacked trees with 100% crown scorch, that eventually might be attacked, as well as trees with <100% scorch that were seldom, or never attacked. Thus, we focused our sampling around trees with and without early pioneering beetle activity and high levels of crown scorch. We noted in some instances small diameter (2–20 cm dbh) saplings with 100% crown scorch were the first being attacked, but then over a period of a few weeks all sizes with severe crown scorch, including larger trees were being attacked.

The Two Bulls wildfire was northwest of Bend, Oregon, USA (44.123077° N lat.; –121.462593° W long., elev. 1128 m) in the Skyline Forest. Trees sampled were from two management units. One unit in 2009, five years prior to burning, had 106 trees ha^{–1} with 76.4% ranging from 22.9 to 53.1 cm diameter and 1 tree ha^{–1} > 53.3 cm diameter. The other unit had 132 trees ha^{–1} with 70.5% ranging from 22.9 to 53.1 cm diameter, plus 7 trees ha^{–1} > 53.3 cm diameter. This fire started 7 June 2014 and burned approximately 2796 ha. Trees were selected on 17 June and all samples collected on 18 June, 11 days post ignition. Trees attacked by *D. valens* were sporadic on selection day, and required extended searching to locate. Attacked trees were sampled as encountered provided there was a nearby (to minimize environmental differences) unattacked tree of similar diameter and fire injury. All sample trees occurred in an area with uniform high fire severity, with insufficient numbers of ≤95% crown scorch trees to

sample. A total of 32 trees were selected, 16 attacked and 16 unattacked.

The prescribed fire was located about 6 km northwest of Sisters, Oregon, USA (44.322614° N lat.; -121.627900° W long., elev. 1036 m). In 2009 there were 180 trees ha⁻¹ with 42.2% 0–12.4 cm and 28.3% 12.7–22.6 cm diameter, with 5 tree ha⁻¹ > 53.3 cm. It was ignited on 3 October 2014 for fuels reduction and density management of seedlings and saplings. These trees were selected 16 October and all sampled the next day, 14 days post ignition. Here again, beetle activity was scattered and often absent in portions of the burn. Groups of three trees growing near one another with similar diameter were selected, one tree each characterized by: (1) one or more *D. valens* attacks with 100% crown scorch, hereafter referred to as attacked 100% scorch; (2) no beetle attack with 100% crown scorch, hereafter unattacked 100% scorch; and (3) no beetle attack with 60–95% crown scorch, hereafter unattacked ≤95% scorch. There were insufficient attacked <95% scorch trees to include as a fourth category. A total of 60 trees were selected, 20 per category. Temperatures for those days between fire ignition and sampling were obtained from weather station measurements near Sisters, Oregon (elev. 969 m) and another near Bend, Oregon (elev. 1116 m).

2.2. Tree measurements

Tree measurements recorded for selected trees in both fires included diameter at breast height (1.4 m), height, crown scorch height, bole scorch height, crown base height pre-fire, and a basal bark char rating. All heights were measured with a laser hypsometer. Crown scorch (%) is calculated as [(crown scorch height – live crown base height pre-fire)/(tree height – live crown base height pre-fire) × 100]. Bole scorch (%) was calculated as [(bole scorch height/tree height) × 100]. Severity of basal bark char, from here on bark char, was rated with five categories (0–4): 0 = no char; 1 = light char evident; 2 = moderate char, bark uniformly black except fissures and charred bark character still intact; 3 = heavy char, including all fissures with bark character lost; and 4 = bark burned off and charred wood showing (Thies et al., 2006). The wildfire bark char severity was rated in each of four quadrants (NE, SE, SW, and NW) around the boles. Attacks by *D. valens* in each quadrant were counted on the day selected and again on 24 July and 22 October. At the prescribed fire, many trees were too small for meaningful quadrant divisions, so north and south sides of each tree were rated as above for basal char severity and presence of beetle attacks. Beetles were extracted from galleries of non-experimental trees in both experiments to confirm their identity.

2.3. Tree sampling

Wildfire trees attacked by *D. valens* were processed first within each pair of neighboring trees. An increment core (5 mm dia.) was removed from 2.0 cm above a gallery entrance hole randomly chosen when more than one was present. After discarding most or all outer bark, each core with phloem and 1 cm of sapwood was sealed in a screw cap vial (4.0 ml) and immediately frozen with dry ice for transport to the laboratory freezer (−36 °C). A second sample was removed on the opposite side of the bole (usually 180°) without a nearby beetle gallery hole, at the same distance above the forest floor as the gallery sample, yielding two cores per tree. The neighboring unattacked tree was then sampled at the same bole location (aspect and height) as the gallery hole on the attacked tree.

In the prescribed burn, attacked 100% scorch trees were processed first within each group of three neighboring trees as described above, and then the other two sampled at the same bole position (aspect and height) yielding one core per tree. Knowing ethanol in stem tissues diffuses outward to the atmosphere, we

suspected bark char could adsorb it like activated charcoal traps (Kelsey et al., 2013). Before drilling each core, black charred bark on the bole surface (2–5 mm depth) was removed with a laboratory stopper bore (13 mm dia. circle), then sealed in a vial and frozen with dry ice for transport to the laboratory freezer. The tissue core was taken from the center of this circle and processed as described for wildfire samples.

