Received: 3 March 2022 | Accepted: 7 August 2022

DOI: 10.1111/afe.12535

ORIGINAL ARTICLE

Agricultural and Forest Entomology

Royal Entomological Society

# Red turpentine beetle primary attraction to β-pinene or 3-carene (with and without ethanol) varies in western US pine forests

Rick G. Kelsey[1] | Douglas J. Westlind[1] | Monica L. Gaylord[2]

[1]United States Department of Agriculture Forest Service, Pacific Northwest Research Station, Corvallis, Oregon, USA

[2]R3 Forest Health Protection, Flagstaff, Arizona, USA

**Correspondence**
Rick G. Kelsey, United States Department of Agriculture Forest Service, Pacific Northwest Research Station, Corvallis, OR, USA.
Email: rick.kelsey@usda.gov

## Abstract

1. Lure attraction strength for red turpentine beetle, *Dendroctonus valens* (Coleoptera: Curculionidae: Scolytinae) observed previously in US Pacific Northwest ponderosa pine forests is (−)-β-pinene+ethanol > (+)-3-carene+ethanol, but untested elsewhere in its western US range. Thus, both were tested with (−)-β-pinene, (+)-3-carene, ethanol, and a blank in Oregon and California sites burned by wildfire, whereas in Arizona the first four lures were tested in a thinned-unburned site.
2. The *D. valens* responses in burned Oregon and California sites were similar, (−)-β-pinene+ethanol > (−)-β-pinene > 3-carene = 3-carene+ethanol > ethanol > blank, whereas in the cut-unburned Arizona site it was 3-carene+ethanol > 3-carene = (−)-β-pinene+ethanol > (−)-β-pinene. Whether this variation was influenced by beetle genetic differences, or chemical and physical parameters in the different environments and remaining stressed host resources 1-year post disturbance warrants additional study.
3. Responses to (−)-β-pinene varied, from a stronger attractant than (+)-3-carene in Oregon and California, to a weaker lure than (+)-3-carene in Arizona. This (−)-β-pinene variability was minimized when released in combination with ethanol, making (−)-β-pinene+ethanol the most consistent attractant of those tested across the three states, and a reliable lure for detection, monitoring, and management projects for *D. valens* in western US pine forests.

**KEYWORDS**
(−)-β-pinene, (+)-3-carene, *Dendroctonus valens*, ethanol, primary attraction

## INTRODUCTION

In North and Central America the red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae) is a nonaggressive, generalist bark beetle colonizing all pine species across its native range (Kelley & Farrell, 1998; Owen et al., 2010). Although they attack stressed hosts their colonization usually does not enhance mortality (Fettig et al., 2008; Westlind & Kelsey, 2019; Zhu et al., 2008). However, its invasion into China during the 1980's was accompanied by an increased aggressive behaviour contributing to extensive mortality of healthy Chinese red pine, *Pinus tabuliformis* (Sun et al., 2013; Yan et al., 2005), attributed in part to their vectored microbial symbionts (Cheng et al., 2016, 2018; Liu et al., 2020; Lu et al., 2010, 2011; Lu, Decock, et al., 2009; Lu, Zhou, et al., 2009; Xu et al., 2016). Subsequent to entering China, the fungal communities vectored by *D. valens* have changed and now include new Ophiostomatoid species not

present in North American pine forests (Lu, Decock, et al., 2009; Lu, Zhou, et al., 2009; Taerum et al., 2013, 2016). This creates a latent threat from re-introduction of *D. valens* to North America with new or potentially more virulent fungal pathogens from China (Lu et al., 2011). Furthermore, although Southern Hemisphere pine forests remain *D. valens* free, the beetle was ranked the highest risk for possible introduction and establishment there among 64 North American Scolytinae species evaluated (Lantschner et al., 2017).

The first line of defence against re-introductions from China has been the implementation of phytosanitary standards to reduce the transport of forest pests by world trade and movement of people, but various issues can weaken their effectiveness (Haack & Petrice, 2009; Meurisse et al., 2019). In the event of any break-throughs, there is an abundance of susceptible fire-stressed pine in western US forests that *D. valens* are known to quickly colonize (Ganz et al., 2003; Kelsey & Westlind, 2017a; Westlind & Kelsey, 2019). There is evidence from China showing some fungi vectored by *D. valens* may produce larger lesions in fire-injured trees than in healthy hosts (Lu, Decock, et al., 2009). Fire-injured pine may also enhance the further spread of native pathogens such as *Leptographium wageneri* vectored by *D. valens* (Owen et al., 2005). Availability of these stressed hosts in western US forests has expanded in recent years in conjunction with climate changes contributing to increases in the number of large wildfires, fire severity and area burned (Abatzoglou & Williams, 2016; Littell et al., 2009, 2016; Singleton et al., 2019; Westerling, 2016; Williams et al., 2019) along with more human-caused ignitions (Balch et al., 2017). The number of fire-injured trees may remain high or increase in the western landscape with further climate changes (Barbero et al., 2015; Peterson & Littell, 2014; Williams et al., 2019), as well as potentially greater use of prescribed fire to manage fuel loads (Kolden, 2019; Melvin, 2018).

Surveillance and early detection are the second level of defence (Liebhold et al., 2017). Early detection programs at ports of entry and nearby pine stands need a strong lure coupled with molecular protocols for identifying vectored exotic symbionts to separate re-introductions from China and native beetles. Ethanol is a likely lure component because its release is a key factor in the rapid *D. valens* detection of fire-stressed trees (Kelsey & Westlind, 2017a). Monoterpene+ethanol mixtures have shown strong *D. valens* responses in Wisconsin (Klepzig et al., 1991) and Oregon (Joseph et al., 2001), with the specific monoterpene(s) released having a major impact on the *D. valens* response. For example, when lures with the three dominant or co-dominant ponderosa pine monoterpenes were tested against one another, the order of attraction strength was (−)-β-pinene+ethanol > (+)-3-carene+ethanol > (-+)-α-pinene+ethanol in Pacific Northwest pine forests of Oregon and Washington (Kelsey & Westlind, 2020). Additional tests found the *D. valens* response was linearly related to the total dose of (−)-β-pinene+ethanol released and changing their release ratios had minimal impact on attraction (Kelsey & Westlind, 2022). (−)-β-Pinene+ethanol and (+)-3-carene+ethanol lures have not been tested outside Pacific Northwest pine forests.

