
Erika L. Eidson
Utah State University

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GREAT BASIN BRISTLECONOE PINE RESISTANCE TO MOUNTAIN PINE BEETLE:
AN EVALUATION OF DENDROCTONUS PONDEROSAE HOST SELECTION
BEHAVIOR AND REPRODUCTIVE SUCCESS IN PINUS LONGAEVA

by

Erika Lynn Eidson

A thesis submitted in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

in

Forestry

Approved:

Karen E. Mock, Ph.D.  James N. Long, Ph.D.
Major Professor  Committee Member

Barbara J. Bentz, Ph.D.  Mark R. McLellan, Ph.D.
Research Advisor  Vice President for Research and
  Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2017
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 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 pine (*Pinus longaeva*), the longest-living non-clonal organism, were not attacked. I assessed Great Basin bristlecone pine resistance to mountain pine beetle by evaluating mountain pine beetle host selection behavior and reproductive success in this species.

To evaluate mountain pine beetle host selection preference for Great Basin bristlecone pine, I used no-choice 48-hour attack box experiments that confined pioneering female beetles onto pairs of living Great Basin bristlecone and limber pine (*P. flexilis*), a susceptible host tree species. To investigate the effect of induced tree defenses on host selection behavior, I repeated the tests on 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. Mountain pine
beetles avoided Great Basin bristlecone pine relative to limber pine, suggesting 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 females from initiating attacks, even when induced defenses are compromised.

To investigate mountain pine beetle reproductive success in Great Basin bristlecone pine, I compared the mating success, fecundity, and brood production of mountain pine beetle parents placed in cut Great Basin bristlecone pine bolts with that of mountain pine beetles placed in cut bolts of limber pine and lodgepole pine (*P. contorta*), two susceptible species. Initial reproductive development was similar in all three tree species, but nearly all brood in Great Basin bristlecone pine died before emerging. The extensive offspring mortality observed in Great Basin bristlecone pine may be a key evolutionary driver behind mountain pine beetle aversion to the species. These findings suggest that Great Basin bristlecone pine is a highly resistant species with low vulnerability to climate-driven increases in mountain pine beetle outbreaks at high elevations.
PUBLIC ABSTRACT

Great Basin Bristlecone Pine Resistance to Mountain Pine Beetle:
An Evaluation of *Dendroctonus ponderosae* Host Selection Behavior and Reproductive Success in *Pinus longaeva*

Erika L. Eidson

The mountain pine beetle (*Dendroctonus ponderosae*) is a native bark beetle that attacks and kills most species of pines (*Pinus*) throughout its range in western North America. Due to the strong relationship between thermal conditions and mountain pine beetle population success, climate change-induced changes in mountain pine beetle outbreaks are a major concern for land managers. Over the past several decades, warmer than average temperatures allowed mountain pine beetle populations to reach epidemic levels across much of the western U.S. and Canada, including high elevations where outbreaks were previously limited by cool temperatures. Many high-elevation pine species experienced extensive mortality in recent outbreaks, but co-occurring Great Basin bristlecone pines (*Pinus longaeva*), the longest-living non-clonal organism, were not attacked.

With the guidance of my committee, my research focused on investigating Great Basin bristlecone pine resistance to mountain pine beetle. Specifically, I compared mountain pine beetle host selection behavior and reproductive success in Great Basin bristlecone pine relative to susceptible host tree species. The results of both studies suggest that Great Basin bristlecone pine is a highly resistant species with low vulnerability to climate-driven increases in mountain pine beetle outbreaks at high elevations. These findings aid forest managers in predicting and managing high-elevation mountain pine beetle impacts and can be applied in future research to cultivate novel tools for forest protection.
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Sustainable forest management in the face of climate change requires predictions of how shifting natural disturbance regimes will impact forest environments. Phytophagous insects are important natural disturbance agents in forested ecosystems, affecting far greater total area than wildfire in the United States (Dale et al., 2001). Insects are especially sensitive to climate change because they are cold-blooded and therefore respond quickly to changes in their thermal environment. Climate change not only has the potential to alter insect population dynamics, but can also change the spatial extent of climatically suitable habitats for insect species. For this reason, many insect herbivores are undergoing geographic range shifts as a result of climate change, often leading to new associations with potential host plant species (Bale et al., 2002; Mason et al., 2015). The vulnerability of plant species encountered by phytophagous insects as a result of climate change is often initially unclear. Understanding insect-plant interactions between species with limited prior contact is vital to evaluating forest susceptibility to insect utilization along shifting geographic range margins.

Plant utilization by phytophagous insects involves both behavioral and physiological factors (Beck, 1965). First, host-searching insects must select a host plant for use, which relies on insect behavioral preferences. Second, the host plant must support insect physiological performance, including growth, survival and development. For many insect species, particularly those with sessile larvae that are unable to switch to other hosts, insect physiological performance depends largely on the host selection preferences of ovipositing females. The preference-performance hypothesis (Jaenike, 1978) predicts that female insects use plant cues (i.e., visual, olfactory, or gustatory stimuli) to assess plant suitability for their offspring and preferentially oviposit on high-quality host plants that will support optimal offspring performance, in turn maximizing their own evolutionary fitness. Although female host preferences are not always
initially well-matched with offspring performance in relatively novel systems (i.e., low
preference-high performance or high preference-low performance relationships), natural selection
is expected to promote increasing correspondence between female host preferences and offspring
performance over time. Preference-performance relationships are therefore a useful tool for
predicting plant vulnerability to phytophagous insects, particularly between species with limited
evolutionary history.

The mountain pine beetle (*Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae, Scolytinae) is a native insect herbivore that is responding to climate change with
geographic range expansion, leading to increased contact with host species that were previously
infested infrequently. Mountain pine beetles attack and kill most species of pine (*Pinus*) in
western North America. Female beetles locate, assess and select host trees for colonization, and
in successful attacks, mountain pine beetles bore through the bark of host trees to feed and
reproduce in the phloem. Lodgepole pine (*Pinus contorta* Douglas) is considered to be a primary
host tree species because it is ubiquitous in the elevation zone that is currently most climatically
suitable for mountain pine beetle. Like all insects, mountain pine beetles are highly sensitive to
temperature (Safranyik and Carroll, 2006; Powell and Bentz, 2009). Warmer than average
temperatures have the potential to improve winter survival and speed lifecycle completion,
particularly in areas where populations are typically limited by cold. Over the past several
decades, increasing temperatures have been linked to mountain pine beetle population irruptions
across the western U.S. and Canada (USDA Forest Service, 2015; Hicke et al., 2015). In addition
to affecting tens of millions of hectares of lodgepole pine at relatively low elevations, recent
outbreaks caused extensive tree mortality at high elevations where cool temperatures previously
limited mountain pine beetle persistence. High-elevation mountain pine beetle outbreaks intersect
landscapes where pine species such as whitebark pine (*P. albicaulis* Engelm.), limber pine (*P.
*flexilis* James), and Foxtail Pines (three closely-related species in *P. subsection Balfourianae*)
occur in greater abundance than lodgepole pine, which may be absent from these sites. Climate change is expected to continue to support mountain pine beetle persistence at high elevations throughout this century (Bentz et al., 2016a; Buotte et al., 2016). Given sustained climate-driven increases in high-elevation mountain pine beetle pressure, the vulnerability of high-elevation pine species depends largely on mountain pine beetle interactions with these hosts, including female host preferences and offspring performance.

High-elevation pines are keystone species in fragile mountain ecosystems, providing important ecosystem services in harsh environments where few other species can grow (Gibson et al., 2008). Although high-elevation pine communities face a number of threats related to climate change, mountain pine beetle outbreaks are considered to be the most serious short-term threat to these ecosystems (Gibson et al., 2008). One reason for concern is that high-elevation pine species are hypothesized to be more susceptible to mountain pine beetle-caused mortality than low-elevation pines (Raffa et al., 2013). This is because pines rely on defenses to resist mountain pine beetle use, and the ability of host trees to invest in these defenses depends in part on heritable traits (Franceschi et al., 2005). Generally, tree species with a long evolutionary history with mountain pine beetle are expected to be well defended, and those with limited prior association are expected to be poorly defended. High-elevation pine species are therefore hypothesized to possess inferior defenses against mountain pine beetles due to historically intermittent contact (Raffa et al., 2013). Given adequate nutritional value, host trees with low defenses are expected to support favorable mountain pine beetle offspring performance, and consequently be preferred by host-selecting females.

High-elevation species including whitebark and limber pine have been shown to support favorable mountain pine beetle offspring performance (Amman, 1982; Langor et al., 1990; Cerezke, 1995; Dooley et al., 2015; Esch et al., 2016). These species were also selected, attacked and killed in large numbers by mountain pine beetles during recent high-elevation outbreaks.
(Macfarlane et al., 2013; Cleaver et al., 2015). However, mountain pine beetle preference and offspring performance have not been evaluated in all high-elevation pine species, therefore the future vulnerability of some high-elevation forest communities remains unclear.

