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Mountain pine beetle host selection behavior confirms high resistance in Great Basin bristlecone pine

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ABSTRACT

Over the last two decades, mountain pine beetle (*Dendroctonus ponderosae*) populations reached epidemic levels across much of western North America, including high elevations where cool temperatures previously limited mountain pine beetle persistence. Many high-elevation pine species are susceptible hosts and experienced high levels of mortality in recent outbreaks, but co-occurring Great Basin bristlecone pines (*Pinus longaeva*) were not attacked. Using no-choice attack box experiments, we compared Great Basin bristlecone pine resistance to mountain pine beetle with that of limber pine (*P. flexilis*), a well-documented mountain pine beetle host. We confined sets of mountain pine beetles onto 36 pairs of living Great Basin bristlecone and limber pines and recorded beetle status after 48 h. To test the role of induced defenses in Great Basin bristlecone pine resistance, we then repeated the tests on 20 paired sections of Great Basin bristlecone and limber pines that had been recently cut, thereby removing their capacity for induced defensive reactions to an attack. In tests on cut trees, we also investigated the potential for population-level differences in mountain pine beetle host selection behavior by testing beetles from two separate geographic regions. Beetles placed on Great Basin bristlecone pine rarely initiated attacks relative to those placed on limber pine in both studies, regardless of the beetle population source. Our results indicate that Great Basin bristlecone pine has a high level of resistance to mountain pine beetle due at least in part to stimuli that repel pioneering attackers from initiating attacks, even when induced defenses are compromised.

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1. Introduction

Sustainable forest management in the face of climate change requires predictions of how shifting natural disturbance regimes will impact forest environments (Dale et al., 2001). Bark beetles (Coleoptera: Curculionidae, Scolytinae), particularly 'aggressive' species that can attack and kill living trees, are important natural disturbance agents in western North American forests (Hicke et al., 2015). Due to the strong relationship between thermal conditions and bark beetle population success (Safranyik and Carroll, 2006; Powell and Bentz, 2009), climate-induced changes in native bark beetle outbreaks are a major concern for land managers. Warmer than average temperatures have the potential to improve winter survival, speed lifecycle completion, and allow for range expansion into areas where outbreaks were previously limited by cold (Bentz et al., 2010; Sambaraju et al., 2012; Weed et al., 2015). In addition to favorable climate conditions, access to host resources is required for bark beetle outbreaks. Host trees that are unable to resist attacks can be killed and used for bark beetle reproduction and proliferation, but sufficiently resistant trees represent resources that are inaccessible for bark beetle use (Lieutier, 2002). Understanding these important relationships, particularly along expanding latitudinal and elevational range margins, is vital to evaluating stand susceptibility, predicting outbreak development, and planning for forest conservation.

The mountain pine beetle (*Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae, Scolytinae), a native bark beetle that infests most species of pine (*Pinus*) throughout western North America, recently experienced population irruptions that resulted in large-scale outbreaks across its range (Raffa et al., 2008; USDA Forest Service, 2015). In addition to killing millions of acres of lower-elevation lodgepole pine (*Pinus contorta* Douglas), a primary host species, mountain pine beetle caused substantial mortality among high-elevation pines. Although outbreaks at high elevations are not unprecedented (Perkins and Swetnam, 1996), their extent

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has previously been limited by cool temperatures (Amman, 1973; Gibson et al., 2008; Bentz et al., 2011). Therefore, high-elevation pines are hypothesized to be especially susceptible to attacks due to insufficiently coevolved resistance mechanisms (Raffa et al., 2013). Keystone high-elevation species such as whitebark (*P. albicaulis* Engelm.) and limber (*P. flexilis* James) pines have experienced high levels of mountain pine beetle-caused mortality over the past several decades (Macfarlane et al., 2013; Cleaver et al., 2015), but susceptibility has not been shown for all high-elevation pine species. Successful mountain pine beetle attacks on Great Basin bristlecone pine (*P. longaeva* Bailey), an extremely long-lived species found at high elevations in Utah, Nevada and California, have not been documented, despite evidence of extensive mountain pine beetle activity occurring in limber pines within the same stands (Bentz et al., 2016b). With the expectation that climate conditions will continue to support mountain pine beetle success at high elevations throughout this century (Bentz et al., 2016a; Buotte et al., 2016), a better understanding of Great Basin bristlecone pine's apparent resistance to mountain pine beetle is needed for insight into managing these ecosystems.

Tree resistance to the mountain pine beetle involves complex interactions between the insect and the potential host. Mountain pine beetle adults emerge from their natal host trees in midsummer to locate and colonize new hosts for reproduction. Synchronous emergence and dispersal are critical for mountain pine beetle success because high numbers of "mass attacking" beetles are required to deplete the defensive resources of new hosts. In successful attacks, adult beetles bore through the bark of new host trees, mate, and females deposit eggs along vertical galleries in the phloem. After egg hatch, larvae feed and develop in the phloem over the next ~ one to three seasons (Bentz et al., 2014), typically killing the host tree, before completing their life cycles and emerging through the bark as adults (Safranyik and Carroll, 2006). Due to this selective pressure, host trees have evolved defense systems to resist bark beetle use. These systems generally involve a combination of pre-formed constitutive defenses and attack-activated induced defenses that reduce insect colonization success and/or prevent brood development and survival. Constitutive defenses may include either mechanical mechanisms such as physically obstructive compounds built into the bark, or chemical mechanisms such as toxic phloem compounds (Franceschi et al., 2005). Important induced defenses for tree resistance to the mountain pine beetle include toxic resin flow that impedes or kills attacking beetles and hypersensitive phloem reactions that entrap beetles in lesions impregnated with inhibitory compounds (Lieutier, 2002). Relative to limber pine, a well-documented susceptible mountain pine beetle host species, Great Basin bristlecone pine has high concentrations of constitutive chemical phloem compounds (Bentz et al., 2016b) that are associated with tree defense (Raffa, 2014). Information about induced defenses in Great Basin bristlecone pine is lacking. Moreover, the direct impact of Great Basin bristlecone pine defense traits on mountain pine beetle attacks is unknown.