Our objective was to test the attraction consistency of *D. valens* to (−)-β-pinene+ethanol and (+)-3-carene+ethanol at three locations in western US pine forests and compare their catch numbers with, (−)-β-pinene, (+)-3-carene.

## MATERIALS AND METHODS

### Lure processing

(+)-3-Carene (>90.0%), and (−)-β-pinene (≥97%) were purchased from Sigma Aldrich (St. Louis, MO) and dispensed from low-density polyethylene (LDPE) bottles (15 mL, The Cary Company, Addison, IL) similar to those used in previous tests (Erbilgin et al., 2007; Kelsey & Westlind, 2020, 2021, 2022; Liu et al., 2013; Sun et al., 2004). They were filled in advance to the threaded neck with a small air bubble under the cap, then grouped by lure type in sealed plastic bags and stored at 5°C. Before initial weighing they were held 5 days at room temperature to saturate the LDPE bottles (Kelsey & Westlind, 2020), then refilled, weighed and immediately returned to the cold room for a few hours until further processing. Ethanol (95%) pouches (approx. 59.6 × 8.4 cm inseams) were purchased from Synergy Semiochemicals Corp., Burnaby, Canada, and stored at 5°C until adjusted to room temperature for initial weighing, then immediately returned to the cold room for further preparation.

Ethanol released from stressed conifer tissues mixes with resin monoterpenes as it vaporizes into the atmosphere (Gara et al., 1993). To simulate comparable vapour mixing the weighed monoterpene and ethanol dispensers were combined in a wide mouth, high-density polyethylene jar (9.3 × 9.7 cm, D × L cap on, 473 mL, Uline, Pleasant Prairie, WI) with five equally spaced ventilation holes drilled in the bottom (0.79 cm dia.) and screw cap (0.95 cm dia.) in an X pattern (Kelsey & Westlind, 2020, 2021). A screen inside the cap prevented insects from entering the top holes, and the outside jar wall was wrapped with aluminium foil to reduce solar heating. To monitor site temperatures one ibutton temperature data logger (model 1922L, Maxim Integrated Corp. San Jose, CA) was secured with tape to the inside wall for three randomly selected jars of each lure type.

Each mixing jar, except blanks, received one ethanol pouch wrapped around the inside wall leaving a center opening for one 3-carene bottle, or two β-pinene bottles to provide similar release rates based on laboratory tests (Kelsey & Westlind, 2020). Arizona 3-carene lures had an empty LDPE bottle added, giving them the same number of bottles in each jar as the β-pinene lures. Loaded mixing jars for Oregon or California were sealed in plastic bags by lure type and stored in separate coolers at 5°C overnight. The next morning they were packed with ice and transported to the study sites. Arizona lures were weighed, loaded into mixing jars, then grouped by lure type in cardboard boxes with 10 frozen cold packs (473 mL each, Uline). Boxes were sealed in a plastic bag, placed in separate coolers and shipped overnight air to Arizona where they were stored in a freezer for less than 48 h before attaching to traps. On the last day, at all sites, the beetles and lures were collected simultaneously. Oregon and

**TABLE 1** Field test locations, prior year disturbances and tree metrics[a]

| Site | Tree means (range) | | | | |
|---|---|---|---|---|---|
| | Species[b]-status | BA ($m^2$/ha) | Diameter (cm) | Height (m) | Age |
| Arizona, thinned June, 2020, no burn 35°12.980′ N; 112°16.243′ W; Elevation (m): 1980 | PP-live | 15.3 | 40.4 (24.1–68.1) | 16.5 (10.2–24.2) | 101[c] |
| | AJ-live | 3.67 | 22.5 (16.8–34.5) | 6.4 (17.8–28.4) | 125 |
| California, burned 6 July, 2020; 41°2.789′ N; 120°50.740′ W; Elevation (m): 1689 | PP-dead | 13.8 | 40.3 (13.7–86.1) | 17.3 (9.7–27.1) | 235 |
| | WJ-dead | 1.8 | 42.4 (31.8–64.8) | 11.3 (8.3–16.0) | - |
| Oregon, burned 16 August, 2020; 44°28.094′ N; 121°33.272′ W; Elevation (m): 1165 | PP-live | 0.7 | 57.7 (49.8–68.8) | 28.3 (20.9–34.0) | 66 |
| | PP-dead | 9.4 | 35.8 (9.7–86.4) | 16.0 (4.0–28.7) | - |
| | DF-live | 0.7 | 55.0 (40.6–68.3) | 26.4 (20.7–32.4) | 105 |
| | DF-dead | 2.5 | 42.8 (5.6–61.0) | 20.0 (4.0–25.7) | - |
| | GF-live | - | - | - | - |
| | GF-dead | 0.7 | 20.2 (15.0–24.4) | 11.4 (8.2–13.7) | 64 |
| | WL-live | 0.9 | 42.2 (29.8–62.2) | 26.9 (23.1–33.5) | 99 |
| | WL-dead | 0.2 | 32.5 | 21.0 | - |
| | WJ-live | - | - | - | - |
| | WJ-dead | 0.2 | 53.9 | 12.6 | - |

[a]Tree metrics are means (ranges) obtained from 10 systematically positioned variable radius plots overlapping the study area using a 10 basal area factor prism. All trees within each plot were measured for diameter at 1.4 m, and height using an Impulse laser range finder (Laser Technology Inc., Englewood, CO). In Arizona, the largest diameter ponderosa pine on each plot was cored to the pith at 1.4 m with a 5 mm diameter increment borer and growth rings counted in the lab. In California and Oregon, the age was estimated for one tree only because xylem tissue in burned trees was dry and crumbly. The California PP-live age is from one 79.8 cm diameter unburned tree outside the fire boundary.
[b]PP, *Pinus ponderosa*, Douglas ex P. Lawson & C. Lawson; AJ, *Juniperus deppeana* Steud.; WJ, *Juniperus occidentalis* Hook.; DF, *Pseudotsuga menziesii* (Mirbel) Franco; GF, *Abies grandis* (Douglas ex D. Don) Lindley; WL, *Larix occidentalis* Nutt.
[c]The Arizona PP-live age ranged from 64 to 353 years from 10 trees, the AJ-live age ranged from 105 to 176 with 5 trees cored.