2.4. Tissue and bark char chemical analysis

Vials holding frozen cores were thawed on ice, then the phloem and sapwood separated into pre-weighed 20 ml headspace vials that were quickly weighed and sealed with a PTFE septa (Agilent Technologies), star washer, and crimp cap (PerkinElmer). Sealed vials were held on ice until an autosampler set was completed, then heated at 102 °C for 30 min to stop any residual enzyme activity. The vial headspace was analyzed using a PerkinElmer Autosystem XL gas chromatograph (GC) with FID detector and a Turbomatrix 110 headspace autosampler. The column was a DBWAX, 30 m × 0.32 mm i.d., 0.25 µm film thickness (J&W Scientific from Agilent) in an oven held at 50 °C for 2.4 min, then increased 45 °C min⁻¹ to 120 °C with a 0.5 min hold, and back to 50 °C, for a 7.46 min total run time. Helium was the carrier gas set to 2.0 mL min⁻¹ throughout the run. Autosampler temperature settings were 110, 102, and 100 °C for the transfer line, needle, and vial oven, respectively, with helium pressure at 123.7 kPa. Vials were heated for 30 min in the autosampler oven, with a 4.0 min pre-injection pressurization, 0.04 min injection, 0.1 min needle withdrawal time, and 20 s vial venting. Quantities of ethanol were determined with a four level, or three level external standard curve for wildfire and prescribed fire samples, respectively; both were linear. Duplicate vials representing each standard level were analyzed with every set of samples on the autosampler. Standards vials contained 5 µL of solution with known quantities of 100% ethanol diluted in deionized water.

α-Pinene in the same samples was quantified from the ethanol standard curve using a relative response factor determined with α-pinene standards. 3-Carene and β-pinene peaks were not quantified because their high concentrations exceeded the maximum instrument limit set for detecting low ethanol levels. Final ethanol and α-pinene concentrations were calculated by the multiple headspace technique after analyzing the same sample twice with 20 s venting between runs (Kolb et al., 1984). Ethanol is reported as µg g⁻¹ fresh mass (fm) because it diffuses into tissue water and solid polymers, providing more relevant concentrations for beetles entering the tissues than those reported on a dry weight basis. The α-pinene concentrations are reported in the same units for consistency.

Thirty-one of the 96 wildfire cores had small (1–6 mm), uncharred, residual pieces of inner most outer bark still attached to the phloem, normally discarded while sampling. These bark pieces were detached, processed and analyzed for ethanol concentrations like the phloem and sapwood samples to verify ethanol diffusion from phloem into adjacent outer bark.

Charred bark samples from trees at the prescribed burn were prepared for analysis by carefully removing black char from light to dark brown bark under the char, when present. Previous tests showed lightly charred or uncharred bark contained minimal ethanol. Black char pieces were returned to the vial and crushed with a spatula to reduce variation in particle sizes. Each char sample was weighed into a headspace vial and sealed as described above, 50.0–50.5 mg for large samples with more than 50 mg, or the entire sample for those with less than 50 mg. Just prior to placement on the headspace autosampler 20 µL of *n*-butanol (reagent grade) was injected through each vial septum to help desorb ethanol from the char. Ethanol concentrations were calculated from a four level

standard curve prepared by adding one standard solution by syringe injection into sealed headspace vials containing 50.0–50.5 mg of activated charcoal (Sigma-Aldrich, 20–60 mesh). We tested and confirmed that activated charcoal was a suitable substitute for ponderosa pine bark char. These samples were analyzed by a single injection, static headspace analysis.

Tissue water contents were determined after headspace analysis by removing vial septa, heating for 16 h at 102 °C, then cooling to room temperature in a desiccator chamber and reweighing.

2.5. Statistical analysis

The two fire types were considered separate experiments and none of their parameters compared statistically. All analyses within fire type were modeled separately for each tissue (char, bark, phloem, sapwood, as they are typically different) using the SAS 9.4 (SAS, 2012) mixed procedure for linear mixed models, while concentration relationships between tissue types were evaluated using the correlation procedure. Tree and fire injury parameters were compared only within each fire, between attacked and unattacked wildfire trees, and among the three prescribed fire tree categories by analysis of variance using the SAS 9.4 (SAS, 2012) mixed procedure.

Wildfire trees were sampled to address two questions regarding how ethanol, α -pinene, and water concentrations in tissues near a *D. valens* attack entrance hole differed from: (1) those at an unattacked position on the same bole; and (2) those in comparable tissues from an unattacked, adjacent tree. Tissue concentrations above gallery entrances were used for comparison in both experiments. Experiment 1 values were modeled as a randomized complete block (tree) design with tree modeled as a random effect and bole side (attacked, unattacked) as a fixed effect. Experiment 2 comparisons were similarly modeled using a randomized complete block (tree pair) design, with tree pair modeled as a random effect and attack status modeled as a fixed effect.

For prescribed fire tissues the ethanol, α -pinene, and water contents were modeled using a randomized complete block (three tree group) analysis, with the tree group modeled as a random effect and tree condition (attacked 100% scorch, unattacked 100% scorch, and unattacked $\leq 95\%$ scorch) modeled as a fixed effect.

Assumptions of normality and equal variance of residuals were checked during analysis with quantile-quantile and residual vs predicted plots, respectively. All ethanol and α -pinene data from both fires were natural log transformed for analysis and their resulting means with 95% confidence intervals (CI) back-transformed to geometric means for presentation. Tree and fire injury parameters, and water contents were not transformed for analysis in either fire type. Denominator degrees of freedom were calculated using the containment method (SAS default) as appropriate for our relatively simple, balanced, random effect models (SAS, 2012). Effects were considered statistically different at $\alpha \leq 0.05$ and marginally different for $\alpha = 0.05$ –0.10.

3. Results

3.1. Tree parameters

Wildfire trees attacked by *D. valens* and their unattacked neighbors (Table 1) were similar in mean height ($F_{1, 30} = 0.35$, $P = 0.560$), diameter ($F_{1, 30} = 0.01$, $P = 0.906$) and scorch injury to their crowns ($F_{1, 30} = 0.12$, $P = 0.732$) and boles ($F_{1, 30} = 0.00$, $P = 0.975$). Mean crown scorch was 95–96%, but in many instances most needles had been consumed and 74–75% of the bole length was blackened, with all predicted to die from their injuries (Thies et al., 2006). Bark char in every quadrant of all trees was rated as 3 with no variation, characterized by uniform charring sufficient to cause loss of the bark surface structure, and into all depths of the bark fissures (Thies et al., 2006).