Mountain pine beetles contend with tree defenses through flexible host selection behavior that enhances their chance of successfully colonizing a favorable host (Raffa et al., 2016). Female beetles are the pioneering attackers and therefore play a central role in selecting susceptible hosts and avoiding resistant or otherwise unsuitable trees. Research has shown that mountain pine beetle females use a combination of visual cues and random landings to locate potential hosts (Hynum and Berryman, 1980; Wood, 1982), but tree volatiles also play an important role in host attraction (Moeck and Simmons, 1991). After landing on a potential host, a female decides whether or not to attack based on several factors including short-range olfactory and gustatory cues (Raffa and Berryman, 1982). If the host tree is accepted, the female will proceed to initiate gallery construction in the phloem, emitting aggregation pheromones that can instigate a mass-attack by attracting other adult mountain pine beetles (Safranyik and Carroll, 2006). Appropriate female host selection decisions are critical because accepting unsuitable trees results in reduced survival and reproduction, but prolonged host searching increases exposure to predators, expends energy, and can lead to high intraspecific competition with earlier attackers. Due to these challenges, host acceptance decision-making is not only driven by an assessment of the potential host tree, it is also mediated by individual beetle and population conditions that influence the likelihood or degree of reproductive success (Boone et al., 2011; Chubaty et al., 2014; Burke and Carroll, 2017). Host selection behavioral traits have been shown to have a heritable component in other bark beetle species (Wallin et al., 2002), which may result in varying behavior between populations. Variation in host selection behavior between geographically-separated populations of the same species has been documented in other insect species (Keeler and Chew, 2008), and in some cases insects have the capacity to locally adapt to highly defended host species (Zovi et al., 2008). Understanding mountain pine beetle host selection choices and how they can vary with population is important for understanding the potential for local adaptation to host defenses and for predicting future host tree vulnerability to attack.

Ultimately, pioneering female mountain pine beetles incorporate both internal and external stimuli to choose a host that provides the greatest likelihood of maximizing their reproductive success. Host acceptance therefore would suggest that a tree is both susceptible to successful mountain pine beetle colonization and can support brood development and survival. Host rejection implies that a tree is either highly resistant, poor quality (i.e., thin or low-nutrient phloem), or incompatible with the biological needs of the insect and unlikely to support the goal of reproductive success. The lack of mountain pine beetle attacks observed on Great Basin bristlecone pine (Bentz et al., 2016b) suggests that it falls into one of the latter categories compared to limber pine in mixed stands. However, Great Basin bristlecone pine susceptibility to attack has not been tested when there are no alternative host species present. Additionally, Great Basin bristlecone pine foliage volatiles have been shown to be unattractive to mountain pine beetle (Gray et al., 2015), but it is unknown if the same repellent qualities are present in short-range stimuli from the bole, where mountain pine beetles land to initiate attacks. Furthermore, the roles of constitutive and induced tree defenses and the importance of mountain pine beetle population trait variation in Great Basin bristlecone pine resistance remain unclear.

The goal of our study was to test and characterize Great Basin bristlecone pine resistance to mountain pine beetle by evaluating mountain pine beetle host selection behavior. We used no-choice attack box tests (Netherer et al., 2015) to compare the host selection responses of pioneering female mountain pine beetles placed on Great Basin bristlecone pine boles with the responses of those placed on co-occurring limber pine boles, a susceptible host species. Specifically, we asked (1) whether female mountain pine beetles have a low preference for Great Basin bristlecone pine relative to limber pine when exposed to the tree boles, (2) whether host tree capacity for induced defensive responses influences host selection behavior, and (3) whether mountain pine beetle populations from different geographic locations exhibit different host selection responses to Great Basin bristlecone and limber pines. We hypothesized that mountain pine beetle females would demonstrate aversive host selection behavior toward Great Basin bristlecone pine relative to limber pine, and that tree capacity for induced defense would play an important role in mountain pine beetle host selection decisions on both tree species. We also pre-

dicted that responses to Great Basin bristlecone and limber pines would be influenced by the geographic origin of the mountain pine beetle population used in tests.

2. Materials and methods

2.1. Tests on live trees

We obtained unmated adult female mountain pine beetles by felling two mass-attacked, mountain pine beetle-infested lodgepole pines in June 2015, prior to seasonal brood emergence. Both infested trees were cut from a lodgepole-subalpine fir (*Abies lasiocarpa* [Hook.] Nutt.) stand in Logan Canyon, Utah (UT) (41°52′30.2″N, 111°29′29.7″W) (Fig. 1a), where the mountain pine beetle population state was incipient-epidemic (Carroll et al., 2006). Cut bolts (~30 to 70 cm in length) of the infested trees were transported to the U.S. Forest Service Rocky Mountain Research Station in Logan, UT and placed in enclosed rearing containers for brood to complete development. We collected newly emerged adults twice daily and stored them without a food source in petri dishes with moistened filter paper at approximately 4 °C for up to 16 days. We used secondary sex characters on the seventh tergite (Lyon, 1958) to select only female beetles for host selection tests. Beetles that had been stored for between four and 16 days were transported in insulated coolers to field sites for testing.

We selected four field sites (Table 1) (Fig. 1a) with accessible, co-occurring Great Basin bristlecone and limber pines. At each site, we identified uninfested living bristlecone-limber pine tree pairs that were proximal (estimated < 0.5 km apart) and similar in size (diameter at breast height) (dbh) and vigor (crown density and live crown ratio). We selected a total of 36 Great Basin bristlecone-limber pine pairs as test trees, all of which were between 28 and 50 cm dbh and free of obvious severe health problems.

Table 1
Study sites for field-conducted mountain pine beetle attack box tests on 36 total pairs of live Great Basin bristlecone and limber pines.