California lures were regrouped by type in a plastic bag in separate coolers with ice and returned to the 5°C cold room later in the day and held overnight. Arizona mixing jars were regrouped by lure type in boxes with frozen cold packs, held overnight and transported by vehicle for 33 h to the laboratory and stored overnight in the cold room. The next day LDPE dispensers and ethanol pouches were adjusted briefly to room temperature and reweighed for calculating release rates.

## Study sites and trial designs

The Oregon trial was located approximately 19 km north of Sisters, Oregon, in a ponderosa pine stand burned by wildfire the previous summer, see Table 1 for details. It was setup in a randomized complete block design with 12 blocks each having six randomly assigned lures, (+)-3-carene, (+)-3-carene+ethanol, (−)-β-pinene, (−)-β-pinene+ethanol, ethanol, or a blank jar attached to 16-unit multiple-funnel traps. Blocks were a line of traps separated by a minimum of 50 m, and each trap was at least 3 m from potential host trees. Blocks were a minimum of 50 m apart. Traps were secured to a metal rod stand, with catch cup bottoms 10–30 cm above the forest floor. Catch cups contained marine antifreeze with propylene glycol (Prime Guard, Highline Warren, Memphis, Tennessee, or Winter-eez, South Win. LTD, Greensboro, NC) to kill and preserve beetles. Lure jars were attached to the 6th funnel above the catch cup on 28 April, 2021, beetles were collected on 11 and 28 May, and 21 June when lures were removed, 54 trap days total.

The California field trial was located approximately 71 km north of Susanville, California, in a ponderosa pine stand burned by wildfire the previous year, see Table 1 for details. It was setup with the same randomized complete block design with randomly assigned lures as in Oregon. All parameters were the same except a 30 m minimum distance between blocks and traps within blocks, to ensure all were located within the burned area. Lure jars were attached on 6 May 2021, beetles were collected 24 May and 15 June when lures were removed, 40 trap days total.

The Arizona test was located approximately 8 km southwest of Williams, Arizona. Unlike Oregon and California, there was no previous year prescribed or wildfire burn sites readily accessible, so the test was conducted in a ponderosa pine stand thinned the previous year, with the woody debris collected and piled throughout the site, see Table 1 for details. The design and distances were the same as in Oregon, but only four randomly assigned lures in each block, (+)-3-carene, (+)-3-carene+ethanol, (−)-β-pinene, (−)-β-pinene+ethanol. The multiple-funnel traps were shorter with five larger diameter funnels (Synergy Semicochemicals Corp.) rather than 16 funnels at the other two sites. Lure jars were attached to the second funnel above the catch cup on 3 May 2021, with beetles collected 13 and 24 May and 9 June when lures were removed, 37 trap days total.

## Statistical analysis

Each site had one or more lure treatments with 11 lure replicates rather than 12 because of interfering factors during the tests. Physical evidence indicated the presence of elk (*Cervus elaphus*) near Oregon traps throughout the test period, resulting in loss of catch data from four separate blocks; one trap with ethanol, one with β-pinene, and two blank traps with no lures. Elk activity in Arizona also caused a loss of catch data from one trap baited with β-pinene. In California, sustained high wind or intermittent wind gusts dislodged catch cups from two traps, one baited with 3-carene and the other with β-pinene.

Due to site differences in lure releases and trapping periods the data from all three sites were separately analysed using SAS software, version 9.4 (SAS, 2014). Assumptions of normality and equal variance of residuals were checked during analysis through quantile-quantile and residual versus predicted plots, respectively. Where necessary to meet these assumptions, the data were natural log transformed. For all analyses the means were compared by Fisher's protected ($\alpha \leq 0.05$) least significant difference. Means were considered significantly different at $p \leq 0.05$, but most were lower and reported in the graph legends.

β-pinene, 3-carene, and ethanol daily release rates within each site were separately analysed as a randomized complete block analysis of variance (ANOVA) using the mixed model procedure (proc MIXED). Block was modelled as a random effect, and lure as a fixed effect, with no data transformation required to meet model assumptions. Lure jar average, minimum, and maximum daily temperatures were analysed similarly by the mixed model procedure, with no transformations necessary.

At each site the total *D. valens* catch by each trap over the entire trap period was analysed by a generalized mixed models ANOVA (proc GLIMMIX) using a Poisson distribution for count data with a log link function to meet model assumptions for normality and equal variance. Block was modelled as a random effect and lure as a fixed effect. The means and 95% confidence limits were back-transformed for presentation.

# RESULTS

## Lure release rates and temperatures

3-Carene 24 h release rates with or without ethanol were greater than β-pinene with or without ethanol at each site and all within site differences were statistically significant (Figure 1). β-Pinene release was faster from the Oregon β-pinene+ethanol lures than from the β-pinene only lure, but not in California, or Arizona (Figure 1). The 3-carene release was also higher from 3-carene+ethanol than 3-carene only lures in Oregon and California, where both differences were statistically significant (Figure 1a,b), but not in Arizona (Figure 1c). Ethanol pouch release rates were similar among lures at each site, with no statistical differences among them (Figure 1).

β-pinene, 3-carene, and ethanol release rates, and mean average daily temperature increased as the site latitude progressed to the south. They were all lowest in Oregon, intermediate in California, and highest in Arizona, but these differences among sites were not compared statistically (Figure 1). Lure temperatures within each site were not statistically different, although the daily average for monoterpene+ethanol lures in Oregon and California were slightly higher (13.0, 15.0°C; respectively), than their corresponding lures with monoterpenes only (12.6, 14.6°C; respectively).