Prescribed fire trees among all three condition groups were similar in mean height ($F_{2, 57} = 0.05$, $P = 0.948$) diameter ($F_{2, 57} = 0.17$, $P = 0.844$), and bark char ($F_{2, 57} = 0.54$, $P = 0.586$; Table 1). The attacked and adjacent unattacked tree groups with 100% scorch did not differ in mean crown ($t_{57} = 0.00$, $P = 1.00$), or bole scorch ($t_{57} = 0.17$, $P = 0.865$), whereas both of them had greater crown (both $t_{57} = 12.89$, $P < 0.001$) and bole scorch (both $t_{57} \geq 2.11$, $P \leq 0.040$) than the unattacked $\leq 95\%$ scorch trees. Prescribed fire trees attacked by *D. valens* and selected for sampling were much shorter than those attacked and sampled in the wildfire, about 6 m tall with 100% crown scorch, but their needles were seldom consumed and bole scorch was confined to the lower 27–38% of the stem height (Table 1). Average bark char ranged from 1.6 to 1.8 indicating they had more than superficial surface blackening, but less than a moderate 2 rating characterized by a uniformly blackened bark except for prominent fissures (Thies et al., 2006). Those trees with 100% scorch were likely to die, whereas some portion of those with $\leq 95\%$ scorch would likely survive (Thies et al., 2006). The prescribed fire did have trees of similar height to those sampled in the wildfire, but due to low fire severity few had sufficient crown injury to match our selection criteria, or were not yet attacked by *D. valens*, or if attacked, there were no similar sized trees nearby with injuries suitable for the other two condition groups.

3.2. Ethanol

In wildfire trees, phloem above *D. valens* galleries contained a mean ethanol content of $252.5 \mu\text{g g}^{-1}$ fm, 18.4 (3.2, 110.1; 95% CI) times greater than in phloem from the opposite bole side without galleries (Fig. 1A). Underlying sapwood contained less ethanol than adjacent phloem, but the same pattern persisted with a mean of $117.0 \mu\text{g g}^{-1}$ fm from above galleries, 35.2 (4.1, 301.8; 95% CI) times greater than on the opposite bole side with no gallery entrances (Fig. 1B). In adjacent unattacked trees, phloem and sapwood from equivalent positions on the bole contained only 4.2 and 6.0%, respectively, the amount of ethanol above galleries in attacked trees (Fig. 2A and B). Sapwood and phloem ethanol

Table 1
Tree diameters, heights, and burn injury at the two fires. Means $\pm$ SE.^a

Tree parameter	Wildfire		Prescribed fire		
	Attacked 100% scorch	Unattacked 100% scorch	Attacked 100% scorch	Unattacked 100% scorch	Unattacked $\leq 95\%$ scorch
DBH (cm)	34.9 $\pm$ 2.1a	35.3 $\pm$ 2.1a	10.6 $\pm$ 1.3A	10.2 $\pm$ 1.3A	11.2 $\pm$ 1.3A
Height (m)	17.5 $\pm$ 0.9a	16.7 $\pm$ 0.9a	5.8 $\pm$ 0.7A	5.6 $\pm$ 0.7A	5.9 $\pm$ 0.7A
Crown scorch (%)	95.4 $\pm$ 1.8a	96.3 $\pm$ 1.8a	100 $\pm$ 1.7A	100 $\pm$ 1.7A	68.4 $\pm$ 1.7B
Bole scorch (%)	74.7 $\pm$ 5.9a	74.4 $\pm$ 5.9a	38.6 $\pm$ 3.6A	37.8 $\pm$ 3.6A	27.2 $\pm$ 3.6B
Bark char (0–4)	3 $\pm$ 0.0a	3 $\pm$ 0.0a	1.8 $\pm$ 0.1A	1.7 $\pm$ 0.1A	1.6 $\pm$ 0.1A

^a For each fire type, the tree parameters values within rows followed by the same letter are not statistically different, see text for P values.