Living trees site	State	Coordinates	Paired tests
Humboldt-Toiyabe National Forest Site 1	NV	39°10′56.7″N, 114°37′5.9″W	4
Humboldt-Toiyabe National Forest Site 2	NV	39°17′24.4″N, 114°13′4.3″W	16
Dixie National Forest Site 1	UT	37°33′54.3″N, 112°51′1.3″W	12
Dixie National Forest Site 2	UT	37°29′45.2″N, 112°45′1.2″W	4

We used attack boxes that confined live beetles to a controlled area of exposed tree bark to evaluate mountain pine beetle host selection responses to each test tree (Fig. 2a). Our attack box design was based on a similar model used by Netherer et al. (2015) to measure *Ips typographus* attacks on Norway spruce (*Picea abies* L. [Karst]). Clear plastic bins (41 cm tall × 20 cm wide × 13 cm deep) were modified so that the open side could fit tightly against a curved tree bole. We cut a nine cm diameter hole in the outward-facing plane of each box and connected a screw-on clear plastic jar extension (the '*exit jar*') to collect mountain pine beetles that moved away from the exposed bark. We attached attack boxes to the north aspect of test tree boles at breast height (~1.4 m). We used lashing straps to tighten the box edges, cushioned by a compressible foam frame, to the tree bole. To improve the seal, we

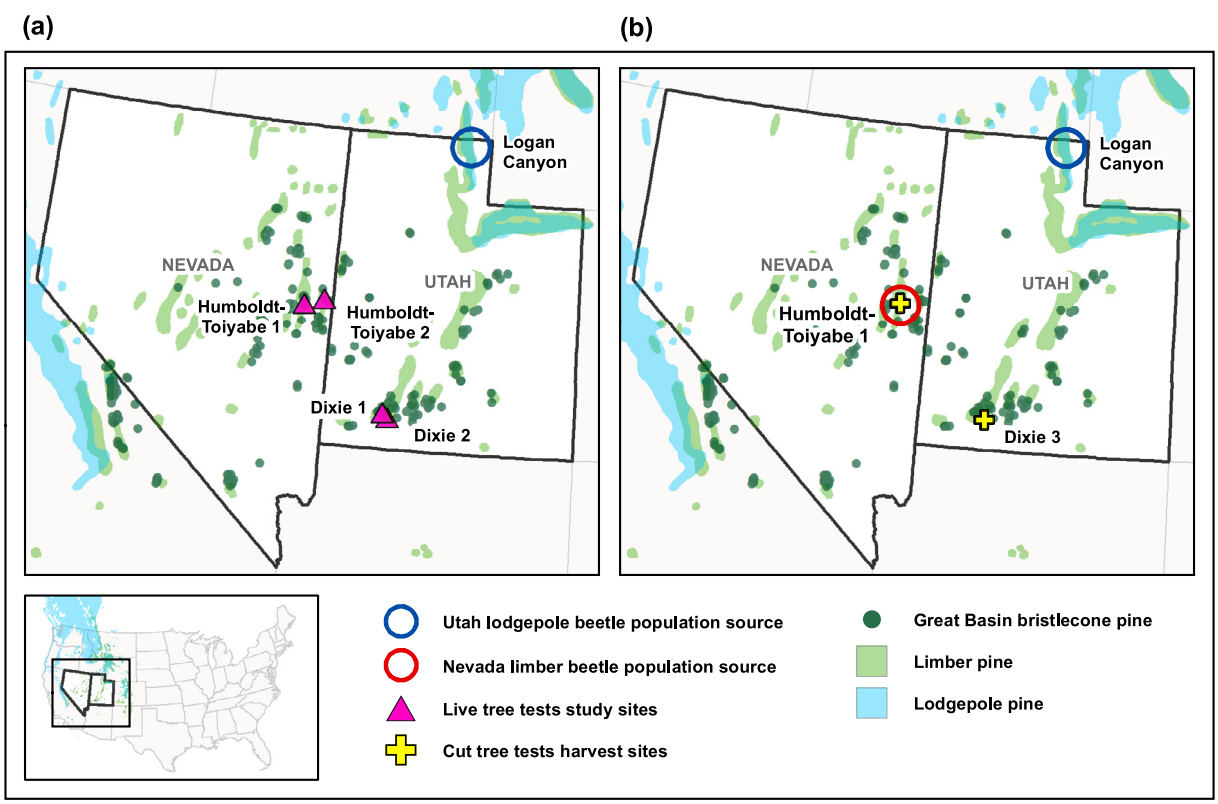

Fig. 1. (a) Locations of one mountain pine beetle population collection site and four study sites for attack box tests on live trees; (b) locations of two mountain pine beetle population collection sites and two tree harvest sites for bolts used in attack box tests on cut tree sections. The collection site for Utah (UT) beetles was a predominantly lodgepole pine stand that did not contain limber or Great Basin bristlecone pine in the stand but was within limber pine range. The Nevada (NV) beetle collection site was located within the ranges of Great Basin bristlecone and limber pines and had both tree species in the stand. Shown on the maps are observed points of Great Basin bristlecone pine occurrence (data from Bentz et al., 2016a) and limber and lodgepole pine distributions (Little, 1971).