Great Basin bristlecone pine (*P. longaeva*), a member of the Foxtail Pine group, is a high-elevation pine species found in Utah, Nevada, and California. Great Basin bristlecone pine is well-known for being the longest-living non-clonal organism, and is valued for its remarkable ability to grow in adverse environments, its picturesque growth form, and its contributions to science (Lanner, 2007). Unlike other high-elevation pine species, successful mountain pine beetle attacks on Great Basin bristlecone pine have not been documented. Bentz et al. 2016b found that in surveys of mixed stands of Great Basin bristlecone and limber pines, mountain pine beetles avoided Great Basin bristlecone pine even when mountain pine beetle-caused mortality in neighboring limber pines was over 34%. Furthermore, Gray et al., 2015 found that the volatile organic compounds of Great Basin bristlecone pine foliage repelled female mountain pine beetles. The findings of both studies suggest low female mountain pine beetle preference for Great Basin bristlecone pine, but more information is needed to understand whether or not mountain pine beetles will attack Great Basin bristlecone pine if no alternative host species are available. Furthermore, mountain pine beetle offspring performance in Great Basin bristlecone pine has not been investigated, but this information could have important consequences on the future vulnerability of this species. If at least some females select Great Basin bristlecone pine as a host and their offspring perform well, increased preference may be anticipated in the future (Wasserman and Futuyma, 1981; Karowe, 1990). On the other hand, if offspring performance in Great Basin bristlecone pine is poor, natural selection should act to maintain low preference. Furthermore, a low preference-low performance relationship would suggest that Great Basin bristlecone pine possesses resistance mechanisms that are highly effective against mountain pine beetles.
The purpose of this research was to investigate Great Basin bristlecone pine vulnerability to climate-driven increases in mountain pine beetle outbreaks at high elevations. To accomplish this, I characterized the host preference-offspring performance relationship between mountain pine beetles and Great Basin bristlecone pine. Specifically, I evaluated mountain pine beetle host selection behavior, reproductive capacity, and offspring performance in Great Basin bristlecone pine relative to known-host species. This research not only aids forest managers in predicting mountain pine beetle impacts in Great Basin bristlecone pine forest communities, but also improves our understanding of the evolution of tree resistance mechanisms against phytophagous insects. Understanding the relationship between Great Basin bristlecone pine and mountain pine beetle also provides an important framework for studying the phylogeography of both species.

References


CHAPTER 2

MOUNTAIN PINE BEETLE HOST SELECTION BEHAVIOR CONFIRMS
HIGH RESISTANCE IN GREAT BASIN BRISTLECONE PINE

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 activity after 48 hours. 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 demonstrated aversive host selection behavior 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.
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 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 (\textit{P. albicaulis} Engelm.) and limber (\textit{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 (\textit{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., 2016a). With the expectation that climate conditions will continue to support mountain pine beetle success at high elevations throughout this century (Bentz et al., 2016b), 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 mid-summer 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 levels of constitutive chemical phloem defenses (Bentz et al., 2016a), although 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 surrenders host resources to 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. For example, mountain pine beetles with reduced energy reserves
have been shown to exhibit less discriminatory host acceptance behavior than beetles without
energy limitation (Chubaty et al., 2014), presumably due to the increased risk of exhaustion with
continued searching, resulting in no chance for reproduction. Furthermore, high numbers of
attacking mountain pine beetles improve the chance of exhausting induced host tree defenses, but
intraspecific competition for phloem resources can reduce larval productivity in high-density
attacks (Raffa and Berryman, 1983). Accordingly, mountain pine beetles from low-density
populations have been shown to preferentially attack weakened trees, despite their inferior
phloem resources, whereas mountain pine beetles from high-density populations have been
observed to preferentially attack vigorous, well-defended trees with better phloem resources
(Boone et al., 2011). Adaptive host selection behavior has been shown to be heritable in other
bark beetle species (Wallin et al., 2002), resulting in persistent between-population differences in
host acceptance behavior following a common rearing environment (Wallin and Raffa, 2004).
Understanding how mountain pine beetle ‘aggressiveness’ can vary with population is essential
for predicting and managing outbreak development.

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, or incompatible with the
biological needs of the insect and is unlikely to support the goal of reproductive success. The lack
of mountain pine beetle attacks observed on Great Basin bristlecone pine (Bentz et al., 2016a)
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 trait variation in Great Basin bristlecone 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 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. Tests were first conducted on paired live trees at four field sites, then the role of induced defenses was assessed by repeating the tests on sections of trees that had been recently cut, thereby removing their capacity for induced defensive responses. In tests on cut trees, we investigated between-population host selection differences by comparing the responses of mountain pine beetles collected from a population in Utah with the responses of beetles collected from a second population in Nevada (only one population of beetles was used in tests on live trees due to logistical constraints). We hypothesized that mountain pine beetle females would demonstrate aversive host selection behavior on living Great Basin bristlecone pine relative to living limber pine and that mountain pine beetle responses would be similar on both species in tests on cut trees without induced defenses. We also predicted that the mountain pine beetles
collected from the Nevada population (obtained from a predominantly limber pine stand) would
demonstrate greater host recognition and acceptance of limber pine relative to mountain pine
beetles from the Utah population (obtained from a lodgepole pine-dominated stand), and that
beetle responses to Great Basin bristlecone pine would be similarly aversive between the two
populations.

2. Methods

2.1 Tests on live trees

We obtained unmated adult female mountain pine beetles by felling two 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, UT (41°52'30.2"N, 111°29'29.7"W) (Fig. 2.1a), where the mountain pine beetle
population was an at endemic level. Cut bolts (~30 – 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 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 were transported in insulated coolers to
field sites for testing.

We selected four field sites (Table 2.1) (Fig. 2.1a) with accessible, co-occurring Great
Basin bristlecone and limber pines. At each site, we identified 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 and all test trees were between 28 and 50 cm dbh and free of
obvious severe health problems.
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. 2.2a). 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 x 20 cm wide x 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 meters). We used lashing straps to tighten the box edges, cushioned by a compressible foam frame, to the tree bole. To improve the seal, we 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 minute 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 an attack box was attached to a test tree, we removed the exit jar and inserted 10 unmated female mountain pine beetles into the bottom of the box. After up-righting all individuals, we reconnected the exit jar to seal all test beetles inside the attack box. 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 hours and found that very few beetles initiated attacks in that period. Test length was increased to ~48 hours 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 activity 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.

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 activity, 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 a generalized linear mixed model with odds ratio estimates. 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 (ranged 5.5 to 16 days), hereafter referred to as 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 were 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 tested the effects of several additional factors on mountain pine beetle response, including temperature within the attack box, test date, test tree dbh, test tree live crown ratio, and test tree crown density, but all were excluded from the model due to weak or inconsistent effects. A multinomial ordered response distribution, with the
cumulative logit link function and residual pseudo likelihood estimation, was 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. Predicted probabilities of response levels by median beetle ‘age’ were calculated using population-averaged estimates on the inverse link scale. Model analyses were computed using the GLIMMIX procedure in SAS Studio version 9.4.

2.2 Tests on cut tree sections

We collected test beetles from populations in two different regions for use in host selection tests on cut trees. For comparison to tests on living trees, one population was obtained from a newly infested lodgepole pine cut from the same lodgepole-subalpine fir stand in Logan Canyon, UT in September, 2015 (Fig. 2.1b). 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 mountain pine beetles from this population likely had a limited insect-host association history 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 infested limber pines cut from a limber-Great Basin bristlecone pine stand near Humboldt-Toiyabe National Forest Site 1 (39°09'41.9"N, 114°36'54.2"W) (Fig. 2.1b) in August, 2015. The mountain pine beetle populations in both locations were at endemic levels. We reared, handled and selected unmated adult female beetles 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 bristlecone-limber pine pairs (~30 – 35 cm dbh) from two sites (Table 2.2) (Fig. 2.1b) 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. 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 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 x 20 cm wide x 13 cm deep) than those used for tests on live trees, but were otherwise
identical in design (Fig. 2.2b). 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 2.2).

Mountain pine beetle response totals and attack box response rates were calculated using
the methods outlined for live tree tests response summaries. Similarly, individual beetle response
value (1 = in the exit jar, 2 = in the attack box container, 3 = on the tree bark, 4 = attacking) was
statistically analyzed using the same model design and software used for live tree tests analyses,
with appropriate fixed and random-effects factor modifications. 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. Fixed-effects factors
used in the analysis of this study 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’ (ranged 5 to 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. Similar to analysis for tests on living trees, each of the 20 Great Basin bristlecone-limber pine bolt pairs were 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.

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 \(F_{1,21}=15.76, P=0.0007, 95\% \text{ CI } [1.478, 3.493] \). 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. 2.3a). 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’ 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. 2.4a).

Average temperatures inside attack boxes were similar across Great Basin bristlecone and limber pines (Fig. 2.5a), and the effect of average temperature on beetle response was not significant \(F_{1,25}=3.80, P=0.0625 \) and therefore was not included in the model.
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. 2.3b). 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, the effect of median ‘age’ on beetle response was significantly positive for both tree species ($F_{1,20}=17.40$, $P=0.0005$) (Fig. 2.4b). Again, beetles that had been out of infested bolts the longest were more likely to be on the cut bolt bark and attacking and were less likely to be in the exit jar.

Interior attack box temperatures were similar on Great Basin bristlecone and limber pine (Fig. 2.5b). 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. 2.6). 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. 2.2c) 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 indicates 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 colonized due to high defenses or cannot adequately meet their needs for reproductive success (i.e., non-hosts). A complete inability of Great Basin bristlecone pine to meet mountain pine beetle needs would be surprising, considering 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 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. Contrary to our predictions, 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 levels of constitutive toxic phloem compounds relative to limber pine (Bentz et al., 2016a) and this quality was presumably retained, at least in part, in cut bolts. The removal of induced defenses increased host acceptance in limber pine, which suggests that induced reactions play an important role in limber pine resistance, but a similar increase in acceptance was not observed in Great Basin bristlecone pine, indicating a less important role for induced reactions in resistance. 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, 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.

