

# Temperature-driven range expansion of an irruptive insect heightened by weakly coevolved plant defenses

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Edited by Rodney B. Croteau, Washington State University, Pullman, WA, and approved December 5, 2012 (received for review September 26, 2012)

Warming climate has increased access of native bark beetles to high-elevation pines that historically received only intermittent exposure to these tree-killing herbivores. Here we show that a dominant, relatively naïve, high-elevation species, whitebark pine, has inferior defenses against mountain pine beetle compared with its historical lower-elevation host, lodgepole pine. Lodgepole pines respond by exuding more resin and accumulating higher concentrations of toxic monoterpenes than whitebark pine, where they co-occur. Furthermore, the chemical composition of whitebark pine appears less able to inhibit the pheromonal communication beetles use to jointly overcome tree defenses. Despite whitebark pine's inferior defenses, beetles were more likely to attack their historical host in mixed stands. This finding suggests there has been insufficient sustained contact for beetles to alter their complex behavioral mechanisms driving host preference. In no-choice assays, however, beetles readily entered and tunneled in both hosts equally, and in stands containing less lodgepole pine, attacks on whitebark pines increased. High-elevation trees in pure stands may thus be particularly vulnerable to temperature-driven range expansions. Predators and competitors were more attracted to volatiles from herbivores attacking their historical host, further increasing risk in less coevolved systems. Our results suggest cold temperatures provided a sufficient barrier against herbivores for high-elevation trees to allocate resources to other physiological processes besides defense. Changing climate may reduce the viability of that evolutionary strategy, and the life histories of high-elevation trees seem unlikely to foster rapid counter adaptation. Consequences extend from reduced food supplies for endangered grizzly bears to altered landscape and hydrological processes.

climate change | coevolution | disturbance | plant-insect interactions | forest insects

Climate warming can have multiple and complex effects on species distributions, community structure, and ecosystem functions (1). These effects are likely to be particularly strong for species interactions, especially those involving ectotherms (2), which comprise the most abundant and diverse animal taxa on the biosphere. For example, there are already well-documented examples of insects colonizing elevations and latitudes beyond their historic limits in response to recent warming trends (1, 2). The resulting new host-plant associations have uncertain long-term consequences, but could potentially alter fundamental ecosystem processes and lead to new dynamics of relationships among species on the landscape. These changes also provide a rare opportunity to examine plant and herbivore factors that drive coevolution and to identify the mechanics of biological invasions along the boundaries of adapted versus naïve ecosystems.

Most herbivorous insects do not cause outbreaks under natural conditions, but populations of several dozen “irruptive” species undergo order-of-magnitude increases that arise from interactions between specific elements of their life histories and exogenous drivers. Bark beetles are perhaps foremost among this group, and are natural disturbance agents that contribute to succession, nutrient dynamics, and carbon cycling in conifer biomes (3–5). Adults bore through the bark, mate, and oviposit, and their larvae feed within the phloem, killing the tree. These native insects undergo landscape-scale outbreaks that pose significant

socioeconomic challenges and draw substantial political attention (2, 6). Over 47 million hectare have been impacted over the last decade (6, 7), and major biomes have been converted from carbon sinks to sources, thereby precipitating additional feedbacks (8). Such outbreaks, however, are actually intermittent exceptions to more stable long-term dynamics, as populations are typically constrained below critical thresholds for lengthy periods (6). Cool temperatures, tree-defense physiology, and natural enemies are foremost among the factors limiting population growth (9–12).

The frequency and severity of outbreaks have increased dramatically as rising temperatures have allowed greater winter survival and accelerated development from semivoltine to univoltine life histories (2, 10, 11, 13). Historically, mountain pine beetle (*Dendroctonus ponderosae*), the foremost tree-killer, primarily occupied lower-elevation lodgepole pine (*Pinus contorta*) habitat of the United States and Canadian Rocky Mountains (14). During occasional warm periods, *D. ponderosae* dispersed above the intervening barrier of nonhost spruce forests and killed whitebark pines (*Pinus albicaulis*), the dominant species in many high-elevation stands in the central and northern Rockies. These were usually small-scale incidents that subsided when normal temperatures resumed. However, recent continuously warm weather has allowed persistent reproduction in this keystone species (2, 14, 15).