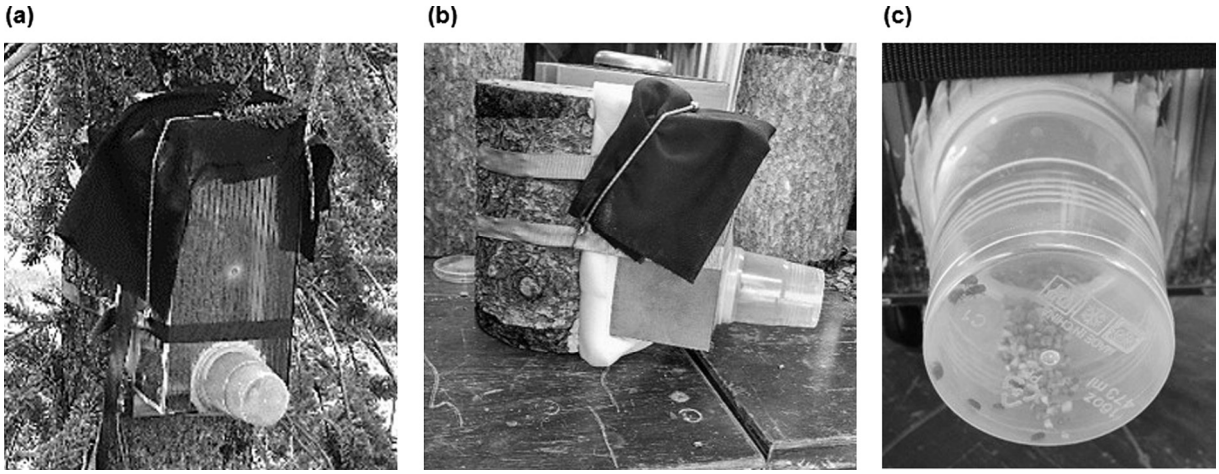

Fig. 2. (a) Attack box on a live Great Basin bristlecone pine; (b) attack box on a cut bolt; (c) mountain pine beetles that moved to the exit jar in a test on Great Basin bristlecone pine, which was interpreted as avoiding the tree. Attack boxes containing 10 live female mountain pine beetles were attached to Great Basin bristlecone-limber pine pairs and the status of individual beetles was recorded after 48 h.

scraped outer bark along the outline of the box edges and filled remaining crevices with caulking putty, but phloem was not damaged. Coverings shaded the top and sides of attack boxes to mitigate a greenhouse effect and interior box temperatures were recorded at 10 min intervals (measured with HOBO Pendant® data loggers, Onset Computer Corp, Bourne, MA). A small amount of litter was placed in the bottom of each box and exit jar to improve beetle traction.

Once attack boxes were attached to test trees, we removed the exit jars and inserted 10 unmated female mountain pine beetles into the bottom of each box. Within each Great Basin bristlecone-limber pine pair, beetles were distributed by the number of days they had been stored prior to testing, and randomly assigned to either Great Basin bristlecone or limber pine. After up-righting all individuals, we reconnected the exit jars to seal all test beetles inside the attack boxes. Confined beetles could then move about inside the box; i.e. they could move toward the test tree and initiate an attack or move away from the test tree into the exit jar. In pre-study trials, we ran attack box tests for 24 h and found that very few beetles initiated attacks during that period. Test length was increased to ~48 h for this study to allow beetles more time to initiate attacks, although we did not attempt longer tests. We ran tests concurrently on four Great Basin bristlecone-limber pine pairs at a time, then all attack box materials were removed and the status of each beetle was recorded. We also monitored exposed bark areas for evidence of abandoned attacks but did not find obvious signs. Tests were never repeated on the same trees and all 36 paired tests were conducted in July and August 2015, concurrent with natural mountain pine beetle flight and attack timing. To prevent attacks outside of our supervised experiments, beetles were recovered and placed in alcohol vials following each test.

2.2. Tests on cut tree sections

To investigate the role of induced tree defenses on mountain pine beetle host selection behavior, we repeated attack box tests on sections of Great Basin bristlecone and limber pines that had recently been cut, thereby removing their capacity to induce defensive reactions to an attack. We collected test beetles from populations in two different geographic regions for use in host selection tests on cut trees. For comparison to tests on living trees, one population was obtained from a newly mass-attacked lodgepole pine cut from the same lodgepole-subalpine fir stand in Logan Canyon, UT in September 2015 (Fig. 1b). The Logan Canyon site was located within limber pine range but did not contain limber pine in the immediate surrounding area and was located outside of Great Basin bristlecone pine range, so this population of mountain pine beetles likely had limited insect-host association with Great Basin bristlecone and limber pines. To test and compare the behavior of a mountain pine beetle population closely associated with Great Basin bristlecone and limber pines, a second population was obtained from two newly mass-attacked, mountain pine beetle-infested limber pines cut from a limber-Great Basin bristlecone pine stand near Humboldt-Toiyabe National Forest Site 1 in Nevada (NV) (39°09′41.9″N, 114°36′54.2″W) (Fig. 1b) in August 2015. The mountain pine beetle population state in both locations (UT and NV) was incipient-epidemic (Carroll et al., 2006). We reared, handled, and selected unmated adult female beetles (stored between five and 15 days) for tests in the same manner as described for the host selection tests on living trees.

Uninfested, cut bolts of Great Basin bristlecone and limber pines were obtained by harvesting two healthy Great Basin bristlecone-limber pine pairs (~30 to 35 cm dbh) from two sites (Table 2) (Fig. 1b) in August 2015 (felled four trees total). We cut ~30 cm long sections from the upper boles of the felled trees and paired Great Basin bristlecone-limber pine bolts based on site and similar height along the bole. Sections were cut to 30 cm to maximize the number of usable test bolts cut from each tree. Bolt ends were sealed with paraffin wax to reduce desiccation and bolts were stored just above 0 °C until use.

Host selection tests on 20 Great Basin bristlecone-limber pine bolt pairs were conducted indoors at ambient temperatures at the U.S. Forest Service Rocky Mountain Research Station in Logan, UT between October 20th and November 23rd, 2015. We used the same attack box method outlined for tests on paired live trees to measure host selection responses to paired cut bolts, with a few minor alterations. To accommodate bolt size, the attack boxes used in this study were slightly smaller (25 cm tall × 20 cm wide × 13 cm deep) than those used for tests on live trees, but were otherwise identical in design (Fig. 2b). We placed sterilized aquarium gravel in the bottom of each box to improve beetle traction since litter was not available. To simulate summer light cycles experienced by beetles in live tree tests, we placed a lamp over the test area and turned it on from 6:00 am to 9:00 pm during tests. Equal numbers of bolts from each site were tested using beetles from UT and NV populations (Table 2).

Table 2
Cut tree sites and mountain pine beetle source populations used for lab-conducted attack box tests on 20 total pairs of cut Great Basin bristlecone and limber pine bolts.