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 adaptive flexibly 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 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. 2.6). Greater acceptance of limber pine by the NV beetles was consistent with our predictions, but higher avoidance of Great Basin bristlecone pine was unexpected. 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, evidence of larval experience affecting adult host selection in insects is rare (Barron, 2001), which contradicts Hopkins’ explanation. Host acceptance 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 a limber pine component, and was located far outside of Great Basin bristlecone pine
range (Fig. 2.1). 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 population density. The mountain pine beetles used in all tests were collected from endemic-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 for 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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### Tables and Figures

#### Table 2.1
Study sites for field-conducted mountain pine beetle attack box tests on 36 total pairs of live Great Basin bristlecone and limber pines.

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<thead>
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<th>Living trees site</th>
<th>State</th>
<th>Coordinates</th>
<th>Paired tests</th>
</tr>
</thead>
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<tr>
<td>Humboldt-Toiyabe National Forest Site 1</td>
<td>NV</td>
<td>39°10'56.7&quot;N, 114°37'5.9&quot;W</td>
<td>4</td>
</tr>
<tr>
<td>Humboldt-Toiyabe National Forest Site 2</td>
<td>NV</td>
<td>39°17'24.4&quot;N, 114°13'4.3&quot;W</td>
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</tr>
<tr>
<td>Dixie National Forest Site 1</td>
<td>UT</td>
<td>37°33'54.3&quot;N, 112°51'1.3&quot;W</td>
<td>4</td>
</tr>
<tr>
<td>Dixie National Forest Site 2</td>
<td>UT</td>
<td>37°29'45.2&quot;N, 112°45'1.2&quot;W</td>
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</table>

#### Table 2.2
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.

<table>
<thead>
<tr>
<th>Cut tree site</th>
<th>State</th>
<th>Coordinates</th>
<th>Paired tests using beetles reared from UT lodgepole pine</th>
<th>Paired tests using beetles reared from NV limber pine</th>
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</thead>
<tbody>
<tr>
<td>Humboldt-Toiyabe National Forest Site 1</td>
<td>NV</td>
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<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Dixie National Forest Site 3</td>
<td>UT</td>
<td>37°28'46.1&quot;N, 112°43'46.6&quot;W</td>
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Fig. 2.1 (a) Map of single mountain pine beetle population collection site and four study sites for attack box tests on live trees; (b) map of two mountain pine beetle population collection sites and two tree harvest sites for bolts used in attack box tests on cut trees. The collection site for UT beetles was a predominantly lodgepole pine stand that did not contain limber pine in the stand but was within limber pine range. The NV beetle collection site was located within the ranges of Great Basin bristlecone and limber pines and had both tree species in the stand.
Fig. 2.2 (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 individual beetle activities were recorded after 48 hours.
Fig. 2.3 (a) Response rates by attack box for paired tests on live trees; (b) response rates by attack box for paired tests on cut bolts. There were 10 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.
Fig. 2.4 (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.
Fig. 2.5 Average interior attack box temperatures were similar on both tree species for (a) tests on live trees and (b) tests on cut bolts.
Fig. 2.6 Mountain pine beetle population differences for response rates by attack box in tests on cut bolts. Limber-reared 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 UT beetles collected from a lodgepole-subalpine fir stand.
CHAPTER 3

HIGH MOUNTAIN PINE BEETLE OFFSPRING MORTALITY IN GREAT BASIN
BRISTLECONE PINE SUGGESTS HOST AVOIDANCE IS ADAPTIVE

Abstract

The preference-performance hypothesis states that ovipositing phytophagous insects will select host plants that are well-suited for their offspring and avoid host plants that do not support offspring development. However, adaptive host selection choices are not always evident in plant and insect populations with limited prior association, and host selection choices may not reflect host plant suitability. The mountain pine beetle (*Dendroctonus ponderosae*), a native insect herbivore in western North America, recently experienced population irruptions in high-elevation pine forests that were previously too cold for mountain pine beetle persistence. Although many high-elevation pine species were successfully attacked, co-occurring Great Basin bristlecone pines (*Pinus longaeva*) were avoided by mountain pine beetles. To clarify whether or not this low preference is adaptive, we investigated mountain pine beetle offspring performance in Great Basin bristlecone pine. We manually inserted mountain pine beetle parent pairs into cut bolts of Great Basin bristlecone pine and two host tree species known to be well-suited for mountain pine beetle offspring: limber pine (*P. flexilis*) and lodgepole pine (*P. contorta*). Additionally, to explore the effects of mountain pine beetle population trait variation on offspring performance, we conducted tests with mountain pine beetle parents collected from two population sources. Initial reproductive development was similar in all tree species, but nearly all offspring in Great Basin bristlecone pine died before completing development, regardless of the beetle population. Our results confirm an adaptive low oviposition preference-low offspring performance relationship between mountain pine beetle and Great Basin bristlecone pine. These findings provide strong evidence that Great basin bristlecone pine has low vulnerability to climate-driven increases in high-elevation mountain pine beetle outbreaks.
Introduction

Many insect herbivores are undergoing geographic range shifts as a result of climate change (Bale et al., 2002; Mason et al., 2015), often leading to new associations with potential host plant species. The preference-performance hypothesis (Jaenike, 1978) predicts that female insects use cues (i.e., visual, olfactory, or gustatory stimuli) to assess plant suitability for their offspring and preferentially oviposit on host plants that will support optimal offspring performance (i.e., growth, survival, and fecundity), in turn maximizing their own evolutionary fitness. Strong correspondence between oviposition preferences and offspring performance is evident in many cases (Gripenberg et al., 2010 and references within). In plant and insect populations with limited prior association, however, the lack of time for insect oviposition preferences to adapt to novel or previously rare host plant species can result in mismatched preference-performance relationships (Thompson, 1988). For example, female insects may initially avoid a recently encountered potential host plant species because they fail to recognize that the plant is suitable for their offspring (Wiklund, 1975; Karowe, 1990; Janz et al., 2001). However, if at least some females choose the new potential host plant species and their offspring perform well, increased preference for the species may be anticipated (Wasserman & Futuyma, 1981; Karowe, 1990). On the other hand, if offspring performance on the new potential host plant species is poor, natural selection should act to maintain low preference if alternative host plant species are available. Preference-performance relationships are therefore useful for predicting plant vulnerability to phytophagous insects, particularly between species with limited evolutionary history.

The mountain pine beetle (*Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae, Scolytinae) is a native insect herbivore that is responding to climate change with geographic range expansion. Mountain pine beetles attack and kill most species of pine (*Pinus*) throughout their current range in western North America, but lodgepole pine (*Pinus contorta*
Douglas) is considered to be a primary host tree species because it is ubiquitous in the elevation zone that is most suitable for mountain pine beetle. Like all insects, mountain pine beetles are highly sensitive to temperature (Safranyik & Carroll, 2006; Powell & Bentz, 2009). Warmer than average temperatures have the potential to improve winter survival, speed lifecycle completion, and alter the spatial extent of climatically suitable habitats (Bentz et al., 2010; Sambaraju et al., 2012; Weed et al., 2015). Over the past several decades, increasing temperatures have been linked to mountain pine beetle population irruptions, resulting in large-scale outbreaks in the western U.S. and Canada (USDA Forest Service, 2015; Hicke et al., 2015). In addition to affecting tens of millions of hectares of lodgepole pine at relatively low elevations, recent outbreaks caused extensive tree mortality at high elevations where cool temperatures previously limited mountain pine beetle persistence. High-elevation mountain pine beetle outbreaks intersect landscapes where pine species such as whitebark pine (P. albicaulis Engelm.), limber pine (P. flexilis James), and Foxtail Pines (three closely-related species in P. subsection Balfourianae) occur in greater abundance than lodgepole pine, which may be absent from these sites. Climate change is expected to continue to support mountain pine beetle persistence at high elevations throughout this century (Bentz et al., 2016a; Buotte et al., 2016). Given sustained climate-driven increases in high-elevation mountain pine beetle pressure, the vulnerability of high-elevation pine species depends largely on mountain pine beetle interactions with these hosts, including preference-performance relationships.

Preference-performance relationships in mountain pine beetles are complex because adult beetles must successfully bore through host tree bark and colonize the phloem before oviposition can occur. Female mountain pine beetles locate, assess, and select host trees for colonization, after which they produce aggregation pheromones to attract more beetles to join the attack (Safranyik & Carroll, 2006). Many conifer species invest in defenses against bark beetles, which generally include targeted, induced reactions that are activated in response to an attack (i.e., resin
flow to ‘pitch out’ attacking beetles) as well as more generalized, constitutive defenses that are pre-formed and always present in the tree (i.e., obstructive bark compounds and phloem toxins) (Lieutier, 2002; Franceschi \textit{et al.}, 2005). Synchronous, high-density mass-attacks are required for mountain pine beetles to exhaust induced host tree defenses, and attacking beetles must also overcome or tolerate constitutive defenses. In successful attacks, adult mountain pine beetles bore through host tree bark, feed and mate, and females oviposit along vertical galleries constructed in the phloem. Offspring develop under the bark at temperature-dependent rates before emerging as adults, which typically occurs after one to three seasons depending on the temperature regime (Bentz \textit{et al.}, 2014). The ability of female mountain pine beetles to choose hosts which will support offspring development is important for mountain pine beetle population success. Host preferences have a highly heritable component in bark beetles (Wallin \textit{et al.}, 2002; Wallin & Raffa, 2004) and increasing correspondence between female mountain pine beetle host preferences and offspring performance would therefore be expected over time in sustained insect-host associations.