Throughout much of the Rocky Mountains, whitebark pine is a critical source of nutrients for a diverse range of wildlife, most notably grizzly bears that rely on its energy-rich seeds for hibernation and gestation reserves. Whitebark pine also plays an important hydrological role for lowland systems by affecting snow melt distribution, provides microhabitat for other subalpine plants, and confers much of the aesthetic and recreational values associated with high-elevation systems (14). Ecologically, lodgepole and whitebark pines represent extremes along a continuum of life-history strategies within the genus *Pinus*, with the former being fast growing, early reproducing, and disturbance-adapted, and the latter having the opposite properties.

Conifers possess sophisticated defenses that can rapidly confine and kill invading bark beetles and their symbionts (16–18). These defenses consist of resin exudation that pitches out or delays attacking adults, and allelochemicals, particularly monoterpenes, that repel or kill the adults, prevent egg hatch, and inhibit or kill their symbionts (18–20). Monoterpenes occur at relatively low concentrations in constitutive (preattack) phloem, but are rapidly biosynthesized at the point of attack and accumulate to toxic levels within a few days (21–23). Bark beetles can overwhelm these barriers through pheromone-mediated mass attacks, in which large numbers of both sexes land on selected trees and tunnel into their tissues. When successful, high densities of beetles jointly deplete resin and reduce toxins to sublethal concentrations within

Author contributions: K.F.R. and P.A.T. designed research; E.N.P. and P.A.T. performed research; P.A.T. analyzed data; and K.F.R. and P.A.T. wrote the paper.

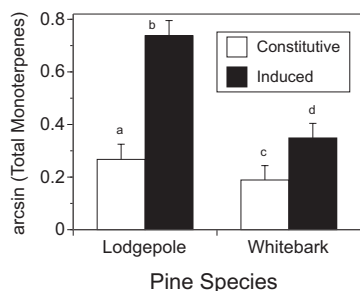
The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1216666110/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1216666110/-DCSupplemental).



**Fig. 1.** Total monoterpene contents of constitutive and induced phloem tissue in lodgepole and whitebark pines. Mature, apparently healthy, unattacked trees were in stands containing both species in Greater Yellowstone Ecosystem. Induction was performed by simulating natural attacks. Data shown as arcsin values. The means labeled with different letters are significantly different at  $P < 0.05$ .

just a few days (9). Bark beetles link their signaling behavior to tree physiology by exploiting some host monoterpenes as precursors and synergists of the pheromones they emit as they bore through the bark (24, 25). However, high concentrations of monoterpenes, as well as other host compounds, can inhibit attraction of flying beetles to signals emitted by tunneling beetles (26–28). If aggregation is not initiated, the tunneling beetles either abandon their attempt or are killed by host encapsulation responses. The ability of bark beetles to overcome tree defenses is further enhanced by their vectoring of moderately phytopathogenic symbiotic fungi, such as *Grosmannia clavigera* (29). This tenuous balance between rates of opposing plant and insect processes at the organismal scale has population- and landscape-scale consequences. At low densities, bark beetles are limited to trees whose defenses are impaired by stresses, such as drought, root disease, and lightning. Once their populations surpass a critical threshold, however, they can become self-driving through positive feedback between their behavior and abundance (30).

Predators and competitors exert additional constraints on bark beetle reproduction (12). The major predators are beetles in the family Cleridae, which are strongly attracted to bark beetle pheromones and associated host-plant volatiles (31), and consume all life stages on and under the bark. The major competitors are secondary bark beetles, such as *Ips pini*, which likewise are attracted to volatiles emitted from attacked trees and typically outcompete tree-killing species after tree death.

We currently do not know whether sustained reproduction by mountain pine beetle in high-elevation whitebark pine stands represents a mere spillover from the current outbreak in lodgepole pine or an incipient regime change. Current plant-defense theory suggests the K-selected whitebark pine should invest more in defense, assuming equal pest pressures (32). Conversely, the relatively sparse degree of historical interaction with bark beetles suggests whitebark pine may have invested less in defense, and hence be more susceptible. These interpretations are confounded by the dimension of host-selection behavior: namely, whether and how insects alter their relative preferences among plant species as environmental conditions change. Similarly, we have little information on how natural enemies respond to mountain pine beetles attacking historical vs. seminaïve host species.

We compared the susceptibility of mountain pine beetle's traditional host, lodgepole pine, with those of the relatively naïve host, whitebark pine, from three perspectives: tree-defense physiology, insect preference, and natural enemy communities. All experiments were conducted in the Greater Yellowstone Ecosystem of North America.