Cut tree site	State	Coordinates	Paired tests using beetles reared from UT lodgepole pine	Paired tests using beetles reared from NV limber pine
Humboldt-Toiyabe National Forest Site 1	NV	39°10′52.9″N, 114°37′11.7″W	5	5
Dixie National Forest Site 3	UT	37°28′46.1″N, 112°43′46.6″W	5	5

2.3. Statistical analysis

After totaling all individual beetle responses, beetles that were dead, stuck upside-down, or missing at the conclusion of a test were excluded from analysis. Remaining beetles were assigned individual response values corresponding to their recorded status, which were ranked in order of advancement toward an attack: 1 = in the exit jar, 2 = in the attack box container, 3 = on the tree bark, 4 = attacking. Beetles classified as 'attacking' were observed to be actively boring into the tree bark (producing boring dust or frass) at the conclusion of a test. To examine variability in beetle responses, Great Basin bristlecone and limber pine attack box response rates were calculated from the sum of individuals (0–10) in each response category per attack box. Individual beetle response values were then analyzed with ordinal logistic regression using generalized linear mixed models with odds ratio estimates. Multinomial ordered response distributions with the cumulative logit link function and residual pseudo likelihood estimation were used for model calculations. Because all beetles within a given attack box experienced the same conditions, denominator degrees of freedom for approximate F tests were estimated to reflect attack boxes as the experimental unit rather than individual beetles. All model analyses were computed using the GLIMMIX procedure in SAS Studio version 9.4.

For tests on living trees, fixed-effect factors were tree species (Great Basin bristlecone pine and limber pine) and the median number of days since emergence from infested bolts in the laboratory for the combined set of beetles used in each beetle's respective attack box (median ranged 5.5–16 days), hereafter referred to as median mountain pine beetle 'age.' Dixie National Forest Sites 1 and 2 were collapsed into a single site category due to their close proximity, and study site (Humboldt-Toiyabe 1, Humboldt-Toiyabe 2, and Dixie 1 & 2) was a random-effects factor. Each of the 36 test tree pairs was assigned a unique identifier, and tree pair nested within study site as well as tree species by tree pair nested within study site were also included as random-effects factors. We also tested the effects of average interior box temperature, cumulative thermal units inside the attack box, test date, and the dbh, live crown ratio, and crown density of the test tree on beetle response by initially including these variables as fixed-effect factors in the model. However, these variables were ultimately excluded from the model due to insignificant or inconsistent effects.

For tests on cut bolts, fixed-effects factors were tree species (Great Basin bristlecone pine and limber pine), mountain pine beetle population source (UT or NV), the interaction of tree species and mountain pine beetle population source, median mountain pine beetle 'age' (median ranged 5–14.5 days), and cut tree site (Humboldt-Toiyabe 1 and Dixie 3). Cut tree site was included as a fixed-effects factor rather than a random-effects factor because there were only two levels, which is arguably too few for estimation of site variance. We tested the effects of temperature within the attack box and test date on beetle response, but these factors were confounded because decreasing fall temperatures reduced indoor lab temperatures over the course of attack box testing. Both factors were therefore excluded from analysis. Similar to analysis for tests on living trees, each of the 20 Great Basin bristlecone-limber pine bolt pairs was assigned a unique pair identifier. To account for bolt pairing and test replicates, random-effects factors were bolt pair nested within both cut tree site and beetle population source. For both tests on living trees and tests on cut bolts, predicted probabilities of response levels by median beetle 'age' were calculated using population-averaged estimates on the inverse link scale.

3. Results

3.1. Tests on live trees

Mountain pine beetles placed on Great Basin bristlecone pine were 2.272 times more likely to be in a lower response category (further away from tree bole) than beetles placed on limber pine ($F_{1,21}$ = 15.76, P = 0.0007, 95% CI [1.478, 3.493]). Differences in mountain pine beetle host selection responses between Great Basin bristlecone and limber pine were especially pronounced for the two extreme categories, "attacking," and "in exit jar" (Fig. 3a). Out of the 720 total beetles used in tests on live trees, there were 69 beetles excluded from these results due to being dead, missing, or stuck upside-down at the end of testing: 25 from tests on Great Basin bristlecone pine and 44 from tests on limber pine.

Median mountain pine beetle 'age' was similar across Great Basin bristlecone and limber pines (Fig. S1a) and had a significant positive effect on response level for both tree species ($F_{1,22}$ = 10.04, P = 0.0045). Older female beetles (i.e., beetles that had been stored the longest before testing) were more likely to be on the tree bark and attacking and were less likely to move to the exit jar (Fig. 4a).

Average temperatures inside attack boxes ranged from a minimum of 12.6 °C to a maximum of 19.3 °C and were similar across Great Basin bristlecone and limber pines (Fig. S2a). The effect of average temperature on beetle response was not significant ($F_{1,25}$ = 3.80, P = 0.0625) and therefore was ultimately not included in model analysis.

3.2. Tests on cut tree sections

Female mountain pine beetles on Great Basin bristlecone pine bolts in the lab were 5.182 times more likely to be in a lower response category (further from the cut bole) than beetles on limber pine bolts ($F_{1,17}$ = 62.14, P < 0.0001, 95% CI [3.336, 8.048]) (Fig. 3b). Twenty-one out of the 400 total beetles used in tests on cut bolts were excluded from analysis because they were dead, missing, or stuck upside-down at the end of testing: 8 beetles from tests on Great Basin bristlecone pine and 13 beetles from tests on limber pine.

Similar to results from live tree tests, median beetle 'age' was comparable between Great Basin bristlecone and limber pines (Fig. S1b) and the effect of median 'age' on beetle response was significantly positive for both tree species ($F_{1,20}$ = 17.40, P = 0.0005) (Fig. 4b). Again, beetles that had been out of infested bolts the longest prior to testing were more likely to be on the cut bolt bark and attacking and were less likely to be in the exit jar.