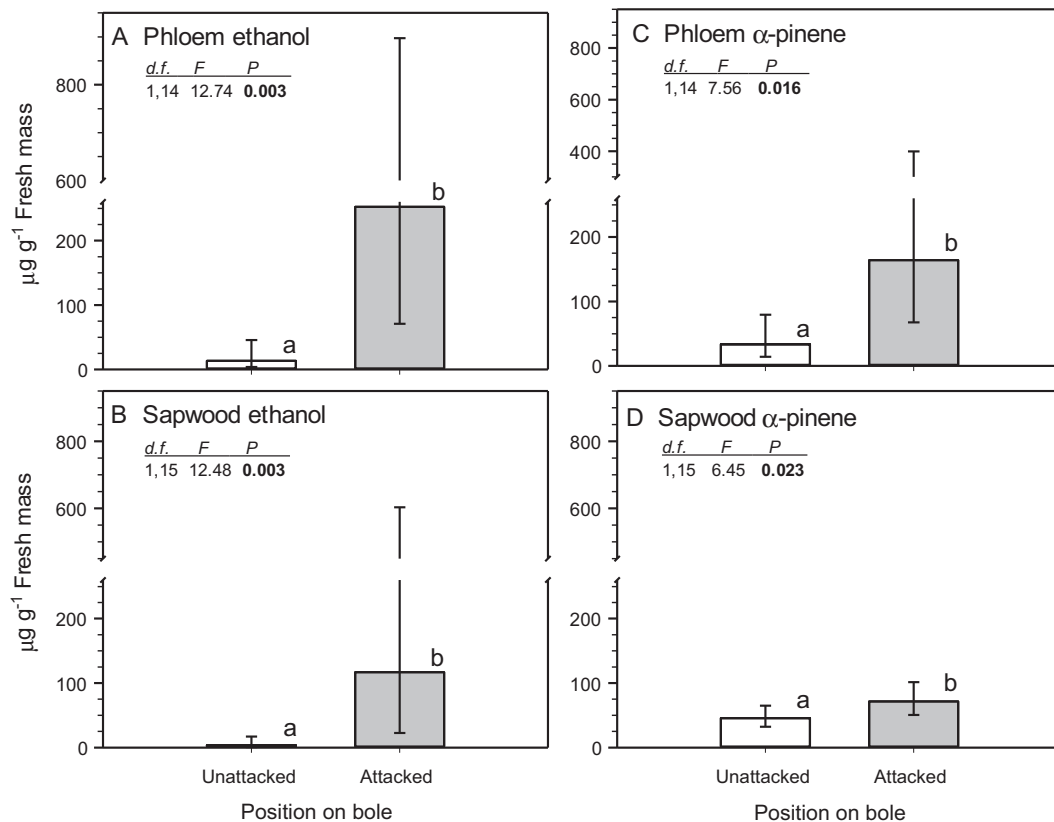


Fig. 1. Ethanol and α -pinene concentrations in phloem and sapwood from wildfire stressed trees. Two tissue cores were sampled from the same tree bole, one above a *D. valens* gallery entrance, and the other at an equivalent height on the opposite bole side without a gallery hole. Bars are back-transformed geometric means with 95% CI. Bold *P* values are statistically different. In graph C note the Y-axis scale difference above the break.

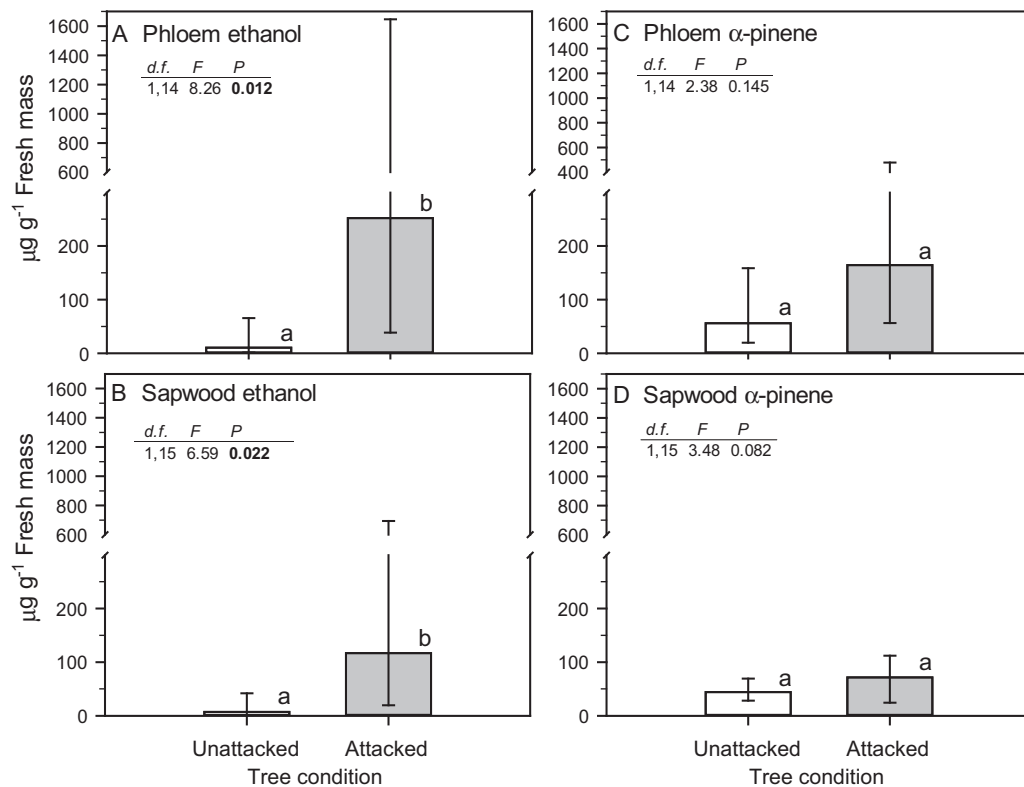


Fig. 2. Ethanol and α -pinene concentrations in phloem and sapwood from two adjacent wildfire stressed trees, one attacked by *D. valens* and the other unattacked. Tissue cores were sampled above a beetle gallery entrance on the attacked trees, and at an equivalent bole height on the adjacent, unattacked trees. Bars are back-transformed geometric means with 95% CI. Bold *P* values are statistically different.

concentrations were very strongly correlated ($r = 0.928$, $P < 0.001$) based on samples pooled from all wildfire trees and bole positions. All outer bark samples (except for one) contained ethanol, with a mean quantity about half the amount ($55.5 \mu\text{g g}^{-1} \text{ fm}$, $\pm 10.8 \text{ SD}$) in adjacent phloem ($97.7 \mu\text{g g}^{-1} \text{ fm}$, $\pm 14.6 \text{ SD}$), but very strongly correlated ($r = 0.953$, $P < 0.001$).

In prescribed fire trees, bark char ethanol concentrations were highest above *D. valens* gallery holes in attacked 100% scorch trees and lowest in the unattacked $\leq 95\%$ scorch trees with a marginal statistical difference (Fig. 3A). Phloem from attacked 100% scorch trees contained a mean ethanol content of $0.036 \mu\text{g g}^{-1} \text{ fm}$, 49.8 (2.4, 1016.4; 95% CI) times greater than in adjacent unattacked $\leq 95\%$ scorch trees (Fig. 3B). It was also higher than in phloem of unattacked 100% scorch trees, but not statistically different. Sapwood ethanol concentrations among trees in the three condition groups were not statistically different (Fig. 3C). Ethanol in the sapwood was strongly correlated with quantities in the phloem ($r = 0.913$, $P < 0.001$). Ethanol in bark char was not correlated with concentrations in the underlying phloem ($r = -0.052$, $P = 0.696$) or sapwood ($r = -0.026$, $P = 0.847$).