## Results

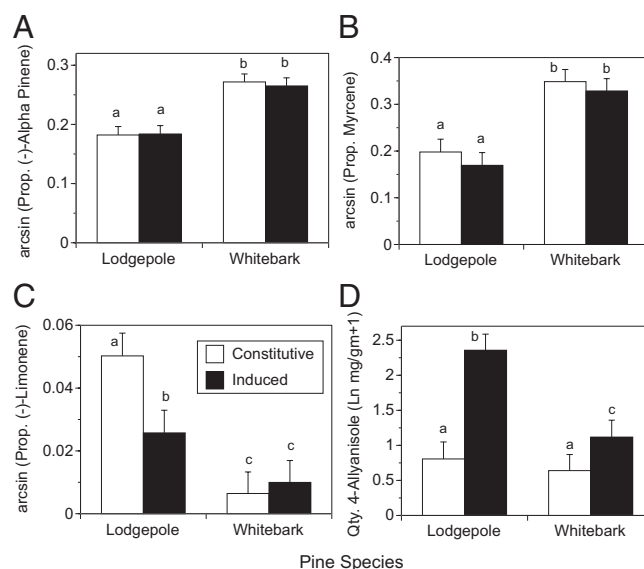
**Tree-Defense Physiology.** Tree species, tissue condition (constitutive vs. induced), and species by tissue condition significantly affected

total monoterpene content, but year, site, and tree size (DBH: diameter at breast height) did not ( $F_{6,248} = 41.62$ ,  $P < 0.0001$ ;  $r^2 = 0.502$ ) (Tables S1–S4). Overall, species, tissue condition, and their interaction explained over 50% of the variation in total monoterpene content in lodgepole and whitebark pines.

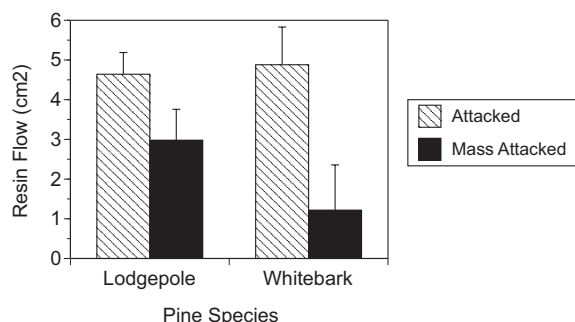
Lodgepole pines had slightly higher quantities of monoterpenes in their constitutive phloem tissue than did whitebark pine. However, these trees differed much more markedly in their responses to simulated attack (Fig. 1). Lodgepole pines responded to simulated attack by undergoing pronounced increases in their total monoterpene concentrations, whereas the induced responses of whitebark pines were comparatively lower.

In addition to differences in total quantities of monoterpenes, lodgepole and whitebark pines differed in their relative proportions of specific constituents (Tables S5–S17). In particular, whitebark pine had a 2.1-times higher (–)- $\alpha$ -pinene ( $F_{6,248} = 29.45$ ,  $P < 0.0001$ ;  $r^2 = 0.416$ ) and a 3.5-times higher concentration of myrcene ( $F_{6,248} = 26.94$ ,  $P < 0.0001$ ;  $r^2 = 0.395$ ) (Fig. 2A and B). Lodgepole pines had higher concentrations of (+)-limonene ( $F_{6,248} = 20.16$ ,  $P < 0.0001$ ;  $r^2 = 0.328$ ) (Fig. 2C). Complete monoterpene profiles are in Table S18 (proportion data used for analysis) and Tables S19 and S20 (raw concentrations by species by year). Lodgepole pines also contained more of the phenylpropanoid 4-allylanisol than did whitebark pine, particularly in induced phloem tissue ( $F_{6,248} = 41.62$ ,  $P < 0.0001$ ;  $r^2 = 0.502$ ) (Fig. 2D).