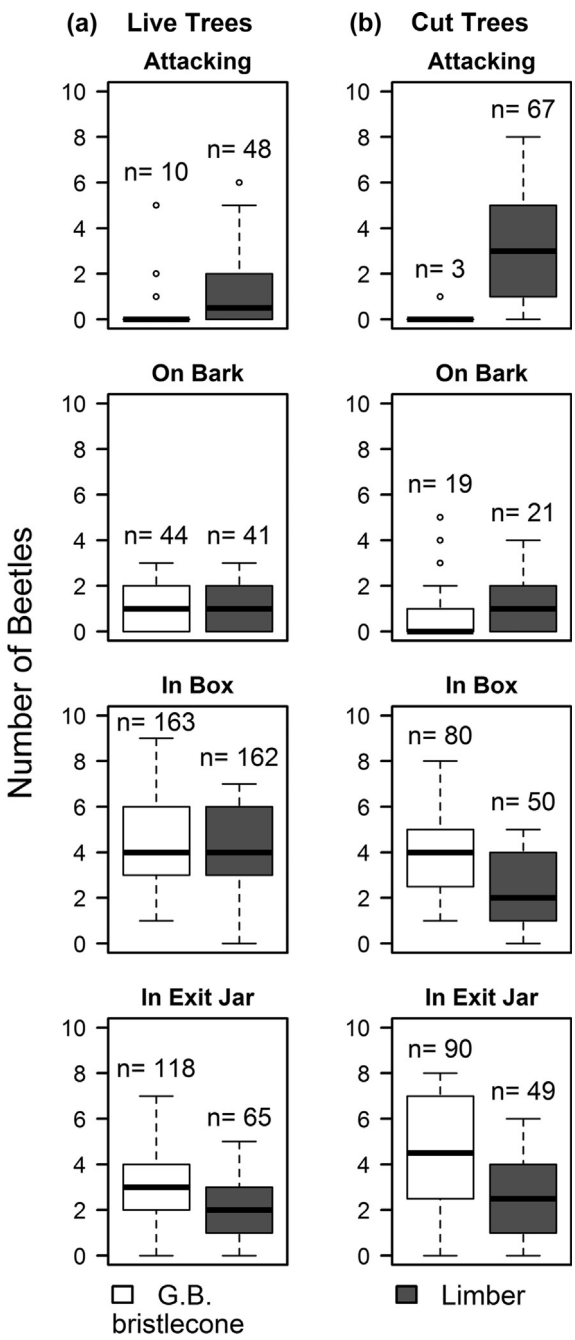

Fig. 3. (a) Response rates per attack box for paired tests on live trees; (b) response rates per attack box for paired tests on cut bolts. There were 10 female beetles per attack box for each test. Mountain pine beetles on Great Basin bristlecone pine were rarely attacking and moved into the exit jar more often than beetles on limber pine in both studies.

Average interior attack box temperatures ranged from a minimum of 16.5 °C to a maximum of 21.7 °C and were similar on Great Basin bristlecone and limber pines (Fig. S2b). We found a positive correlation between warmer temperature and increasing beetle response level (i.e., closer to the cut bolt) on both tree species, and the relationship was significantly stronger on limber pine ($F_{1,20} = 18.28$, $P = 0.0004$ for the interaction between the average temperature inside the attack box and tree species). However, removing temperature from the model due to its confounding with test date did not affect the significance of other covariates and interactions or the odds ratio estimate.

There was a significant interaction between tree species and mountain pine beetle population source ($F_{1,20} = 12.35$, $P = 0.0022$). Mountain pine beetles from NV were more likely to be attacking limber pine and were more likely to be in the exit jar of Great Basin bristlecone pine compared to mountain pine beetles from UT (Fig. 5). The site from which trees were cut did not significantly influence mountain pine beetle response ($F_{1,17} = 1.12$, $P = 0.3043$).

4. Discussion

On both live trees and cut bolts, pioneering female mountain pine beetles placed on Great Basin bristlecone pine rarely initiated attacks relative to beetles placed on limber pine. Additionally, beetles placed on Great Basin bristlecone pine moved to the exit jar (Fig. 2c) more often than beetles placed on limber pine. The very low acceptance and the high avoidance of Great Basin bristlecone pine in no-choice tests suggests that its resistance is driven by repellent stimuli, not simply weaker attraction than other co-occurring pine species. These results are consistent with the absence of mountain pine beetle attacks on Great Basin bristlecone pine *in situ* found by Bentz et al. (2016a) and the findings of Gray et al. (2015) that pioneering beetles avoid the volatile organic compounds of Great Basin bristlecone pine foliage. Research suggests that mountain pine beetle host rejection of well-defended lodgepole pines is based on contact with the phloem, not initial feeding on outer bark (Raffa and Berryman, 1982). Because gustatory cues in outer bark may not be responsible for eliciting host rejection, and we did not observe evidence of abandoned attacks that had reached the phloem in our tests, perhaps volatiles emitted from the bole of Great Basin bristlecone pine have similar properties to the unattractive compounds of its foliage.

Aversive host selection behavior suggests that there is a low compatibility between the insect and the tree. It is maladaptive for insects to colonize a host tree in which they or their brood will fare poorly, so pioneering attackers are expected to avoid trees that either cannot be successfully exploited due to high defenses/toxicity or cannot adequately meet their needs for reproductive success (i.e., poor quality or non-hosts). A complete inability of Great Basin bristlecone pine to meet mountain pine beetle needs would be surprising, considering that Great Basin bristlecone pine has been shown to have thicker phloem than limber pine (Bentz et al., 2016b; Eidson, 2017) and that mountain pine beetles can successfully reproduce in other western North American pines, several species of exotic pines, and even some species of spruce (*Picea*) (Wood, 1963; Furniss and Schenk, 1969; Mckee et al., 2013). Female mountain pine beetle avoidance of Great Basin bristlecone pine may therefore result from stimuli reflecting high defenses/-toxins rather than absolute ecological incompatibility.