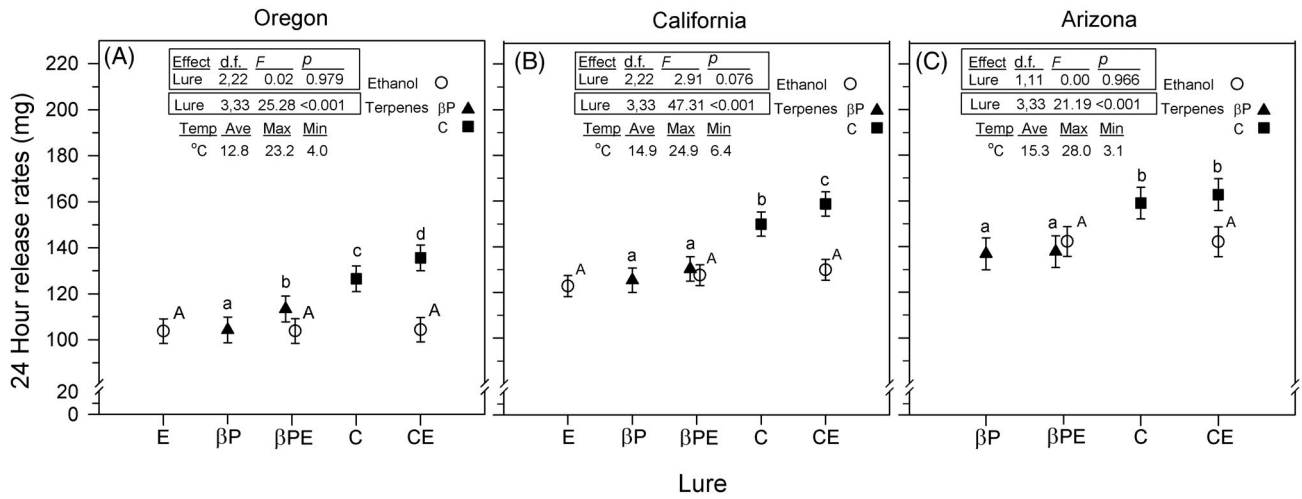


**FIGURE 1** Twenty-four-hour release rates (means ±95% CI) for ethanol, β-pinene and 3-carene from each lure type. Box inserts show separate ANOVA results for ethanol and monoterpenes, E, ethanol; βP, β-pinene; C, 3-carene. (a) Oregon monoterpene symbols with different letters indicate significant statistical differences (SSD) at $p \leq 0.026$ for all comparisons. (b) California monoterpene symbols with different letters indicate SSD at $p \leq 0.011$ for all comparisons, and those with the same letters are not different at $p = 0.139$. (c) Arizona monoterpene symbols with different letters indicate SSD at $p \leq 0.001$ for all comparisons, and those with the same letters are not different at $p \geq 0.385$. Ethanol release rates within each site were not statistically different among any lure types as indicated by the ANOVA table $p$ values. Ethanol symbols for βPE lures were offset slightly for clarity. Temperatures are the mean daily average, maximum, and minimum across the four lures releasing monoterpenes at each site.

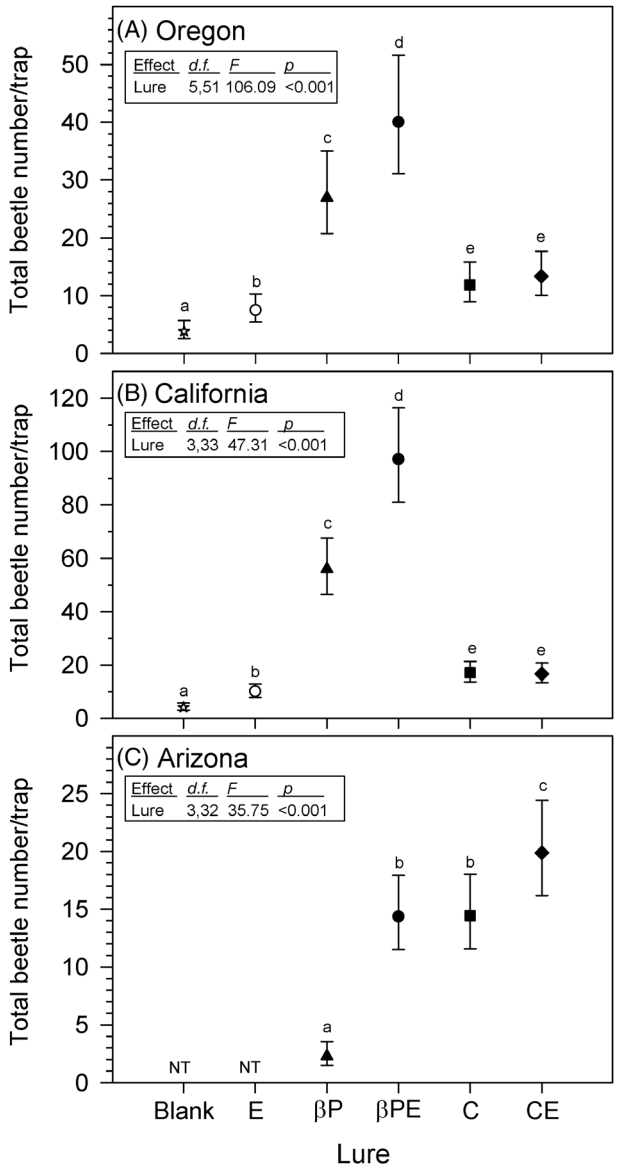


**FIGURE 2** The *Dendroctonus valens* total beetle number/trap (means ±95% CI) for lures at each test site plotted on different scales. Box inserts show the ANOVA results, E, ethanol; βP, β-pinene; C, 3-carene; NT, not tested. (a) Oregon lure symbols with different letters indicate significant statistical differences (SSD) at $p \leq 0.001$ for all comparisons, and those with the same letters are not different at $p \geq 0.297$. (b) California lure symbols with different letters indicate SSD at $p \leq 0.001$ for all comparisons, and those with the same letters are not different at $p = 0.798$. (c) Arizona lure symbols with different letters indicate SSD at $p \leq 0.003$ for all comparisons, and those with the same letters are not different at $p \geq 0.958$

### Beetle responses

Total beetle catch was lowest in Arizona (625), intermediate in Oregon (1305) and highest in California (2415). The *D. valens* responses in Oregon (Figure 2a) and California (Figure 2b) were very similar, where β-pinene+ethanol was the most attractive lure with catch numbers statistically higher than all the others. At both sites β-pinene was the second most attractive, its beetle catch was also statistically greater than 3-carene and 3-carene+ethanol, with no difference in the low beetle numbers for the latter two. Traps baited with ethanol caught more beetles than the blank, but lower numbers statistically than lures with either monoterpene present. Arizona *D. valens* responses were distinctly different from Oregon and California because 3-carene+-ethanol attracted greater beetle numbers statistically than either 3-carene or β-pinene+ethanol with nearly identical catches, whereas β-pinene lures attracted the fewest beetles (Figure 2c).