Although high-elevation mountain pine beetle outbreaks are not unprecedented (Perkins & Swetnam, 1996), high-elevation pine species and mountain pine beetles are hypothesized to be weakly coevolved due to historically intermittent contact (Raffa \textit{et al.}, 2013). Suboptimal preference-performance relationships between mountain pine beetles and some high-elevation pine species may support this prediction. For example, field studies in mixed stands show that mountain pine beetles prefer to attack lodgepole pine over whitebark pine (Raffa \textit{et al.}, 2013; Bentz \textit{et al.}, 2015), even though whitebark pine supports mountain pine beetle offspring performance that is at least commensurate with that of lodgepole pine (Amman, 1982; Dooley \textit{et al.}, 2015; Esch \textit{et al.}, 2016). Furthermore, Raffa \textit{et al.} (2013) found that whitebark pine may have lower defenses than lodgepole pine, and Bentz \textit{et al.} (2015) found that whitebark pine resists
mountain pine beetle attacks less frequently than lodgepole pine, although both species were killed equally owing to the greater number of attempts on lodgepole pine.

Similar to whitebark pine, high-elevation limber pine has also been shown to be a favorable host for mountain pine beetle offspring performance (Amman, 1982; Langor et al., 1990; Cerezke, 1995), and both whitebark and limber pines experienced high levels of mortality during recent outbreaks (Macfarlane et al., 2013; Cleaver et al., 2015). However, mountain pine beetle preference-performance relationships have not been evaluated for all high-elevation pine species. Great Basin bristlecone pine (\textit{P. longaeva} Bailey), a member of the Foxtail Pine group, is a high-elevation species in Utah, Nevada, and California that seems to be avoided by mountain pine beetles (Gray et al., 2015; Bentz et al., 2016b). The cause for this low preference has not been identified and could have important implications on the future vulnerability of this species.

Great Basin bristlecone pine is well-known for being the longest-lived non-clonal organism in the world, with the oldest documented individual reaching ≥5,062 years of age (RMTRR OLDDLST, Tom Harlan, 2012). Currently, Great Basin bristlecone pine occupies high-elevation islands between ~2,800 and 3,500 meters, but its elevational range likely fluctuated over time, expanding down slope during cool ice ages and retreating upslope during interglacial warming (Lanner, 2007). These elevational transitions between glacial periods may have provided windows where the range of areas that were climatically favorable for bark beetles overlapped Great Basin bristlecone pine forests. Evidence suggests that Great Basin bristlecone pine most recently existed at low elevations between 25,000 and 11,000 years ago, forming extensive low-elevation forests with limber pine before retreating upslope in the early Holocene epoch (Cohen, 1998; Lanner, 2007). Low mountain pine beetle preference for Great Basin bristlecone pine has been documented in several recent studies. In surveys of mixed stands of Great Basin bristlecone and limber pines, mountain pine beetles avoided Great Basin bristlecone pine even when mountain pine beetle-caused mortality in neighboring limber pines was > 34%
(Bentz et al., 2016b). The volatile organic compounds of Great Basin bristlecone pine foliage repelled pioneering female mountain pine beetles in two-way choice tests even when the alternative offered choice was just clean air (Gray et al., 2015). Furthermore, Bentz et al. (2016b) found that Great Basin bristlecone pine has over eight times the concentration of constitutive phloem compounds relative to limber pine. The concentrated compounds identified by Bentz et al. (2016b) have been associated with tree defense (Raffa, 2014), but the direct effects of these Great Basin bristlecone pine traits on mountain pine beetle adults and their offspring have not been tested.

The purpose of this study was to investigate mountain pine beetle oviposition and offspring performance in Great Basin bristlecone pine. Specifically, we focus on reproductive development following successful mountain pine beetle colonization, which was simulated by manually infesting sections of trees that had recently been cut. We evaluated mountain pine beetle mating, fecundity, brood production, and offspring fitness in Great Basin bristlecone pine relative to limber and lodgepole pines, two host species known to be well-suited for mountain pine beetle offspring performance (Langor et al., 1990; Cerezke, 1995). Additionally, to explore the effects of mountain pine beetle population trait variation on offspring performance, we conducted tests with mountain pine beetle parents collected from two population sources: one population was collected from two infested limber pines in Nevada (NV) and a second population was collected from an infested lodgepole pine in Utah (UT). Although the cut host trees used in our study had no capacity for induced defensive reactions, the bolts presumably retained many pre-formed constitutive qualities. We predicted that the high constitutive defenses in Great Basin bristlecone pine (Bentz et al., 2016b) would result in low reproduction in Great Basin bristlecone pine relative to known-hosts lodgepole and limber pines. However, because the mountain pine beetles from NV had several potential fitness advantages over those from UT, we predicted that the NV beetles would perform better than the UT beetles. This research clarifies the preference-
performance relationship between mountain pine beetle and Great Basin bristlecone pine and aids in predicting future Great Basin bristlecone pine vulnerability to mountain pine beetle.

Methods

Sourcing and handling of parent mountain pine beetles and host tree materials

We obtained uninfested, cut bolts of Great Basin bristlecone, limber, and lodgepole pines to be manually infested with mountain pine beetles by harvesting two live Great Basin bristlecone-limber pine pairs in August, 2015 and one healthy lodgepole pine in September, 2015 (cut five trees total, all trees ~30 – 35 cm dbh). One Great Basin bristlecone (BR-HT) and one limber pine (LM-HT) were cut from the Humboldt-Toiyabe National Forest, NV (39°10’52.9"N, 114°37’11.7"W), a second bristlecone-limber pine pair (BR-DX and LM-DX, respectively) was cut from the Dixie National Forest, UT (37°28’46.1"N, 112°43’46.6"W) and the lodgepole pine (LP-UWC) was cut from the Uinta-Wasatch-Cache National Forest, UT (41°52’30.2"N, 111°29’29.7"W) (Fig. 3.1). We cut ~30 cm long bolts from the lower boles of each tree and sealed bolt ends with paraffin wax to reduce desiccation. For each bolt, we recorded the average phloem thickness (mm) calculated from two measurements taken at opposite sides of the bolt cross section. Bolts were stored just above 0° C for between 26 and 81 days before use.

To assess the effects of mountain pine beetle population trait differences on reproductive success in contrasting host trees, we collected unmated adult mountain pine beetles from two population sources from different geographic regions and natal host tree species. The first population was collected by felling two infested limber pines from a mixed stand of Great Basin bristlecone and limber pine on the Humboldt-Toiyabe National Forest, NV (39°09’41.9"N, 114°36’54.2"W) (Fig. 3.1) in August, 2015. The two infested NV limber pines had been attacked over the past two seasons and contained mountain pine beetle brood of mixed life stages. The second population was obtained from one newly infested lodgepole pine cut from a lodgepole-
subalpine fir (*Abies lasiocarpa* Hook) stand on the Uinta-Wasatch-Cache National Forest, UT (41°52′30.2″N, 111°29′29.7″W) (Fig. 3.1) in September, 2015. Unlike the NV site, the UT site was located outside of Great Basin bristlecone pine range.

Mountain pine beetles infesting the NV limber pines had several potential advantages for reproductive success in Great Basin bristlecone pine over those infesting the UT lodgepole pine. Relative to mountain pine beetles raised in lodgepole pine, beetles that develop in limber pine have been found to be larger, more fecund, and are hypothesized to have superior detoxification systems (Langor *et al.*, 1990; Cerezke, 1995), which may improve their ability to contend with the high phloem compounds in Great Basin bristlecone pine. Furthermore, the NV beetles may have had increased opportunity for adaptation to Great Basin bristlecone pine because they were collected from a mixed stand within Great Basin bristlecone pine range. Although little is known about the spatial and temporal scales required for mountain pine beetles to adapt to a relatively new host species, local adaptation (i.e., increased preference and performance) to recently introduced exotic host plant species has been demonstrated among populations of other insects that occur where the new plant species is present (Keeler & Chew, 2008). Our rationale for choosing these two populations of mountain pine beetles was to investigate whether the potentially superior fitness and increased opportunity for adaptation to Great Basin bristlecone would allow the NV beetles to achieve greater offspring performance in Great Basin bristlecone pine cut bolts as compared to the UT beetles.

Cut bolts (~30 – 70 cm in length) of the infested trees from NV and UT were labeled by site and transported to the U.S. Forest Service Rocky Mountain Research Station in Logan, UT. Bolt ends were sealed with paraffin wax to reduce desiccation and bolts were placed in enclosed rearing containers for brood to complete development. Newly emerged adults were collected twice daily and stored in petri dishes with moistened filter paper at approximately 4°C until use, up to ~ two weeks. Male and female beetles were identified and separated using secondary sex
characters on the seventh tergite (Lyon, 1958). The pronotal width (mm), hereinafter referred to as the ‘size’ of each intended parent beetle was measured using an ocular microscope scale to facilitate subsequent investigations of the relationship between parent size and offspring performance in contrasting host tree species.