3.3. α -Pinene

In wildfire trees, phloem and sapwood above *D. valens* galleries contained mean α -pinene quantities of 164.2 and $71.5 \mu\text{g g}^{-1} \text{ fm}$, respectively, that were 4.9 (1.4, 16.8; 95% CI) and 1.6 (1.1, 2.3; 95% CI) times greater than in their respective tissues from opposite bole sides without a nearby gallery entrance (Fig. 1D). Adjacent unattacked wildfire tree tissues also had lower α -pinene contents than the corresponding tissues above galleries of attacked trees (Fig. 2C), but phloem quantities were not statistically lower, whereas sapwood concentrations were marginally lower (Fig. 2D). Sapwood and phloem α -pinene concentrations were strongly correlated ($r = 0.624$, $P < 0.001$) based on pooled samples from all wildfire trees and bole positions.

In prescribed fire trees, phloem α -pinene concentrations above *D. valens* galleries were highest in attacked 100% scorch trees and lowest in the unattacked $\leq 95\%$ scorch trees, but the difference was only marginally significant (Fig. 3D). Sapwood α -pinene concentrations were similar among all three tree condition groups (Fig. 3E), but weakly correlated ($r = 0.384$, $P = 0.002$) with

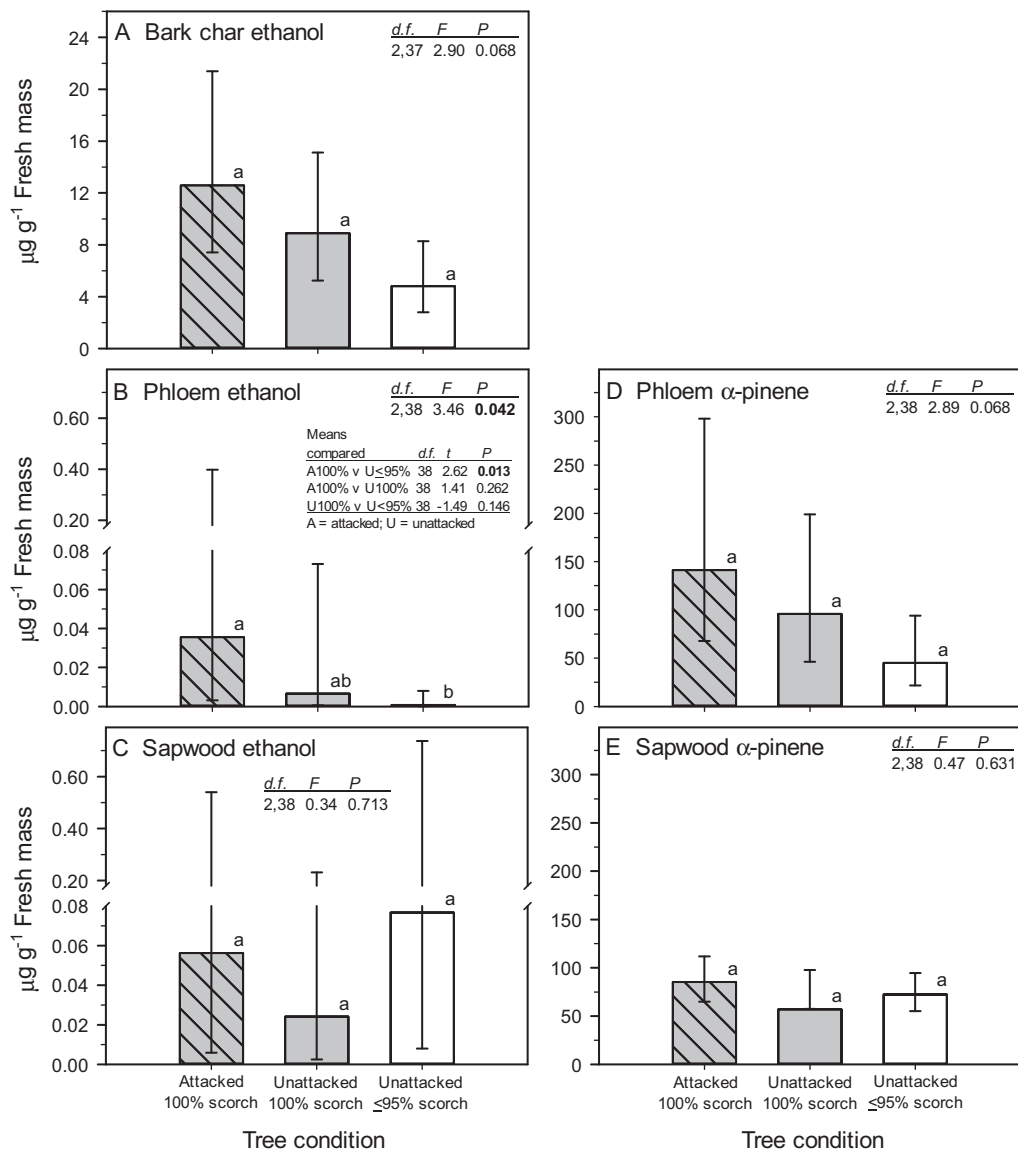


Fig. 3. Ethanol and α -pinene concentrations in tissues from prescribed fire injured trees. Tissue cores were sampled above *D. valens* gallery entrances on attacked trees and at equivalent bole heights and aspects on nearby unattacked trees with the same, or less severe crown scorch as the attacked trees. Bars are back-transformed geometric means with 95% CI. Bold P values are statistically different. Note the differences in Y-axis scales.