## DISCUSSION

The *D. valens* trap catch increases as β-pinene release rates rise (Hobson et al., 1993), or the combined release rates of β-pinene+ethanol increase by changing either one or both components (Kelsey & Westlind, 2022). Therefore, when comparing monoterpene lure attraction strengths the release rates need to be considered. 3-Carene was released 1.2 times faster than β-pinene at all sites when matching both lures without ethanol, or both with ethanol; a result of 3-carene diffusing more than two times faster than β-pinene through the LDPE bottle (Kelsey &Westlind, 2020). Although all β-pinene lures had two bottles to compensate, their combined releases were still slightly lower than one bottle of 3-carene. The monoterpene release from β-pinene+ethanol lures in Oregon, and 3-carene+ethanol lures in Oregon and California was 1.1 times higher than from their corresponding lures without ethanol. This is attributed to their additional mass from ethanol pouches keeping the lure mixing jars mean daily average temperatures slightly higher, in the same way, release rates for all lure components increased as the mean daily average site temperatures climbed. Although lure release rate differences within sites could have influenced some beetle responses they are not considered a major contributing factor to the large catch differences among lures at any site, except for between each respective monoterpene with or without ethanol.

Variability in the *D. valens* responses among lures was most notable in Arizona compared to Oregon and California where their behaviours toward all lures were similar. In Arizona β-pinene was a weaker attractant than both 3-carene lures; 3-carene+ethanol captured the most beetles, 1.4 times more than β-pinene+ethanol or 3-carene with similar catches. This contrasts with Oregon and California where 3-carene+-ethanol and 3-carene had similar low beetle catches to both β-pinene lures, just 33% and 30% of the Oregon β-pinene+ethanol catch, respectively, and 17% and 18% of the numbers attracted to California β-pinene+ethanol, respectively. These variable responses could be due to the presence of other influential chemical or physical signals in the environment, olfactory detection differences in the sensilla on the antennae, and/or dissimilar higher-order neuron processing in the brain.

The environment and type of host materials in Arizona were distinctly different. It was ponderosa thinned the previous year and unburned, with stumps and piles of woody slash as host materials. This contrasts with burned Oregon and California ponderosa pine sites with fire-injured host trees only, with no stumps or slash. The Arizona environment and beetle response were similar to an earlier Oregon test, also thinned the prior year with stumps and piles of

woody slash scattered throughout, where beetles likewise responded more strongly to 3-carene+ ethanol than 3-carene; β-pinene and β-pinene+ethanol were not tested (Kelsey & Westlind, 2017b). Similar geographic variability was observed for α-pinene attraction to wood-boring beetles (Miller, 2006) and several conifer bark beetles when tested against α-pinene+ethanol and ethanol alone in southeastern forests (Miller & Rabaglia, 2009). They proposed site differences, such as the primary host species, possible competing sources of ethanol or α-pinene, local weather, or dampening of lure emissions by associated vegetation as potential influences on beetle responses. For *D. valens*, the contrast between burned and thinned-unburned ponderosa pine forests with distinctly different stressed hosts materials provide an opportunity to gain further insight into whether such disparate environments influence their primary attraction to 3-carene and β-pinene, with or without ethanol. However, both recent and historical wild and prescribed fires in Arizona (Covington & Moore, 1994; Singleton et al., 2019) should have provided similar selective pressure on *D. valens* for attraction to recently burned trees as observed in Pacific Northwest forests (Kelsey & Westlind, 2017a; Westlind & Kelsey, 2019).

Regional differences in host ponderosa pine resin monoterpenes might influence the Arizona responses but seems unlikely, as they do not align with the beetles corresponding response changes. Monoterpenes have been analysed for 10 populations across northeastern Washington, central Oregon and northeastern California in the general vicinity of our trap sites (Hobson et al., 1993; Smith, 1977; Sturgeon, 1979). 3-Carene is the most abundant ranging from about 37% to 64% and β-pinene 9% to 29% (Smith, 1977), with phloem proportions for both in the same ranges at one Washington and one Oregon site (Kelsey & Westlind, 2020; Table S3). Yet, β-pinene or β-pinene+-ethanol attracts more beetles than 3-carene or 3-carene+ethanol across this region (Hobson et al., 1993; Kelsey & Westlind, 2020, results here). In Arizona, α-pinene is typically the most abundant xylem resin monoterpene in ponderosa pine (Smith, 1977). In two populations nearby the Arizona test site it ranges from 43% to 50%, 3-carene from 27% to 35%, and β-pinene at 5% to 6% (Smith, 1977). Phloem proportions for α-pinene and β-pinene are about the same, and 3-carene lower at 19% in another nearby stand (Kolb et al., 2019). Although 3-carene does occur at higher amounts than β-pinene in some Arizona trees and populations, the greater abundance of α-pinene and substantial variation in proportions of all three compounds makes it seem unlikely the host resin composition is a critical element for stronger *D. valens* attraction to lures with 3-carene over β-pinene. Furthermore, at sites with different pine host species and resin components in California, Wisconsin, Pennsylvania, and China, 3-carene was a stronger attractant than β-pinene, (+)-α-pinene, and (−)-α-pinene (Erbilgin et al., 2007; Sun et al., 2004).

Genetic differences in Arizona *D. valens* are another factor potentially influencing response changes in Oregon and California. Recently four genetically and geographically distinct *D. valens* populations were recognized in North America (West, Central, Northeast and Mexico) using microsatellite markers to determine population structure (Taerum et al., 2016). Beetles in the West population were collected from Washington, Idaho, Montana, Oregon, California, and Arizona. Although Arizona was included, the principal components analysis plots indicated it was the least similar to beetles at the other five western sites.

Our Oregon site here is the first β-pinene test in a Pacific Northwest forest, with a catch 2.3 times higher than 3-carene, whereas in California it was 3.2 times higher than 3-carene, and similar to results by Hobson et al. (1993) in California. Their β-pinene catches were 5.4 and 10.7 times greater than 3-carene in separate tests, and the response was log linear with increasing doses of β-pinene. But β-pinene in Arizona was a weak attractant here, with 3-carene capturing 8.6 times more beetles, a relationship also observed at multiple sites across California, Wisconsin, Pennsylvania, and China (Erbilgin et al., 2007; Sun et al., 2004).