Manual infestation of tree bolts

We manually infested eight cut bolts of each of the five host trees (bristlecone pines BR-HT and BR-DX, limber pines LM-HT and LM-DX, and lodgepole pine LP-UWC) with mountain pine beetle adults according to protocols established by Bentz et al. (2001). Four bolts of each tree were infested with NV beetles and the remaining four were infested with UT beetles. Using a power drill (bit size ~five mm diameter), we initiated parent galleries to a depth of approximately two cm along one cross sectional plane of each bolt (Fig. 3.2a). We avoided placing galleries directly over wounds or branch stubs, but otherwise maintained an equal gallery spacing of ~six cm in an effort to standardize intra-specific competition among offspring for each bolt. Owing to this spacing protocol, between 13 and 19 galleries were initiated for each bolt depending on bolt condition and circumference. One female followed by one male mountain pine beetle were manually inserted into each initiated gallery, after which the hole was screened to prevent the pair from exiting (Fig. 3.2b). After a bolt was completely infested (Fig. 3.2c), it was oriented so that the screened entrance holes were at the base and the inserted beetles were facing upwards as in natural attacks. Infested bolts were placed in incubator cabinets (Percival Scientific, Inc., Perry, Iowa) at 22.5°C and low humidity to facilitate brood development. Infested bolts were grouped so that each incubator included a mix of bolts from different host trees.

Mating success and fecundity

After egg hatch, developing mountain pine beetle larvae feed horizontally outward from the parent gallery, often in increasingly meandering patterns that intersect other larval galleries.
Over time, this feeding pattern, in addition to fungal growth, can obscure individual larval galleries and eggs in the phloem. As development progresses, fecundity, i.e., the total number of eggs laid per parent pair, becomes difficult to measure. For this reason, to assess rates of mating success and fecundity we followed a protocol established by Bracewell et al. (2010) and arrested reproductive development through refrigeration for one NV and one UT beetle-infested bolt of each of the five host trees on day 26 after manual infestation (refrigerated 10 bolts total, six infested bolts of each host tree remained at 22.5°C). We peeled the bark and phloem from the 10 refrigerated bolts to expose galleries and eggs in the phloem. Twenty-six days following infestation, although oviposition may have been ongoing, many parent galleries had reached the “top” of the bolt but larval galleries and eggs were still clearly visible in the phloem. For each manually infested parent gallery we recorded (1) mating success or failure, (2) total parent gallery length (cm), and (3) the total sum of hatched eggs (larval galleries) plus eggs that had not yet hatched. Mating was recorded as ‘unsuccessful’ for parent pairs that had either died, emerged through the bark without constructing a vertical gallery, or constructed a vertical gallery with no evidence of oviposition (i.e., no larval galleries or eggs). Mating was recorded as ‘successful’ for parent galleries with evidence of larval galleries or eggs. Unhatched eggs were often found at the end of the parent gallery and were assumed to be oviposited later than the hatched eggs at the beginning of the parent gallery.

**Offspring emergence**

The remaining three UT and three NV beetle-infested bolts of each host tree were individually caged with screen (Fig. 3.3) and remained at 22.5°C. Adult offspring began emerging from the infested bolts approximately 50 days after manual infestation. We collected newly emerged offspring from each bolt once daily for days 50 – 100 after infestation and continued to do so until either five consecutive days had passed with no new offspring emergence
from the bolt or day 150 was reached. At that time, bolts were moved to refrigeration to halt any additional development and fungal growth.

We recorded the size and sex of each offspring beetle. After bolts were moved to refrigeration, we peeled the bark and phloem to expose beetle galleries and recorded individual parent gallery lengths (cm). Parent gallery scoring of xylem was easily visible, but offspring development left minimal xylem scoring and individual larval galleries were difficult to distinguish in the degraded phloem. In bolts that were peeled 26 days after infestation in the mating and fecundity study described above, >93% of unsuccessful parent galleries were <10 cm in length. Therefore, in this study parent galleries that were <10 cm were recorded as ‘unsuccessful’ and parent galleries >10 cm were recorded as ‘successful.’

**Statistical Analyses**

Data were analyzed with generalized linear mixed models using the GLIMMIX procedure in SAS Studio version 9.4. All models used Laplace maximum likelihood estimation and least squared means estimates for multiple comparisons were calculated using Tukey’s range test on the inverse link scale (Littell *et al.*, 1996). Because one Great Basin bristlecone pine and one limber pine each were cut from two sites, but the lodgepole pine was the only host tree cut from the third site, we had an unbalanced factorial design for considering host tree species and host tree site. For this reason, rather than grouping host trees by species, we considered all five host trees individually in analyses to create a balanced, one-way factorial design with five levels. This approach also allowed us to assess intraspecific differences between the two Great Basin bristlecone pines and the two limber pines.

We tested for size differences between parent beetles from NV and parent beetles from UT using mountain pine beetle population source (NV or UT) as a fixed-effect factor and a negative binomial response distribution on the log scale. Due to female-biased sexual size dimorphism in mountain pine beetles (Safranyik, 1976), male and female parent beetle sizes were
tested separately. To verify that similarly-sized parents within each population had been used to infest all host trees, we used the same model design, substituting individual host tree (bristlecone pines BR-HT and BR-DX, limber pines LM-HT and LM-DX, and lodgepole pine LP-UWC) as the fixed-effect factor, to test for parent beetle size differences across host trees for each beetle population. Studies have shown that mountain pine beetle brood production is positively related to phloem thickness (Amman, 1972a; Berryman, 1976; Amman & Cole, 1983; Bentz et al., 2015), therefore we tested for differences in the average phloem thickness of each bolt with host tree as the fixed-effect factor and a gamma response distribution on the log scale. Due to missing data, only measurements from four of the eight total lodgepole pine bolts were used in phloem thickness tests.

Analyses of mating and fecundity study data were conducted using independent individual parent galleries because only one bolt replicate of each host tree-beetle population combination was used. Fixed-effects factors in all models were individual host tree (bristlecone pines BR-HT and BR-DX, limber pines LM-HT and LM-DX, and lodgepole pine LP-UWC) and parent mountain pine beetle population source (NV or UT), but different error distributions and link scale functions were used in model calculations for each response variable tested as indicated. We tested for differences in (1) the probability of parent mating success (binary error distribution; logit link), (2) individual parent gallery length (Gaussian error distribution; identity link), and (3) total fecundity (sum of larval galleries and unhatched eggs) per parent gallery (negative binomial error distribution; log link). All initiated parent galleries were used in test (1), but only successful parent galleries were used in tests (2) and (3). We initially investigated the effects of parent beetle sizes on the response variables but found weak and/or inconsistent relationships. Therefore, parent beetle sizes were ultimately excluded from final model analyses for these tests.
Similar to mating and fecundity study data analyses, results from offspring emergence experiments were analyzed using individual host tree (bristlecone pines BR-HT and BR-DX, limber pines LM-HT and LM-DX, and lodgepole pine LP-UWC) and parent mountain pine beetle population source (NV or UT) as fixed-effect factors in all model calculations. To account for offspring grouping within bolts, host tree bolt nested within individual host tree and mountain pine beetle population source was also included as a random-effects factor in all models. Offspring emergence continued longer in some bolts than others and relatively few offspring emerged after day 100 (Fig. 3.7), so to standardize offspring emergence collection periods across all bolts, only offspring that emerged between day 50 and day 100 after infestation were included in model calculations. Error distributions and link scale functions differed for each response variable tested as indicated. We tested for differences in (1) individual parent gallery length (Gaussian error distribution; identity link), (2) the total number of emerged offspring / the number of successful parent galleries for each bolt, hereinafter ‘average total number of emerged offspring per successful parent gallery’ (Poisson error distribution; log link), and (3) offspring size; tested separately for each sex (Gaussian error distributions; identity links). For tests (1) and (2), only parent galleries marked as ‘successful’ were used, and no information on parent galleries was required for test (3). Again, parent beetle sizes did not strongly influence the response variables, so parent beetle sizes were ultimately excluded from final model calculations for these tests.

**Results**

*Parent beetle sizes and bolt phloem thickness*

Parent mountain pine beetles from the NV population were significantly larger than parent beetles from the UT population for both male ($F_{1,613} = 115.28, P < .0001$) and female ($F_{1,613} = 156.80, P < .0001$) beetles (Fig. 3.4). Statistical tests confirmed that parent beetle sizes
within each population were similar across infested host trees ($F_{4,289} = 0.73, P = 0.5698$ for NV male beetles; $F_{4,289} = 0.12, P = 0.9744$ for NV female beetles; $F_{4,316} = 1.59, P = 0.1756$ for UT male beetles; $F_{4,316} = 1.50, P = 0.2009$ for UT female beetles), meaning there were no unintended size biases in parent beetles used to infest cut bolts of each host tree.

Although all host trees had a similar dbh of ~30-35 cm, phloem thickness differed significantly among host trees ($F_{4,31} = 62.53, P < .0001$). Phloem was the thickest in the Great Basin bristlecone pines. Phloem in bristlecone BR-HT was significantly thicker than phloem in bristlecone BR-DX (adjusted $P < .0001$), and both bristlecone pines had significantly thicker phloem than all other host trees (adjusted $P$ values range 0.0272 to <.0001 for multiple comparisons). Phloem thickness did not differ between limber pines LM-HT and LM-DX (adjusted $P = 0.6871$) and was significantly thinner in the lodgepole pine LP-UWC relative to all other host trees (adjusted $P$ values range 0.0006 to <.0001 for multiple comparisons) (Fig. 3.5).

*Mating success and fecundity*

All mountain pine beetle parent pairs were equally likely to mate successfully regardless of the host tree ($F_{4,127} = 0.90, P = 0.4645$) or the beetle population source ($F_{1,127} = 1.70, P = 0.1949$).