Table 2Tissue water contents, g g⁻¹ dry mass in wildfire trees. Means ± 95% CI.^a

Statistical comparison	Tissue	Bolt side, or tree	
		Attacked	Unattacked
Within tree	Phloem	1.49 ± 0.23a	1.64 ± 0.24a
	Sapwood	1.33 ± 0.10a	1.34 ± 0.10a
Adjacent trees	Phloem	1.46 ± 0.27a	1.43 ± 0.26a
	Sapwood	1.33 ± 0.09a	1.42 ± 0.09a

^a For each tissue row within a comparison group, those values followed by the same letter are not statistically different, see text for *P* values.

Table 3Tissue water contents, g g⁻¹ dry mass in prescribed fire trees. Means ± 95% CI.

Tissue	Tree condition		
	Attacked 100% scorch ^a	Unattacked 100% scorch	Unattacked ≤95% scorch
Phloem	1.66 ± 0.19a	1.66 ± 0.19a	1.69 ± 0.19a
Sapwood	1.42 ± 0.10a	1.43 ± 0.10a	1.45 ± 0.10a

^a In each tissue row the values followed by the same letter are not statistically different, see text for *P* values.

phloem quantities based on pooled samples across all three tree groups.

3.4. Water contents

Within *D. valens* attacked wildfire trees there were no differences in phloem (Table 2; $F_{1, 14} = 0.93$, $P = 0.351$) or sapwood ($F_{1, 15} = 0.04$, $P = 0.842$) mean water contents for tissues above galleries and those from opposite side bole positions without a gallery. Similarly, phloem and sapwood water contents in adjacent unattacked trees were the same as near galleries in attacked trees ($F_{1, 14} = 0.09$, $P = 0.772$; $F_{1, 15} = 3.13$, $P = 0.097$, respectively). In prescribed fire trees phloem and sapwood water contents, did not differ statistically among the three tree condition groups ($F_{2, 38} = 0.06$, $P = 0.941$; $F_{2, 38} = 0.10$, $P = 0.906$, respectively; Table 3).

3.5. Trees attacked by *D. valens*

Wildfire trees attacked by *D. valens* prior to sampling were preferentially reattacked over those unattacked nearby during the subsequent summer and early fall (Table 4). These differences were not compared statistically. In the prescribed fire, attacks on 100% scorch trees were all near the forest floor with a basal char rating of 2 (15/20 trees), while the remaining five trees had char ratings of 1. These differences were not compared statistically.

4. Discussion

4.1. Ethanol

Ethanol concentrations were much higher above *D. valens* gallery entrances than in corresponding tissues from adjacent trees without galleries in each fire, or the unattacked bole position of wildfire trees. Greater ethanol concentrations in more severely

burned wildfire trees, and lower quantities in less damaged prescribed fire trees, is consistent with previous observations within a single wildfire where concentrations increased with fire injuries (Kelsey and Joseph, 2003). It is unknown if ethanol concentrations 2 cm above galleries were influenced by the mechanical wound and the associated *D. valens* microbial symbiont community in the first week or two post-attack. Evidence that ethanol accumulated before beetles arrived include our previous study where all trees sampled were unattacked (Kelsey and Joseph, 2003), and in this study where some trees, unattacked at time of sampling, had concentrations comparable to those above galleries. In addition, ethanol accumulated in Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, stem segments heated to 35 °C (Joseph and Kelsey, 2004) and in fruit, or leaves of crop plants at temperatures between 40 and 50 °C (Anderson, 1994; Fan et al., 2005; Song et al., 2001). Also, there are plausible physiological mechanisms proposed to explain the process without beetles (Kelsey and Westlind, 2017). Lastly, variation in wildfire ethanol concentrations in both tissues, as indicated by their confidence intervals, was greater among unattacked adjacent trees than among unattacked bole positions within trees. This might result from a combination of genetic variation, indicated when seedlings were grown in uniform greenhouse conditions (Kelsey, 1996; Kelsey et al., 1998), plus slight environmental and physiological differences impacting ethanol in adjacent trees that were not factors for tissues within trees.

Uniform bark charring on all wildfire trees suggests their underlying live tissues experienced similar levels of heat stress and injury. But variability in ethanol concentrations suggests otherwise, given their proposed relationship to physiological responses to heat mentioned below (Kelsey and Westlind, 2017). Hood et al. (2008) examined four bark char categories, unburned, light, moderate, or deep, to predict cambium mortality, and found the latter two strongly associated with dead cambium in thin-bark conifers. But in thick-bark conifers, like ponderosa pine, these codes were less useful, especially the moderate category that corresponds with our bark char 3 rating observed in all quadrants of the wildfire trees. The high variability in phloem and sapwood ethanol concentrations supports uneven fire injury to the cambium, like Hood et al. (2008) observed.

4.2. Wildfire and prescribed fire heat stress and ethanol accumulation

Mechanistic explanations of cellular physiological stress and ethanol accumulation in responses to sub-lethal (30–60 °C) temperatures associated with heat from fire have been proposed for tree stems and other woody tissues (Kelsey and Westlind, 2017). Three physiological processes have been proposed: (1) O₂ supply stress, ~30–40 °C; (2) membrane function stress, ~40–50 °C; and (3) enzyme activity stress, across the 30–60 °C range associated with both above. Other intervening physiological factors may include seasonal changes in aerobic respiration rates influencing O₂ supplies, and enzyme activity levels regulating ethanol production. Ethanol synthesis initiates accumulation, but concentrations are mitigated by three dissipation mechanisms: (1) diffusion, (2) sapflow, and (3) metabolism, each impacted also by the sub-lethal stress mechanisms and injury to the tissues and whole

Table 4Wildfire trees attacked by *D. valens* during the post-sampling field season.^a

Tree condition when sampled 17 June	No. of trees with new attacks on		Total attacks on all trees 22 October	Final no. of trees attacked
	24 July	22 October		
Attacked (<i>n</i> = 16)	13	8	73	16
Unattacked (<i>n</i> = 16)	2	4	9	5

^a Distance of gallery entrance above the forest floor ranged from –2 to 21 cm, with mean 5.4 cm ± 1.6 SE.

tree (Kelsey and Westlind, 2017). These interacting processes are considered below to explain what we suspect influenced tissue ethanol concentration analyzed here.