β-Pinene+ethanol produced the most consistent beetle response across all three test sites, although in Arizona the 3-carene+ethanol caught more beetles. Oregon and California catches were 3 and 5.8 times higher than 3-carene+ethanol, respectively, a response pattern observed before in Oregon and Washington (Kelsey & Westlind, 2020). As in a previous test (Kelsey & Westlind, 2022) the higher combined release of β-pinene+ethanol improved attraction strength over β-pinene alone by 1.5, 1.5, and 6.2 times in Oregon, California, and Arizona, respectively, and all were likely synergistic increases. As mentioned, a similar attraction consistency by (−)-α-pinene+ethanol over (−)–αpinene was detected for numerous wood-boring beetles (Miller, 2006) and conifer bark beetles (Miller & Rabaglia, 2009) at multiple trap sites in southeastern US forests.

Ethanol alone produces a weak *D. valens* response, attracting a few more beetles than a blank trap with no lure (Kelsey & Westlind, 2017b; results here), and consistently enhanced the *D. valens* capture of β-pinene+ethanol over β-pinene at all sites. It remains unknown whether ethanol influences β-pinene electrophysiological detection in hairlike sensilla on antennae, or some other higher-order neuron processing in the brain. But comparable responses have been examined more thoroughly for fruit flies, *Drosophila melanogaster*, that exhibit a variety of ethanol-influenced behaviours (reviewed by Park et al., 2017). The flies responded more strongly to farnesol (a component of citrus odour) +- ethanol than to either chemical alone (Park et al., 2020), similar to *D. valens* responses to pine kairomones+ethanol. These investigators showed that brief ethanol exposure activated neuronal detection of subsequent ethanol or farnesol exposure by chemosensory neurons sensitive to farnesol. They also revealed similar effects of ethanol on the activity of neurons tuned to the detection of a fly pheromone. This ethanol-enhanced neuronal activity for both compounds was considered olfactory rather than systemic (Park et al., 2020). The mechanism by which ethanol causes these effects remains a mystery. Furthermore, ethanol in pines, aside from contributing to *D. valens* host detection and selection may provide these beetles other benefits such as increased larval fitness as demonstrated for fruit flies (Schumann et al., 2021) or increased brood production as shown for an ambrosia beetle (Ranger et al., 2018), and are worthy of further investigation.

In conclusion, our three study sites were in the genetically similar *D. valens* West population (Taerum et al., 2016) and beetles at all sites exhibited a strong, consistent response to β-pinene+ethanol, confirming this behaviour is not localized to the Pacific Northwest. This

makes it a reliable lure for detection, monitoring, and management projects for *D. valens* across its West population range, and a reasonable initial choice for any rapid detection programs elsewhere as in the Southern hemisphere where pine plantations could be at risk of invasion from North America. Whether this same response occurs in China, because its invasion was likely by beetles from the West population (Cai et al., 2008; Cognato et al., 2005; Taerum et al., 2016; Yan et al., 2005), or for members in the other three North American populations, awaits testing. If there are differences it might provide new insight into what North American population was the original source of invaders to China, as genetic studies of the beetles and their symbiont assemblages support opposing invasion scenarios (Cai et al., 2008; Cognato et al., 2005; Taerum et al., 2013, 2016). Further studies are needed to evaluate whether host ethanol contents provide *D. valens* with increased larval fitness or greater brood production, in addition to how it is detected by neurons in sensilla or brain. Additional trials with lures in thinned vs burned pine stands can provide insight into what extent, if any, such extreme environmental and host condition differences contributed to observed variation in their primary attraction to 3-carene and β-pinene with or without ethanol.

## ACKNOWLEDGMENTS

The authors thank R. Wolfenberger at the Ponderosa Land & Cattle Company for access to the Green Ridge Fire study site in Oregon, A. Shoaf at the Big Valley Ranger District in the Modoc National Forest for access to the California study site, and J. Ouzts at the Williams Ranger District in the Kaibab National Forest for access to the Arizona site. We also thank D. Mikowski, J. McMillin, and D. DePinte for assistance in the field, E. Sautter for field and dendrochronology assistance in Arizona, and D. Shaw for a review of the manuscript.

## CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

## DATA AVAILABILITY STATEMENT

Data available on request from the authors.

## REFERENCES

Abatzoglou, J.T. & Williams, A.P. (2016) Impact of anthropogenic climate change on wildfire across western US forests. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 11770–11775.

Balch, J.K., Bradley, B.A., Abatzoglou, J.T., Nagy, R.C., Fusco, E.J. & Mahood, A.L. (2017) Human-started wildfires expand the fire niche across the United States. *Proceedings of the National Academy of Sciences of the United States of America*, 114, 2946–2951.

Barbero, R., Abatzoglou, J.T., Larkin, N.K., Kolden, C.A. & Stocks, B. (2015) Climate change presents increased potential for very large fires in the contiguous United States. *International Journal of Wildland Fire*, 24, 892–899.

Cai, Y.W., Cheng, X.Y., Xu, R.M., Duan, D.H. & Kirkendall, L.R. (2008) Genetic diversity and biogeography of red turpentine beetle *Dendroctonus valens* in its native and invasive regions. *Insect Sci.*, 15, 291–301.

Cheng, C., Wickham, J.D., Chen, L., Xu, D., Lu, M. & Sun, J. (2018) Bacterial microbiota protect an invasive bark beetle from a pine defensive compound. *Microbiome*, 6, 1–16.

Cheng, C., Xu, L., Xu, D., Lou, Q., Lu, M. & Sun, J. (2016) Does cryptic microbiota mitigate pine resistance to an invasive beetle-fungus complex? Implications for invasion potential. *Scientific Reports*, 6, 1–12.

Cognato, A.I., Sun, J.-H., Anducho-Reyes, M.A. & Owen, D.R. (2005) Genetic variation and origin of red turpentine beetle (*Dendroctonus valens* LeConte) introduced to the People's Republic of China. *Agricultural and Forest Entomology*, 7, 87–94.

Covington, W.W. & Moore, M.M. (1994) Postsettlement changes in natural fire regimes and forest structure. Ecological restoration of old-growth ponderosa pine forests. *Journal of Sustainable Forestry*, 2, 153–181.