The capacity for induced defensive reactions has been shown to be a more important predictor of tree resistance than levels of constitutive compounds in other pine species (Boone et al., 2011), but our results suggest that high constitutive defenses play a major role in Great Basin bristlecone pine resistance. The magnitude of host selection differences between Great Basin bristlecone and limber pine was greater in tests on cut bolts (odds ratio = 5.182) than on live trees (odds ratio = 2.272), meaning that beetles showed increased host acceptance of cut versus live limber pine, but avoided both cut and live Great Basin bristlecone pine. Although there are resource requirements for the maintenance of constitutive defenses as well as the deployment of induced defenses (Franceschi et al., 2005), tree injury does not significantly reduce the quantity of many important constitutive compounds (Powell and Raffa, 2011), but induced defenses are considered to be absent in cut trees (Lieutier, 2002). Great Basin bristlecone pine is known to have higher concentrations of constitutive toxic phloem com-

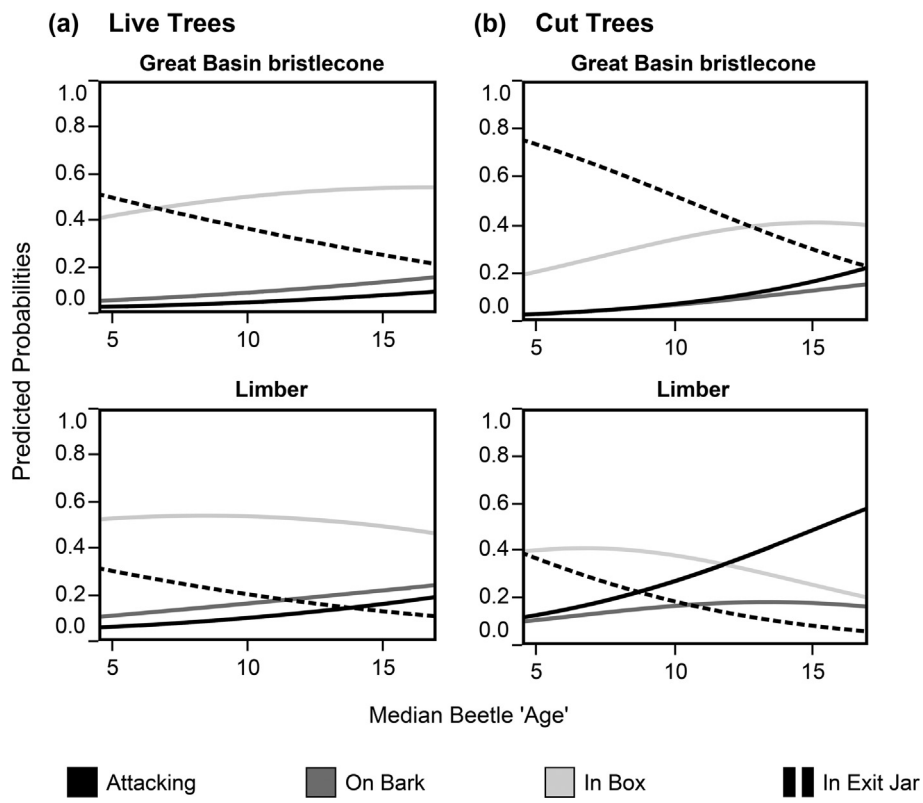

Fig. 4. (a) Predicted probabilities for response levels by median mountain pine beetle 'age' for tests on live trees; (b) response-level predicted probabilities by mountain pine beetle 'age' for tests on cut bolts. 'Older' mountain pine beetles were more likely to be attacking and on the bark and less likely to be in the exit jar in both studies.

pounds relative to limber pine (Bentz et al., 2016b) and this quality was presumably retained, at least in part, in cut bolts. The removal of potential induced defenses increased host acceptance on cut limber pine, which suggests that induced reactions play an important role in limber pine resistance, but a similar increase in acceptance was not observed on cut Great Basin bristlecone pine. Although induced defenses, including resin flow reactions, have not been directly tested in Great Basin bristlecone and limber pines, Bentz et al. (2016a) found that these two species do not differ in resin duct size or total resin duct area. These traits are strong predictors of resin flow in *P. ponderosa* (Hood and Sala, 2015), suggesting similar resin flow between Great Basin bristlecone and limber pines. Our observations of persistent host avoidance on cut Great Basin bristlecone pine, therefore, suggest that constitutive defenses may play a more important role in resistance than induced defenses. This finding is consistent with the Resource Availability Hypothesis, which theorizes that slow-growing organisms like Great Basin bristlecone pine invest heavily in constitutive defenses, which are energy intensive but require no lag time to activate, in order to avoid the high cost of replacing damaged tissue (Coley et al., 1985). However, while comparisons between tests on live trees and tests on cut trees are interesting, there are limits to our conclusions due to fundamental differences in the experimental designs of these two studies. In tests on live trees, each attack box was placed on a new individual tree, whereas attack box tests on cut trees were conducted on multiple sections of the same individual trees and used an additional mountain pine beetle population. More research to directly test for the capacity of an induced response in Great Basin bristlecone and limber pines is needed to better understand how these species invest in tree defense.

In addition to host tree characteristics, internal stimuli were important predictors of mountain pine beetle host selection behavior. On both live trees and cut bolts, mountain pine beetles that had been stored longer between their emergence from infested bolts and their use in attack box tests were more likely to be on the tree bark and attacking and less likely to avoid the tree bole by moving to the exit jar. Decreased host 'choosiness' over time can be advantageous due to the risks associated with exposure during host searching. Our findings support the results of other research showing reduced host discrimination in bark beetles with lower energy reserves (Chubaty et al., 2014) and in bark beetles that had previously rejected potential hosts (Wallin and Raffa, 2002). The influence of age on beetle response highlights the flexibility of bark beetle host selection behavior and its effect on tree resistance. The consistently very low acceptance rate of Great Basin bristlecone pine, regardless of decreased beetle choosiness with increasing age, emphasizes its high level of resistance.