Wildfire stem tissues may have accumulated high ethanol concentrations because they were stressed in late spring near peak physiological conditions for synthesis. These stems would have high volumes of metabolically active cells from new growth, with fast respiration rates (Ryan, 1990; Stockfors and Linder, 1998) that can create seasonally low internal O_2 levels (Eklund, 1990, 2000). O_2 supply stress may begin quickly when tissues reach ~ 30 – $40^\circ C$, if rapid respiration depletes O_2 enough to induce ethanol synthesis, especially if already at seasonally low levels. Wildfire bark char, bole and crown scorch, indicates these trees experienced high temperature, possibly for extended periods. Thus, high volumes of stem tissues likely reached 40 – $50^\circ C$ where membrane function stress induces ethanol synthesis. Synthesis will be rapid for either stress mechanism, since spring tissues have seasonally high fermentation enzyme activity (Kimmerer and Stringer, 1988). Tissue with ~ 40 – $50^\circ C$ membrane function stress likely continues to synthesize ethanol post-burn (Kelsey and Westlind, 2017); as the proportion of tissue with this stress type increases, so may ethanol concentrations.

Diffusion and metabolism in wildfire trees are likely the main ethanol dissipation processes, but not sapflow because of nearly 100% crown scorch (Kelsey and Joseph, 2003; Kelsey and Westlind, 2017). Wildfire tree diameters were relatively large, over three times the prescribed fire trees, with substantial internal tissue volume, or sink for inward diffusion. They also have longer outward diffusion paths for ethanol release to a limitless atmospheric sink than smaller trees, because ponderosa pine bark thickness increases with diameter (Thies et al., 2013; van Mantgem and Schwartz, 2003). Diffusion can be influenced by tissue water, but not here since water contents for each tree group were similar within and across the two fire types. Metabolisms impact on ethanol post-burn in wildfire stems depends on the proportion of heat damage and lost activity of enzymes converting it to other components (Kelsey and Westlind, 2017), especially in tissues reaching 40 – $50^\circ C$. As metabolism activity declines, less ethanol is eliminated. Overall, dissipation mechanisms in wildfire trees likely had less influence on decreasing ethanol, than the mechanisms controlling its synthesis and accumulation.

Prescribed fire trees were opposite of wildfire trees for nearly all factors influencing ethanol. First, bark char, bole scorch, and crown consumption indicate low temperature exposure, and for short durations. Second, fall prescribed fire trees have seasonally low volumes of metabolically active cells with slow respiration rates (Ryan, 1990; Stockfors and Linder, 1998) and seasonally high tissue O_2 levels (Eklund, 1990, 2000). This could delay or prevent O_2 supply stress, postponing ethanol induction until tissues experience 40 – $50^\circ C$ membrane function stress. Third, fall tissues have seasonally low fermentation enzyme activity (Kimmerer and Stringer, 1988), so ethanol synthesis rates are slow for either stress type. Any ethanol synthesis by O_2 supply stress likely occurs only with elevated fire temperatures, and stops quickly after returning to ambient temperatures. These combined factors limited ethanol synthesis.

Diffusion and metabolism would dissipate ethanol in all three prescribed fire tree groups, with some sapflow in the $\leq 95\%$ crown scorch trees (Kelsey and Joseph, 2003). Sapflow could explain why the $\leq 95\%$ crown scorch trees had the lowest phloem ethanol concentrations among all trees in either fire. Yet their sapwood concentrations were not similarly low, possibly from sapflow moving some ethanol from heat stressed roots into the stem. Diffusion in all prescribed fire trees is likely influenced by their small stem diameters and bark thickness. They have less tissue sink volume for inward movement, and short outward diffusion paths

through thin bark, allowing quick atmospheric release of ethanol (Kelsey et al., 2013). Ethanol metabolism to organic acids, protein, sugars, or starch (MacDonald and Kimmerer, 1993) was likely strong given stem tissues were exposed to low temperatures, thus limiting the proportion of lost enzyme activity by heat deactivation. Low ethanol in prescribed fire tree tissues probably results from minimal synthesis, in conjunction with moderate to strong post-burn dissipation processes.

Evidence of ethanol diffusion was detectable from relationships between tissue concentrations, like very strong correlations between sapwood and phloem for both wildfire ($r = 0.928$) and prescribed fire ($r = 0.913$) trees. Plus, in wildfire stems, ethanol in nonliving outer bark correlated very strongly ($r = 0.953$) with concentrations in the adjacent phloem where it was synthesized. In prescribed fire trees, outward diffusing ethanol was adsorbed and concentrated by bark char, similar to charcoal traps (Kelsey et al., 2013), reaching quantities 2–4 orders of magnitude greater than in underlying phloem. But, char and underlying phloem concentrations were not correlated ($r = -0.052$), in part because of sampling protocol, and a potential for independent changes prior to sampling. Char samples covered a 13 mm diameter surface area, while the phloem was a 5 mm diameter core underlying the char center, creating a disproportionate comparison. Also, they each could change independently post-burn. Char initially adsorbed and concentrated ethanol diffusing outward from underlying phloem, but concentrations could likely increase if it adsorbs atmospheric ethanol released from adjacent areas on the same tree, or possibly nearby trees. At the same time, post-burn cambium and phloem concentrations may decline if they quickly recover from heat stress, and metabolize ethanol. These factors would eliminate any correlation between char and underlying phloem ethanol.

Concluding this section, we propose that the ethanol concentrations in wildfire and prescribed fire trees were primarily a function of the duration and rates of its synthesis, both determined by their tissues seasonal physiological status and the heat stress mechanisms they experienced while at sub-lethal temperatures. Ethanol dissipation processes influenced ethanol accumulation also, but not to the extent of synthesis. Ethanol accumulation in tree stems from sub-lethal heat stress by fire is a dynamic process, involving many interactive factors that create variable concentrations across short tissue distances that can change constantly over time (Kelsey and Westlind, 2017).