Erbilgin, N., Mori, S.R., Sun, J.H., Stein, J.D., Owen, D.R., Merrill, L.D. et al. (2007) Response to host volatiles by native and introduced populations of *Dendroctonus valens* (Coleoptera: Curculionidae, Scolytinae) in North America and China. *Journal of Chemical Ecology*, 33, 131–146.

Fettig, C.J., Borys, R.R., McKelvey, S.R. & Dabney, C.P. (2008) Black mountain experimental forest: bark beetle responses to differences in forest structure and the application of prescribed fire in interior ponderosa pine. *Canadian Journal of Forest Research*, 38, 924–935.

Ganz, D.J., Dahlsten, D.L. & Shea, P.J. (2003) The post-burning response of bark beetles to prescribed burning treatments. In: Omi, P.N. & Joyce, L.A. (Eds.) *Fire, fuel treatments, and ecological restoration: conference proceedings, Rocky Mountain Research Station proceedings RMRS-P-29*. Fort Collins, CO: U. S. Department of Agriculture Forest Service, pp. 143–158.

Gara, R.I., Littke, W.R. & Rhoades, D.F. (1993) Emission of ethanol and monoterpenes by fungal infected lodgepole pine trees. *Phytochemistry*, 34, 987–990.

Haack, R.A. & Petrice, T.R. (2009) Bark- and wood-borer colonization of logs and lumber after heat treatment to ISPM 15 specifications: the role of residual bark. *Journal of Economic Entomology*, 102, 1075–1084.

Hobson, K.R., Wood, D.L., Cool, L.G., White, P.R., Ohtsuka, T., Kubo, I. et al. (1993) Chiral specificity in responses by the bark beetle *Dendroctonus valens* to host kairomones. *Journal of Chemical Ecology*, 19, 1837–1846.

Joseph, G., Kelsey, R.G., Peck, R.W. & Niwa, C.G. (2001) Response of some scolytids and their predators to ethanol and 4-allylanisole in pine forests of Central Oregon. *Journal of Chemical Ecology*, 27, 697–715.

Kelley, S.T. & Farrell, B.D. (1998) Is specialization a dead end? The phylogeny of host use in *Dendroctonus* bark beetles (Scolytidae). *Evolution*, 52, 1731–1743.

Kelsey, R.G. & Westlind, D.J. (2017a) Ethanol and primary attraction of red turpentine beetle in fire stressed ponderosa pine. *Forest Ecology and Management*, 396, 44–54.

Kelsey, R.G. & Westlind, D.J. (2017b) Attraction of red turpentine beetle and other Scolytinae to ethanol, 3-carene or ethanol+3-carene in an Oregon pine forest. *Agricultural and Forest Entomology*, 20, 272–278.

Kelsey, R.G. & Westlind, D.J. (2020) Red turpentine beetle primary attraction to (−)-β-pinene+ethanol in US Pacific northwest ponderosa pine forests. *PLoS One*, 15, e0236276.

Kelsey, R.G. & Westlind, D.J. (2021) Key structural features in *cis*-carane, (+)-3-carene, *cis*-pinane, (+)-α-pinene, and (−)-β-pinene influencing red turpentine beetle primary attraction when released with ethanol. *Agricultural and Forest Entomology*, 23, 243–249.

Kelsey, R.G. & Westlind, D.J. (2022) Red turpentine beetle primary attraction increases linearly with (−)-β-pinene+ethanol dose regardless of component ratios, and no change in response with addition of high-release frontalin. *Agricultural and Forest Entomology*, 24, 63–71.

Klepzig, K.D., Raffa, K. & Smalley, E. (1991) Association of an insect-fungal complex with red pine decline in Wisconsin. *Forest Science*, 37, 1119–1139.

Kolb, T., Keefover-Ring, K., Burr, S.J., Hofstetter, R., Gaylord, M. & Raffa, K.F. (2019) Drought-mediated changes in tree physiologicl processes weaken tree defenses to bark beetle attack. *Journal of Chemical Ecology*, 45, 888–900.

Kolden, C.A. (2019) We're not doing enough prescribed fire in the Western United States to mitigate wildfire risk. *Fire*, 2, 30.

Lantschner, M.V., Atkinson, T.H., Corley, J.C. & Liebhold, A.M. (2017) Predicting North American Scolytinae invasions in the Southern Hemisphere. *Ecological Applications*, 27, 66–77.

Liebhold, A.M., Brockerhoff, E.G., Kalisz, S., Nuñez, M.A., Wardle, D.A. & Wingfield, M.J. (2017) Biological invasions in forest ecosystems. *Biological Invasions*, 19, 3437–3458.

Littell, J.S., McKenzie, D., Peterson, D.L. & Westerling, A.L. (2009) Climate and wildfire area burned in western US ecoprovinces, 1916–2003. *Ecological Applications*, 19, 1003–1021.

Littell, J.S., Peterson, D.L., Riley, K.L., Liu, Y. & Luce, C.H. (2016) A review of the relationships between drought and forest fire in the United States. *Global Change Biology*, 22, 2353–2369.

Liu, F., Wickham, J.D., Cao, Q., Lu, M. & Sun, J. (2020) An invasive beetle–fungus complex is maintained by fungal nutritional-compensation mediated by bacterial volatiles. *The ISME Journal*, 14, 2829–2842.

Liu, Z., Xu, B., Miao, Z. & Sun, J. (2013) The pheromone frontalin and its dual function in the invasive bark beetle *Dendroctonus valens*. *Chemical Senses*, 38, 485–495.

Lu, Q., Decock, C., Zhang, X.Y. & Maraite, H. (2009) Ophiostomatoid fungi (Ascomycota) associated with *Pinus tabuliformis* infested by *Dendroctonus valens* (Coleoptera) in northern China and an assessment of their pathogenicity on mature trees. *Antonie Van Leeuwenhoek*, 96, 275–293.

Lu, M., Wingfield, M.J., Gillette, N.E., Mori, S.R. & Sun, J.H. (2010) Complex interactions among host pines and fungi vectored by an invasive bark beetle. *New Phytologist*, 187, 859–866.

Lu, M., Wingfield, M.J., Gillette, N. & Sun, J.H. (2011) Do novel geno-types drive the success of an invasive bark beetle–fungus complex? Implications for potential reinvasion. *Ecology*, 92, 2013–2019.