Mountain pine beetle host selection behavior may also vary locally. In our population source comparisons of mountain pine beetle response on cut tree bolts, limber-reared NV mountain pine beetles attacked limber pine and moved to the exit jar on Great Basin bristlecone pine more frequently than lodgepole-reared UT mountain pine beetles (Fig. 5). The higher acceptance rates of limber pine by the NV beetles as compared to the UT beetles supports Hopkins' host-selection principle, which suggests that oligophagous insects will prefer to colonize their natal host species (Hopkins, 1916). However, other research has demonstrated similar host selection preferences in mountain pine beetles regardless of their natal host tree species (West et al., 2016), which contradicts Hopkins' explanation. Host selection behavior in bark beetles has, however, been shown to have a heritable component (Wallin et al., 2002). Mountain pine beetles from the NV population source were collected from a limber-Great Basin bristlecone pine stand that was well within the ranges of these two tree species. The UT mountain pine beetles originated from a lodgepole-subalpine fir stand that was within the range of limber pine but did not contain

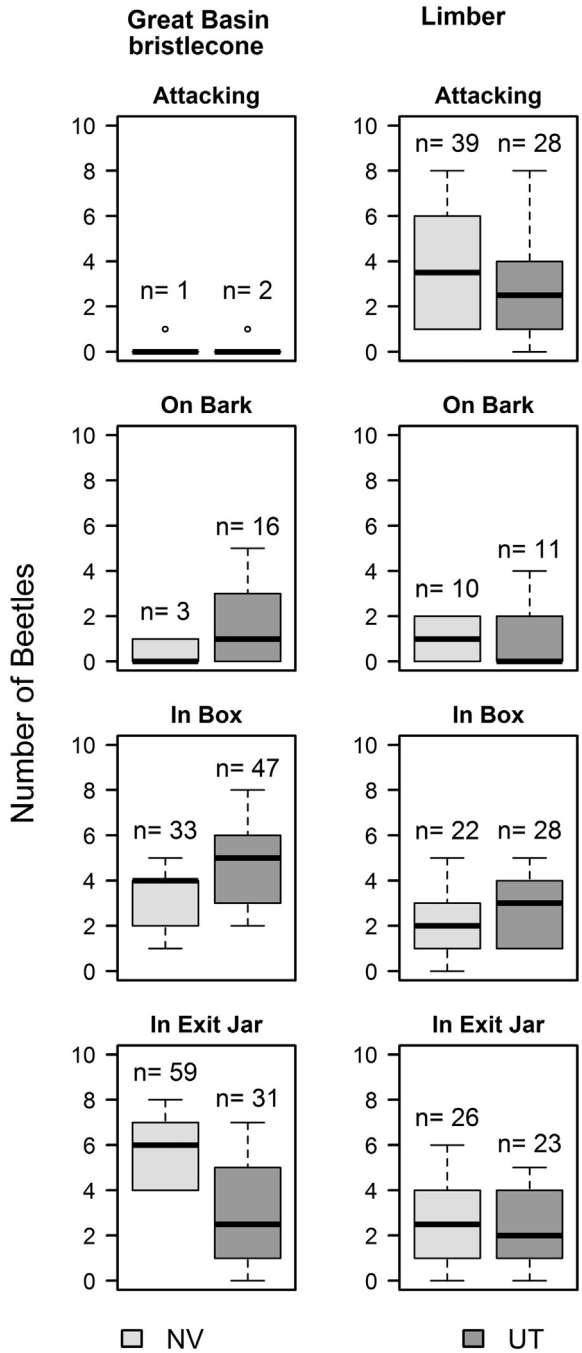

Fig. 5. Mountain pine beetle population differences for response rates per attack box in tests on cut bolts. Limber-reared Nevada (NV) beetles from a mixed stand of limber pine and Great Basin bristlecone pine attacked limber pine and avoided Great Basin bristlecone pine more often than lodgepole-reared Utah (UT) beetles collected from a lodgepole-subalpine fir stand.

a limber pine component, and was located far outside of Great Basin bristlecone pine range (Fig. 1b). The increased recognition of limber pine as a susceptible host species and of Great Basin bristlecone pine as a resistant species in NV beetles may be an adaptive result of selective pressure due to presumably closer associations between the NV mountain pine beetles and these two tree species.

Additional mountain pine beetle population factors that may be important but were not investigated in this study include population phase and attack density. The mountain pine beetles used in all tests were collected from incipient-epidemic-phase populations, not epidemic-phase populations, and only 10 beetles were used in each attack box. Host defenses that cause tree resistance in low-level mountain pine beetle populations are less effective and sometimes detrimental to tree resistance when mountain pine beetle populations are high (Boone et al., 2011). Testing mountain pine beetles from epidemic-level populations or increasing the number of mountain pine beetles in each attack box may have produced different results. However, Bentz et al. (2016a) reported evidence of epidemic-level mountain pine beetle populations in mixed stands with Great Basin bristlecone pine, resulting in up to 34.4% limber pine mortality and no Great Basin bristlecone pine mortality, suggesting Great Basin bristlecone pine resistance is reliable regardless of high mountain pine beetle pressure. The constitutive defenses likely responsible for Great Basin bristlecone pine resistance may not be 'exhaustible' by high numbers of attacking beetles in the same way that induced defenses can be overwhelmed by high-density attacks in other tree species.

5. Conclusions

Our results confirm Great Basin bristlecone pine resistance to mountain pine beetle and suggest that short-range repellent stimuli originating from the tree bole effectively deter pioneering attackers, regardless of the induced defensive capabilities of the tree or the prior insect-host associations of the mountain pine beetle population. These findings suggest that Great Basin bristlecone pine has low vulnerability to climate-driven increases in mountain pine beetle outbreaks at high elevations, which aids forest managers in predicting and managing high-elevation mountain pine beetle impacts. Although mountain pine beetle avoidance of Great Basin bristlecone pine is clearly demonstrated in our results, the evolutionary drivers behind this behavior remain unclear. To discover the cause of mountain pine beetle avoidance, more research is needed on the direct impacts of Great Basin bristlecone pine defenses on mountain pine beetle survival and reproduction. Future applications of this research could be used to identify new beetle deterrents and cultivate new ways to protect forest communities that are susceptible to mountain pine beetle-caused mortality.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2017.06.034.

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