4.3. α -Pinene

α -Pinene concentrations for both fires were also higher in phloem and sapwood above *D. valens* galleries than in tissues without a nearby gallery entrance within the same tree, or from an adjacent tree, but some were only marginally significant. No statistical difference for phloem samples from adjacent attacked vs. unattacked wildfire trees results, in part, from greater tree to tree variability among unattacked trees, as indicated by their wider confidence intervals, for the same reasons as mentioned for their ethanol concentrations above. It appears α -pinene concentrations were not impacted much by the trees heat exposure, as the magnitude of the differences between attacked wildfire and attacked prescribed fire trees were small, relative to the magnitude for ethanol.

We propose higher α -pinene concentrations above *D. valens* gallery entrances were the trees post-attack response to mechanical injury from initial tunnel construction and on-going maintenance that creates the entrance resin tube, plus an initial chemical defense induced response to growth of microbial symbionts. Although tissues were only 2 cm from gallery entrances, they probably were not within tissue lesions caused by wounding or symbiont growth. Tunnels we observed were excavated horizontally or downward prior to egg gallery widening, and other

investigators have measured lesion lengths of only 0.5 cm at 2–3 weeks post fungal inoculation in Arizona ponderosa pine (Gaylord et al., 2011), and less than 0.6 cm at two weeks post inoculation in stems of *P. tabuliformis* seedlings (Lu et al., 2010). Increases in α -pinene and other monoterpenes would be expected had our samples been within lesion boundaries, since ponderosa pine lesion tissue formed in response to mechanical wounds, or wounds + fungal inoculation contained 5.1 and 11.4 times more α -pinene, respectively, than constitutive tissues 17 days after treatment with a pathogenic fungus vectored by mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Keefover-Ring et al., 2016). Their total monoterpene increases were 5.2 and 22.3 times, respectively, but no systemic changes occurred in tissues sampled 30 cm away from a wound. Although our samples were probably not within lesions they were sufficiently close, less than 2 cm accounting for lesion lengths above, to suspect some increases in monoterpene defenses.

4.4. Pioneering *D. valens* host selection

Initial pioneering *D. valens* selected and attacked trees and bole positions within fire injured trees where ethanol concentrations in underlying tissues, or surface char were high. Stem diameters did not contribute to the beetle's selection as they were the same across treatment groups within both fires. *D. valens* may prefer black scorched stems over lighter colored, unscorched stems given other bark beetles prefer black colored traps rather than white (Campbell and Borden, 2006), but this was not a factor in selecting wildfire trees, or bole positions as their stems were equally black, as indicated by the bole scorch and bark char. Trapping studies have demonstrated primary attraction of *D. valens* to ethanol lures combined with host monoterpene lures (Dodds, 2014; Fettig et al., 2004; Gandhi et al., 2010; Joseph et al., 2001; Klepzig et al., 1991; Vité and Gara, 1962). When outwardly diffusing ethanol is released to the atmosphere in heat stressed trees some quantity of monoterpenes will also be released as detected from boles of healthy or diseased lodgepole pine, *P. contorta* Dougl. ex Loud. (Gara et al., 1993; Rhoades, 1990). This creates an attractive mixture drawing pioneering beetles to trees and bole positions where ethanol concentrations are highest. Once beetles enter the phloem, pheromones are released (Liu et al., 2013) and mix with the monoterpenes and ethanol creating a stronger secondary attraction for beetles arriving later, causing the same trees to be re-attacked, as observed in the wildfire. There were some trees and bole positions with high enough ethanol concentrations to be suitable hosts when sampled, but we suspect they remained unattacked, in part, because there were not enough pioneering beetles immediately post-burn to attack all available hosts, and those arriving later responded to the secondary pheromone from previously attacked trees.

Ethanol in bark char might play some role in *D. valens* initial host selection. Beetles were able to discriminate between prescribed fire trees with high and low ethanol, even though their phloem concentrations were three orders of magnitude lower than in wildfire trees. But ethanol trapped in char on prescribed fire trees reached levels 354–6882 times greater than in the underlying phloem for attacked 100% scorched and $\leq 95\%$ scorched unattacked trees, respectively. Monoterpenes would also be adsorbed by char, similar to using activated charcoal to trap their emissions from lodgepole pine stems (Gara et al., 1993; Rhoades, 1990). If *D. valens* can detect these trapped compounds they might influence host selection. Bark char may contribute also to some ground level attacks. Beetles were observed falling from charred stems, then walking back to the tree base. Surface char dislodges with a light touch, and may make it difficult for these heavy beetles to walk across without falling.

Attacks by *D. valens* within 24 h of fire ignition (Ganz et al., 2003) can be explained by heat stress induction of ethanol synthesis and accumulation within hours, similar to applying non-heat stress to tree tissues (Kelsey et al., 1998, 2011; Kreuzwieser et al., 2000). Our observations of small trees being attacked first at some fires, is likely related to their more rapid ethanol release than large trees, discussed above. The association of ethanol with heat stress and fire injury provides a physiological explanation for why bole scorch height and crown scorch height to ponderosa pine can estimate their likelihood of attack by *D. valens* (Negron et al., 2016). Furthermore, release of monoterpenes from stumps or woody residues after thinning, pruning, or other mechanical injuries prior to, or immediately after a fire, would contribute to attacks on healthy and fire injured trees (Fettig et al., 2006; Six and Skov, 2009), although many of the woody residues are likely also to synthesize ethanol at some point and contribute to beetle attraction. In concluding, this new information strongly indicates that the accumulation of ethanol in heat stressed tissues in burned ponderosa pine and its subsequent atmospheric release with tissue monoterpenes is the critical physiological process driving initial host tree and bole position selection by pioneering *D. valens*.

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