The total length of individual galleries constructed by parent mountain pine beetles was significantly influenced by host tree ($F_{4,97} = 3.43, P = 0.0114$), and parent beetles from the NV population constructed significantly longer galleries than those from the UT population ($F_{1,97} = 17.77, P < 0.0001$). The longest successful parent galleries were constructed in bolts from Great Basin bristlecone pine, although only the long successful parent galleries in bristlecone BR-DX (mean 56.4 cm for NV beetles; 36.33 cm for UT beetles) and the short successful parent galleries constructed in limber LM-HT (mean 38.6 cm for NV beetles; 31.0 cm for UT beetles) were significantly different (adjusted $P = 0.0187$).

Total fecundity (hatched + unhatched eggs) per successful mountain pine beetle parent pair was also significantly influenced by host tree ($F_{4,97} = 2.78, P = 0.0310$), and parent mountain
pine beetles from the NV population had significantly higher total fecundity than parent beetles from the UT population ($F_{1,97} = 6.19, P = 0.0146$) (Fig. 3.6). Total fecundity was lowest in the Great Basin bristlecone pines, although the only significant difference was between the high total fecundity of mountain pine beetle parents placed in limber LM-DX and the low total fecundity of parent beetles placed in bristlecone BR-DX (adjusted $P = 0.0422$) (Fig. 3.6).

Offspring emergence

Extremely few offspring emerged from Great Basin bristlecone pine host trees (108 total from BR-HT; 33 total from BR-DX) relative to the other host tree species (2,802 total from LM-HT; 1,833 from LM-DX; 2,314 from LP-UWC) (totals reflect the complete offspring emergence period recorded for each bolt; totals are unadjusted for differences in the number of successful parent galleries) (Fig. 3.7). Although offspring emergence generally continued longer in Great Basin bristlecone pine relative to the other host tree species, post-emergence peeling of the phloem revealed evidence of many dead offspring. However, degradation of the phloem and the deceased offspring over the course of the experiment obscured individuals and their larval galleries, so we were not able to quantify percent brood mortality in any of the host trees.

When adjusting for the number of successful parent galleries in each bolt and standardizing the offspring emergence period to days 50-100 after infestation for all bolts, the average total number of emerged offspring per successful parent gallery was influenced by the host tree ($F_{4,24} = 175.94, P < .0001$), with bolts from the Great Basin bristlecone pine trees producing significantly fewer offspring than the other host trees (Fig. 3.8). There were no significant differences in the average total number of emerged offspring per successful parent gallery between the limber and lodgepole pine host trees (adjusted $P$ values range 0.0852 to 0.9021 for multiple comparisons). Average total offspring emergence per successful parent gallery was not significantly influenced by the mountain pine beetle population ($F_{1,24} = 0.06, P = 0.8124$).
Parent gallery length was significantly influenced by host tree ($F_{4,24} = 4.23, P = 0.0099$), and similar to results in the mating and fecundity study, parent mountain pine beetles from the NV population constructed significantly longer galleries than those from the UT population ($F_{1,24} = 9.46, P = 0.0052$). Also similar to results from the mating and fecundity study, the only significant difference was between the longest successful parent galleries in bristlecone BR-DX (mean 44.3 cm for NV beetles; 46.3 cm for UT beetles) and the shortest successful galleries in limber LM-HT (mean 38.7 cm for NV beetles; 30.7 cm for UT beetles) (adjusted $P = 0.0038$).

Similar to the parent beetles, both male and female offspring from the NV mountain pine beetle population were significantly larger than those from the UT beetle population ($F_{1,20} = 113.33, P < .0001$ for males; $F_{1,20} = 152.16, P < .0001$ for females) (Fig. 3.9). Host tree significantly affected female offspring size ($F_{4,20} = 7.10, P = 0.0008$) but not male offspring size ($F_{4,20} = 2.41, P = 0.4812$). Female offspring from both Great Basin bristlecone pines were significantly smaller than those from limber pine (adjusted $P$ values range 0.0031 to 0.0296 for multiple comparisons), and females from bristlecone BR-HT were also significantly smaller than females from the lodgepole pine (adjusted $P = 0.0453$) (Fig. 3.10).

**Discussion**

Our findings demonstrate that Great Basin bristlecone pine is not a suitable host for mountain pine beetle offspring. The low mountain pine beetle preference for Great Basin bristlecone pine documented in recent studies (Gray *et al.*, 2015; Bentz *et al.*, 2016b) is therefore not mismatched with adequate offspring performance (i.e., a low preference-high performance relationship), but rather reflects a strongly correlated low oviposition preference-low offspring performance relationship in this host.

We anticipated low rates of mating success and fecundity in Great Basin bristlecone pine due to high constitutive phloem defenses (Bentz *et al.*, 2016b), but we found that parent mountain pine beetles were equally likely to mate and successfully oviposit in Great Basin bristlecone,
limber, and lodgepole pines. However, despite similarities among host trees in the early stages of reproduction, extremely few offspring survived to complete development in Great Basin bristlecone pine relative to lodgepole and limber pines (Figs. 3.7 and 3.8). Furthermore, offspring that did survive in Great Basin bristlecone pine were less fit than those from the other host tree species: females were smaller in size (Fig. 3.10) and emerged asynchronously (Fig. 3.7). Small female size is hypothesized to reflect lower energy reserves for dispersal and host-searching (Graf et al., 2012) and may be linked to lower fecundity (Mcghehey, 1971; Amman, 1972b), although the extent of the relationship between female beetle size and fecundity is unclear. Asynchronous emergence hinders the ability of mountain pine beetle to maintain seasonality and for offspring to carry out mass-attacks on new host trees. Meanwhile, although other studies have found that limber pine is a superior host species for mountain pine beetle brood (Langor et al., 1990; Cerezke, 1995), we found that brood production and offspring traits were similar between lodgepole and limber pines (Figs. 3.7, 3.8, and 3.10). Moreover, phloem thickness, regardless of host tree species, has often been considered the most important factor in mountain pine beetle brood production (Amman, 1972a; Berryman, 1976; Amman & Cole, 1983; Bentz et al., 2015).

In our study, however, Great Basin bristlecone pine had the thickest phloem and produced the fewest offspring, and lodgepole pine had the thinnest phloem and produced as many offspring as the limber pines (Figs. 3.5 and 3.8). Both intraspecific and interspecific variability in host tree quality likely have important effects on mountain pine beetle offspring performance, and it is important to consider both types of variability when drawing conclusions about relative host suitability.

We predicted that mountain pine beetles from the NV limber pines would demonstrate superior offspring performance relative to those from the UT lodgepole pine in all host trees due to several potential fitness advantages. Parent beetles from NV were larger (Fig. 3.4), laid more eggs (Fig. 3.6), and constructed longer parent galleries than those from UT, which may have been
to accommodate their higher fecundity and/or because they needed to consume more phloem to support their larger body size. The larger size and higher fecundity of NV parent beetles is consistent with previous studies suggesting that beetles raised in limber pine are larger and more fecund (Langor et al., 1990; Cerezke, 1995), although we did not observe size differences between offspring raised in limber versus lodgepole pine in our study.

Despite evidence of superior fecundity, however, offspring emergence was not higher for the NV population relative to the UT population. Although more NV offspring than UT offspring emerged from the lodgepole and limber pine hosts (Fig. 3.7), when offspring emergence was adjusted for the number of successful parent galleries in each bolt (and the emergence period was standardized to 50 – 100 days), there were no significant differences in total offspring emergence between the two beetle populations in any of the host trees. This finding was also inconsistent with our predictions that the NV beetles, which were collected from a mixed stand within Great Basin bristlecone pine range, would be more locally adapted to utilize the species than the UT beetles, which were collected from outside of Great Basin bristlecone pine range. Furthermore, the hypothesized superior detoxification abilities of mountain pine beetles raised in limber pine (NV parents) as compared to lodgepole pine (UT parents) (Langor et al., 1990) evidently did not affect offspring survival in Great Basin bristlecone pine. In fact, although differences were not significant, fewer NV beetles than UT beetles emerged from Great Basin bristlecone pine, suggesting that opportunities for mountain pine beetle adaptation to utilize Great Basin bristlecone pine are limited.

Because fecundity was higher in NV beetles compared to UT beetles, but offspring emergence was similar between the two populations, we may infer a higher rate of mortality during brood development in the NV population relative to the UT population. Similar to their parents, offspring from the NV population were larger than those from the UT population in all host trees (Fig. 3.9). Perhaps their larger size combined with initially higher numbers of larvae led
to higher intraspecific competition between developing brood within bolts, leading to lower overall percent survivorship from egg to adulthood.

Why did mountain pine beetle offspring develop successfully in lodgepole and limber pine, but not in Great Basin bristlecone pine? A number of factors could lead to poor mountain pine beetle offspring performance in a given host tree species, including unsuccessful colonization by parent beetles, toxic host tree defenses, competition, or external factors, but our study focused specifically on the effects of the passive phloem environment. We simulated successful colonization through manual infestation of parent beetles, we removed the capacity for induced host tree defenses by cutting host trees prior to infestation, and we controlled for competition and external effects in laboratory experiments. Host tree bolts presumably retained many constitutive qualities, including nutrients, physical traits, and pre-formed chemical compounds. Given these circumstances, we propose three hypotheses to explain poor offspring performance in Great Basin bristlecone pine phloem.