Lu, M., Zhou, X., De Beer, Z.W., Wingfield, M.J. & Sun, J.H. (2009) Ophiostomatoid fungi associated with the invasive pine-infesting bark beetle, *Dendroctonus valens*, in China. *Fungal Diversity*, 38, 133–145.

Melvin, M. (2018) *Prescribed fire use survey report. National prescribed fire use survey report 03–18*. Newton, GA: Coalition of Prescribed Fire Councils, Inc, p. 23.

Meurisse, N., Rassati, D., Hurley, B.P., Brockerhoff, E.G. & Haack, R.A. (2019) Common pathways by which non-native forest insects move internationally and domestically. *Journal of Pest Science*, 92, 13–27.

Miller, D.M. (2006) Ethanol and (-)-α-pinene: attractant kairomones for some large wood-boring beetles in southeastern USA. *Journal of Chemical Ecology*, 32, 779–794.

Miller, D.R. & Rabaglia, R.J. (2009) Ethanol and (−)-α-pinene: attractant kairomones for bark and ambrosia beetles in the southeastern US. *Journal of Chemical Ecology*, 35, 435–448.

Owen, D.R., Smith, S.L. & Seybold, S.J. (2010) *Red turpentine beetle*. *USDA Forest Service*, *Forest insect and disease leaflet 55*, USDA Forest Service, Pacific Northwest Region (R6), Portland, OR.

Owen, D.R., Wood, D.L. & Parmeter, J.R. (2005) Association between *Dendroctonus valens* and black stain root disease on ponderosa pine in the Sierra Nevada of California. *The Canadian Entomologist*, 137, 367–375.

Park, A., Ghezzi, A., Wijesekera, T.P. & Atkinson, N.S. (2017) Genetics and genomics of alcohol responses in Drosophila. *Neuropharmacology*, 122, 22e35.

Park, A., Tran, T., Scheuermann, E.A., Smith, D.P. & Atkinson, N.S. (2020) Alcohol potentiates a pheromone signal in flies. *eLife*, 9, e59853.

Peterson, D.L. & Littell, J.S. (2014) Risk assessment for wildfire in the western United States. In: Peterson, D.L., Vose, J.M. & Patel-Weynand, T. (Eds.) *Climate change and United States forests, advances in global change research*, Vol. 57. New York, NY: Springer, pp. 232–235.

Ranger, C.M., Biedermann, P.H.W., Phuntumart, V., Beligala, G.U., Ghosh, S., Palmquist, D.E. et al. (2018) Symbiont selection via alcohol benefits fungus farming by ambrosia beetles. *The Proceedings of the National Academy of Sciences of the United States of America*, 115, 4447–4452.

SAS. (2014) *Base SAS 9.4 procedures guide*. Cary, NC: SAS Institute Inc.

Schumann, I., Berger, M., Nowag, N., Schäfer, Y., Saumweber, J., Scholz, H. et al. (2021) Ethanol-guided behavior in *Drosophila* larvae. *Scientific Reports*, 11, 12307.

Singleton, M.P., Thode, A.E., Meador, A.J.S. & Iniguez, J.M. (2019) Increasing trends in high-severity fire in the southwestern USA from 1984 to 2015. *Forest Ecology and Management*, 433, 709–719.

Smith, R.H. (1977) *Monoterpenes of ponderosa pine xylem resin in western United States*. Technical Bulletin No. 1532. U.S. Department of Agriculture Forest Service.

Sturgeon, K.B. (1979) Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. *Evolution*, 33, 803–814.

Sun, J., Lu, M., Gillette, N.E. & Wingfield, M.J. (2013) Red turpentine beetle: innocuous native becomes invasive tree killer in China. *Annual Review of Entomology*, 58, 293–311.

Sun, J., Miao, Z., Zhang, Z., Zhang, Z. & Gillette, N.E. (2004) Red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Scolytidae), response to host semiochemicals in China. *Environmental Entomology*, 33, 206–212.

Taerum, S.J., Duong, T.A., de Beer, Z.W., Gillette, N., Sun, J.H., Owen, D.R. et al. (2013) Large shift in symbiont assemblage in the invasive red turpentine beetle. *PLoS One*, 8, e78126.

Taerum, S.J., Konečný, A., De Beer, Z.W., Cibrián-Tovar, D. & Wingfield, M.J. (2016) Population genetics and symbiont assemblages support opposing invasion scenarios for the red turpentine beetle (*Dendroctonus valens*). *Biological Journal of the Linnean Society*, 118, 486–502.

Westerling, A.L. (2016) Increasing western US forest wildfire activity: sensitivity to changes in the timing of spring. *Philosophical Transactions of the Royal Society B Biological Sciences*, 371, 20150178.

Westlind, D.J. & Kelsey, R.G. (2019) Predicting post-fire attack of red turpentine or western pine beetle on ponderosa pine and its impact on mortality probability in Pacific Northwest forests. *Forest Ecology and Management*, 434, 181–192.

Williams, A.P., Abatzoglou, J.T., Gershunov, A., Guzman-Morales, J., Bishop, D.A., Balch, J.K. et al. (2019) Observed impacts of anthropogenic climate change on wildfire in California. *Earth's Future*, 7, 892–910.

Xu, L.T., Lu, M. & Sun, J.H. (2016) Invasive bark beetle-associated microbes degrade a host defensive monoterpene. *Journal of Insect Science*, 23, 183–190.

Yan, Z., Sun, J., Don, O. & Zhang, Z. (2005) The red turpentine beetle, *Dendroctonus valens* LeConte (Scolytidae): an exotic invasive pest of pine in China. *Biodiversity and Conservation*, 14, 1735–1760.

Zhu, J., Rasmussen, J.G., Møller, J., Aukema, B.H. & Raffa, K.F. (2008) Spatial-temporal modeling of forest gaps generated by colonization from below- and above-ground bark beetle species. *Journal of the American Statistical Association*, 103, 162–177.

**How to cite this article:** Kelsey, R.G., Westlind, D.J. & Gaylord, M.L. (2023) Red turpentine beetle primary attraction to β-pinene or 3-carene (with and without ethanol) varies in western US pine forests. *Agricultural and Forest Entomology*, 25(1), 111–118. Available from: https://doi.org/10.1111/afe.12535