First, Great Basin bristlecone pine may have low-quality phloem that lacks the nutrients necessary for mountain pine beetle offspring to complete development. For example, low availability of phloem nutrients such as nitrogen can limit insect development (Raffa et al., 2015). The length of mountain pine beetle parent galleries is hypothesized to reflect phloem quality (Langor et al., 1990), with longer galleries indicating low-quality phloem because a greater quantity of phloem is needed to meet nutritional needs. In our study, limber pine LM-HT had the shortest parent galleries and the highest offspring emergence, and Great Basin bristlecone pine BR-DX had the longest parent galleries, the lowest fecundity, and the lowest offspring emergence, suggesting low phloem nutrition in BR-DX relative to LM-HT. However, although offspring performance in bristlecone BR-HT was also very low, parent galleries in BR-HT were not significantly longer than galleries in the other host trees, so our evidence is inconclusive. Given the larger size and presumably higher nutritional needs of the NV offspring, low-nutrition
phloem could also explain the lower offspring emergence from Great Basin bristlecone in the NV population relative to the UT population. However, the inability of mountain pine beetle offspring to obtain sufficient nutrition from Great Basin bristlecone pine phloem would be surprising considering that phloem thickness was greatest in Great Basin bristlecone pine (Fig. 3.5), and therefore was available in the highest abundance. Moreover, mountain pine beetle offspring can obtain enough nutrients to successfully develop in the majority of western North American pines, several species of exotic pines, and even some species of spruce (*Picea*) (Wood, 1963; Furniss & Schenk, 1969; Mckee et al., 2013).

A second hypothesis is that Great basin bristlecone pine possesses constitutive phloem defenses that inhibit developing mountain pine beetle offspring. Bentz *et al.* (2016b) found that Great Basin bristlecone pine has over eight times the constitutive phloem compounds found in limber pine, and these compounds have been associated with high toxicity and tree defense (Raffa, 2014). Xu *et al.* (2016) found that adult *Dendroctonus valens* have highly resilient gut microflora and can quickly adapt to tolerate high levels of host tree defensive chemicals during parent gallery construction. If mountain pine beetle adults also possess these adaptive detoxification traits, this hypothesis could further explain why parent beetles were successful in mating and oviposition in Great Basin bristlecone pine (Fig. 3.6). Perhaps a lack of transfer of gut microflora from parent adults to larvae resulted in the inability of offspring to detoxify the chemicals found by Bentz *et al.* (2016b), or to digest other possible phloem defenses such as high lignin concentration (Franceschi *et al*., 2005). Larval development requires more time than oviposition, and differences in exposure time to toxins in Great Basin bristlecone pine phloem is another possible explanation for mating and oviposition success in adults but high mortality in offspring. Finally, the Resource Availability Hypothesis predicts that slow-growing plants will invest heavily in anti-herbivore defenses due to the high cost of replacing tissue (Coley *et al*.,
1985). Great Basin bristlecone pine, which is a slow-growing tree, is therefore expected to have high defenses that negatively affect phytophagous insects.

Lastly, perhaps Great Basin bristlecone pine possesses traits that inhibit the establishment of mutualistic fungi and bacteria associated with mountain pine beetle. Microbial symbionts, including ‘blue-stain’ fungi, play an important role in mountain pine beetle nutrient acquisition and aid in surviving host tree defensive chemicals (Raffa et al., 2015). Fungal basidiomycetes can provide nutrients by being directly consumed by bark beetle larvae, and symbiotic bacteria can contribute to improved nutrition for developing larvae via nitrogen fixation in the gut (Morales-Jiménez et al., 2012). Mountain pine beetle microbial communities can also degrade and metabolize toxic compounds in host tree phloem (Adams et al., 2013). The lack of establishment of microbial mutualists could therefore exacerbate the effects of either low phloem nutrition or high phloem toxins on mountain pine beetle offspring. However, while we did not test for the presence or absence of fungal and bacterial symbionts in infested Great Basin bristlecone pine, we did observe blue coloration in the sapwood of Great Basin bristlecone pine, which is associated with the presence of some species of symbiotic microbes. Although there is not currently enough evidence to conclusively determine the reason for high offspring mortality in Great Basin bristlecone pine, we favor the second hypothesis that mountain pine beetle offspring cannot tolerate high constitutive phloem defenses in Great Basin bristlecone pine.

If higher constitutive phloem defenses are responsible for low mountain pine beetle offspring performance in Great Basin bristlecone pine, why aren’t these same defense traits present in co-occurring limber pine? Constitutive defenses in pines are closely linked with their macroevolutionary history, and the absence of shared selective pressures over long time scales can result in differences in plant defenses (Carrillo-Gavilán et al., 2015). Tree species in the Foxtail Pine group, including the ancestors of modern Great Basin bristlecone pine, have been growing in the western United States for at least 40 million years (Lanner, 2007). Although it is
possible that limber pine was also present in this area millions of years ago, the oldest limber pine
fossils to date are fewer than 50,000 years old (Mitton et al., 2000 and references within). It is
difficult to infer which selective agents may have interacted with these tree species over their
long evolutionary histories, but perhaps longer or more intense past herbivore pressure in Great
Basin bristlecone pine gave rise to a unique arsenal of defenses against phytophagous insects.
Furthermore, the greater longevity in Great Basin bristlecone pine relative to limber pine may aid
in the maintenance of highly effective defense traits over time despite interrupted contact with
selective phytophagous agents (Hamrick, 1979).

The highly adaptive low oviposition preference-low offspring performance relationship
between Great basin bristlecone pine and mountain pine beetle is interesting considering that cool
temperatures may have limited contact between mountain pine beetle and high-elevation Great
Basin bristlecone pine in recent history. ‘Ecological traps,’ whereby female insects select host
plants for oviposition that are not suitable for their offspring, have been documented between
other insects and plant species with limited prior association (Robertson et al., 2013). Given the
longstanding presence and previously extensive range of Great Basin bristlecone pine in the
Western United States, perhaps mountain pine beetle shares an evolutionary history with this
species and maintains behavioral avoidance traits that evolved during a past time of close
association. Alternatively, mountain pine beetles may avoid Great Basin bristlecone pine due to
ecological fitting, i.e., as a result of traits they already possess that are unrelated to prior
encounters with a given potential host species (Agosta, 2006). Mountain pine beetles possess
complex host assessment capabilities and integrate numerous lines of sensory information at
multiple scales to select optimal hosts (Raffa et al., 2016). Perhaps the stimuli that pioneering
female beetles detect from Great Basin bristlecone pine are similar to the stimuli of highly-
defended tree individuals within other host species, and these familiar cues indicating low
offspring performance cause low preference for Great Basin bristlecone pine. Another possible
explanation is that Great Basin bristlecone pine lacks the stimuli necessary for host recognition by mountain pine beetle, and low mountain pine beetle preference occurs by lucky chance. However, the direct cause for mountain pine beetle avoidance of Great Basin bristlecone pine is beyond the scope of this research, and these hypotheses are highly speculative.

We did not investigate potential barriers to mountain pine beetle colonization in Great Basin bristlecone pine (i.e., the potential for induced defenses and constitutive bark defenses), but future herbivore defenses. Furthermore, the exact cause of high offspring mortality in Great Basin bristlecone pine was not identified in our experiments, but understanding the specific traits that are responsible for the death of developing larvae/pupae may facilitate the development of new tools to protect forest communities that are susceptible to mountain pine beetle outbreaks.

Earlier studies have documented low mountain pine beetle preference for Great Basin bristlecone pine (Gray et al., 2015, Bentz et al., 2016b) and our results indicate that most mountain pine beetle offspring die before completing development in Great Basin bristlecone pine. The strongly correlated low oviposition preference-low offspring performance relationship between mountain pine beetle and Great Basin bristlecone pine indicates that barriers to host plant use in this system involve both behavioral and physiological factors. The number of mountain pine beetle offspring that emerged from Great Basin bristlecone pine was fewer than the number of parent beetles that successfully mated in this host species, therefore our findings suggest that there is strong selective pressure to maintain low mountain pine beetle preference for Great Basin bristlecone pine. Our results provide strong evidence that Great basin bristlecone pine has low vulnerability to climate-driven increases in high-elevation mountain pine beetle outbreaks.

References


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Fig. 3.1 Locations of parent mountain pine beetle population sources and host tree harvest sites. Two mountain pine beetle-infested limber pines were cut from Nevada (NV) and one infested lodgepole pine was cut from Utah (UT) to obtain two populations of unmated mountain pine beetle adults for use in tests. Live, uninfested Great Basin bristlecone and limber pines were cut from the Humboldt-Toiyabe National Forest and the Dixie National Forest sites (a single tree of each species from each site), and one live, uninfested lodgepole pine was cut from the Uinta-Wasatch-Cache National Forest site.
Fig. 3.2 Photos of manually infesting cut tree bolts with mountain pine beetle parent pairs. (a) We initiated ~2 cm deep parent galleries with a drill at equal spacing along the circumference of a bolt and (b) we manually inserted parent beetles into galleries and covered gallery openings with screen. Photo (c) shows the bottom of a fully infested bolt.
Fig. 3.3 Photo of infested tree bolt caged in screen to facilitate daily collections of emerged offspring. Infested bolts were held in incubator cabinets at 22.5°C and emerging offspring were collected from day 50 up to day 150 following infestation.
Fig. 3.4 Male (M) and female (F) parent mountain pine beetle size from Nevada (NV) and Utah (UT) populations. Shown are the median (solid middle line), 25th and 75th percentiles (top and bottom of box), ± 1.5 x the interquartile range (whiskers), and all outliers (points). N = number of beetles in each category.
Fig. 3.5 Phloem thickness of bolts cut from each of the five host trees that were manually infested with mountain pine beetle parents. HT = Humboldt-Toiyabe National Forest site, DX = Dixie National Forest site, UWC = Uinta-Wasatch-Cache National Forest site. All eight bolts from each of the Great Basin bristlecone and limber pine host trees were used in calculations but only four of the lodgepole pines bolts were used due to missing data. Shown are the median (solid middle line), 25th and 75th percentiles (top and bottom of box), ± 1.5 x the interquartile range (whiskers), and all outliers (points).
Fig. 3.6 Total fecundity measured by the sum of larval galleries (hatched eggs) and eggs that had not yet hatched for each parent gallery. HT = Humboldt-Toiyabe National Forest site, DX = Dixie National Forest site, UWC = Uinta-Wasatch-Cache National Forest site; NV = parent beetles from Nevada, UT = parent beetles from Utah. Shown are the median (solid middle line), 25th and 75th percentiles (top and bottom of box), ± 1.5 x the interquartile range (whiskers), and all outliers (points). N = number of parent galleries in each category.
Fig. 3.7 Cumulative total numbers of emerged offspring by host tree and mountain pine beetle population for the entire emergence period, unadjusted for slightly differing numbers of successful parent galleries in each host tree. BR = Great Basin bristlecone pine, LM = limber pine, LP = lodgepole pine; HT = Humboldt-Toiyabe National Forest site, DX = Dixie National Forest site, UWC = Uinta-Wasatch-Cache National Forest site. Emerging offspring were collected from six individually infested bolts of each host tree starting at day 50 through day 100 after infestation and until five consecutive days had passed with no newly emerged offspring from the bolt or day 150 was reached. Note Y axis limit is only 100 for Great Basin bristlecone pine graphs but Y axis limit is 1,500 for graphs for other tree species.
Fig. 3.8 Average total number of emerged offspring per successful parent gallery by host tree for days 50-100 after infestation. There was no difference in average total number of emerged offspring per successful parent gallery between mountain pine beetle populations. HT = Humboldt-Toiyabe National Forest site, DX = Dixie National Forest site, UWC = Uinta-Wasatch-Cache National Forest site. Shown are the median (solid middle line), 25th and 75th percentiles (top and bottom of box), ± 1.5 x the interquartile range (whiskers), and all outliers (points). There were six bolts of each
Fig. 3.9 Male (M) and female (F) offspring mountain pine beetle size from Nevada (NV) and Utah (UT) populations. To standardize the emergence period across all bolts, only mountain pine beetles that emerged days 50-100 were used in size calculations. Shown are the median (solid middle line), 25th and 75th percentiles (top and bottom of box), ± 1.5 x the interquartile range (whiskers), and all outliers (points). N = number of beetles in each category.
Fig. 3.10 Female offspring size by host tree and mountain pine beetle population. HT = Humboldt-Toiyabe National Forest site, DX = Dixie National Forest site, UWC = Uinta-Wasatch-Cache National Forest site; NV = Nevada beetle population, UT = Utah beetle population. Female offspring from the Great Basin bristlecone pines were significantly smaller than those from the limber pines, and females from HT Great Basin bristlecone pine were significantly smaller than females from lodgepole pine. Male offspring size was not significantly different between host trees. Shown are the median (solid middle line), 25th and 75th percentiles (top and bottom of box), ± 1.5 x the interquartile range (whiskers), and all outliers (points). N = number of beetles in each category.
The goal of my thesis research was to investigate Great Basin bristlecone pine vulnerability to climate-driven increases in high-elevation mountain pine beetle outbreaks. To accomplish this, I evaluated female mountain pine beetle host selection behavior and offspring performance in Great Basin bristlecone pine relative to known host species. Results provided strong evidence indicating that mountain pine beetle has low preference for Great Basin bristlecone pine and that this important high-elevation species is not suitable for mountain pine beetle offspring development.

In no-choice tests of female host selection behavior, mountain pine beetles avoided the bole of Great Basin bristlecone pine even though no alternative host options were available. Aversive host selection behavior toward Great Basin bristlecone pine was evident in tests on living trees and in tests on cut tree sections that had no capacity for induced defensive reactions to an attack. Relative to known-host limber pine, mountain pine beetles were about two times more likely to be farther away from the bole of Great Basin bristlecone pine in tests on living trees, and about five times more likely be farther away from the bole of Great Basin bristlecone pine in tests on cut trees. These results support the findings of earlier studies suggesting that mountain pine beetle avoids Great Basin bristlecone pine (Bentz et al., 2016; Gray et al., 2015), and further indicate that low preference is due at least in part to stimuli that repel pioneering females from initiating attacks. Moreover, the increased aversion toward Great Basin bristlecone pine observed in tests on cut tree sections versus tests on living trees suggests that pre-formed constitutive defenses likely play a major role in the cause behind low mountain pine beetle preference for Great Basin bristlecone pine. This finding provides evidence to support the Resource Availability Hypothesis, which predicts that slow-growing plants will invest heavily in constitutive defenses due to the high cost of replacing tissue (Coley et al., 1985).
In tests of mountain pine beetle offspring performance in cut sections of Great Basin bristlecone pine, parent mountain pine beetles successfully carried out mating and oviposition, but nearly all offspring died before completing development. Mountain pine beetle parents were equally likely to mate in Great Basin bristlecone pine relative to known-host species lodgepole and limber pines, and fecundity was mostly similar between all three host tree species. Despite similarities in initial reproductive development, however, the number of mountain pine beetle offspring that emerged from Great Basin bristlecone pine was fewer than the number of parent beetles that successfully mated in this host species, whereas parent beetles in lodgepole and limber pine produced many offspring. Interestingly, phloem thickness has often been considered the most important factor in mountain pine beetle brood production (Amman, 1972; Berryman, 1976; Amman and Cole, 1983; Bentz et al., 2015). In this study, however, Great Basin bristlecone pine had the thickest phloem and produced the fewest offspring. Furthermore, the offspring that did emerge from Great Basin bristlecone pine were less fit than those that emerged from lodgepole and limber pine. Bentz et al. (2016) found that Great Basin bristlecone pine has over eight times the concentration of constitutive phloem toxins relative to limber pine. Perhaps these toxins are not tolerated by developing mountain pine beetle larvae, which could explain the poor offspring performance observed in this study. These findings suggest that Great Basin bristlecone pine is not a suitable host species for mountain pine beetle reproduction. Because of this, there is strong selective pressure to maintain low female mountain pine beetle preference for Great Basin bristlecone pine.

To investigate the effects of mountain pine beetle population trait differences on the host preference-offspring performance relationship in Great Basin bristlecone pine, mountain pine beetles from two different populations were tested in both studies. One population of mountain pine beetles was collected from infested limber pine growing in a mixed stand with Great Basin bristlecone pines in Nevada. The second population was collected from infested lodgepole pine
growing outside of Great Basin bristlecone pine range in Utah. The mountain pine beetles infesting the Nevada limber pines had several potential advantages for host utilization of Great Basin bristlecone pine over those infesting the Utah lodgepole pine. Relative to mountain pine beetles raised in lodgepole pine, beetles that develop in limber pine have been found to be larger, more fecund, and are hypothesized to have superior detoxification systems (Langor et al., 1990; Cerezke, 1995). Furthermore, the Nevada beetles may have had increased opportunity for adaptation to Great Basin bristlecone pine because they were collected from a mixed stand within Great Basin bristlecone pine range. Although little is known about the spatial and temporal scales required for mountain pine beetles to adapt to a relatively new host species, local adaptation (i.e., increased preference and performance) to recently introduced exotic host plant species has been demonstrated among populations of other insects that occur where the new plant species is present (Keeler and Chew, 2008). The rationale for choosing these two populations of mountain pine beetles was to investigate whether the potentially superior fitness and increased opportunity for adaptation to Great Basin bristlecone in the Nevada beetles would result in higher female host preference or offspring performance in Great Basin bristlecone pine. However, in this study, mountain pine beetles from the Nevada population avoided Great Basin bristlecone pine more frequently relative to the Utah beetles, and although differences were not significant, fewer Nevada offspring versus Utah offspring emerged from lab-infested Great Basin bristlecone pine. These results suggest that opportunities for mountain pine beetle populations to adapt to use Great Basin bristlecone pine as a host species may be limited.

Although high-elevation pine species are hypothesized to have low defenses against mountain pine beetles due to historically intermittent contact (Raffa et al., 2013), the results of this study suggest that high-elevation Great Basin bristlecone pine is resistant to mountain pine beetle, likely due to high constitutive defenses. This finding is surprising considering that mountain pine beetles can successfully attack and 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). These results raise interesting questions about the evolutionary history between Great Basin bristlecone pine and phytophagous insects. Perhaps longer or more intense past herbivore pressure resulted in a unique arsenal of defenses in Great Basin bristlecone pine relative to other pine species, and the extreme longevity of Great Basin bristlecone pine aids in the maintenance of these defense traits (Hamrick, 1979).

The strongly correlated low oviposition preference-low offspring performance relationship between mountain pine beetle and Great Basin bristlecone pine indicates that barriers to host plant use in this system involve both behavioral and physiological factors. This evidence of Great Basin bristlecone pine resistance to mountain pine beetle, regardless of induced defensive capability or mountain pine beetle population source, suggests that Great Basin bristlecone pine has low vulnerability to climate-driven increases in high-elevation mountain pine beetle outbreaks. These findings aid forest managers in predicting mountain pine beetle impacts in Great Basin bristlecone pine forest communities. 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.

**References**