Lodgepole pines exuded more resin in response to mass attack by mountain pine beetle than did whitebark pines (Fig. 3). Using a general linear model (GLM), there was a significant attack status (attacked vs. mass attacked) by species interaction with area of resin flow for 167 pitch tubes measured in 2011 ( $F_{4,162} = 10.05$ ,  $P < 0.0001$ ). Specifically, mass attacked whitebark pines exuded 40.8% of the resin of mass attacked lodgepole pines (least-squares difference of means  $P = 0.0093$ ), with no significant differences in resin flow on nonmass attacked trees of each species. On a tree-



**Fig. 2.** Chemical constituents of lodgepole and whitebark pine phloem affecting the behavior of mountain pine beetle. (A–C) Monoterpenes, with each expressed as percentage of total monoterpene fraction. (–)- $\alpha$ -pinene and myrcene enhance attraction to mountain pine beetle pheromones; limonene is repellent and toxic. Complete monoterpene profiles are in the SI Tables. (D) The phenylpropanoid 4-allylanisol inhibits attraction of mountain pine beetle to its pheromone and inhibits bark beetle fungal symbionts. Mature apparently healthy, unattacked trees were in stands containing both tree species in Greater Yellowstone Ecosystem. The means labeled with different letters are significantly different at  $P < 0.05$ . Complete profiles in Table S18.



**Fig. 3.** Resin flow from lodgepole and whitebark pines attacked by mountain pine beetle. Mature trees were in stands containing both tree species, and were exposed to natural attacks.

by-tree basis, ordinary least-squares regression showed a significant decrease in pitch tube size with increasing attack density for whitebark pine ( $F_{1,6} = 10.07$ ,  $P = 0.0193$ , adjusted  $r^2 = 0.5643$ ), but no such relationship was present for lodgepole pine ( $F_{1,18} = 1.11$ ,  $P = 0.306$ ). Resin flow increased with tree diameter ( $F = 26.86$ ,  $P < 0.0001$ ). There were no significant differences in diameter between trees sampled of the two species (Kruskal–Wallis  $\chi^2 = 3.1992$ ,  $P = 0.0737$ ).

**Mountain Pine Beetle Host-Species Preference.** In the field, mountain pine beetles were more likely to attack lodgepole than whitebark pines in each year. In mixed stands in 2011, lodgepole pines ( $n = 30$  attacked of 50 total) were 184% more likely to be attacked than whitebark pine ( $n = 11$  attacked of 52 total) ( $\chi^2 = 16.0$ ,  $P < 0.0001$ ) (Fig. 4). The beetles had equal access to each species, as 45% of the trees larger than 20-cm DBH were lodgepole pines, 44% were whitebark pines, and 11% were other species. In 2012, lodgepole pines were 75% more likely to be attacked than whitebark pines (Fig. 4). However, if lodgepole pine was absent or at low density in a location, whitebark pines were more likely to be attacked than when lodgepole pines were present. In contrast, rates of attack on lodgepole pine were not affected by the presence or absence of whitebark pine (Fisher's Exact Test,  $\chi^2 = 3.0$ ,  $P = 0.0833$ ). Although the overall sample of attacked trees was low, it is noteworthy that only one of the attacked whitebark pines had any sizable (>20 cm) live lodgepole pines nearby, but live whitebark pines were proximal to four of the seven attacked lodgepole pines. In neither year was attack status related to tree size or age, and tree sizes and age did not differ between species at each site (Kruskal–Wallis,  $P > 0.5$  for both tree diameter and age between species for both years). Among trees that were attacked, 32.1% of lodgepole and 35.7% of whitebark pines were killed.

When beetles were placed directly onto bark discs under controlled no-choice conditions, they readily entered each tree species at equivalent rates, 76% (SEM = 5.81) and 80% (SEM = 5.96) in lodgepole and whitebark pine, respectively ( $t = 0.480$ ,  $P > 0.2$ ). Similarly, among those beetles that entered, the distances of the galleries they constructed within 3 d did not vary with tree species ( $t = 1.194$ ,  $P > 0.1$ ). Beetles tunneled 2.99 (SEM = 2.85) cm and 2.36 (SEM = 1.69) cm in lodgepole and whitebark pine, respectively.

**Natural Enemy Responses.** The most abundant predator obtained in our traps was the checkered beetle *Thanasimus dubius* (Coleoptera: Cleridae). The zero-inflated general Poisson model to predict *T. dubius* arrival as a function of species and condition (attacked vs. unattacked) was not significant at  $P = 0.05$ , although attack condition of the tree was significant. Notably, the difference in means between attacked lodgepole pine and the other treatments was statistically significant at  $P < 0.05$  (Fig. 5A). This finding indicates that until trees were attacked, arrival rates were equivalent in lodgepole and whitebark pines. However, once

mountain pine beetles entered trees, the combination of insect and plant volatiles emitting from attacks was more attractive in lodgepole pine.

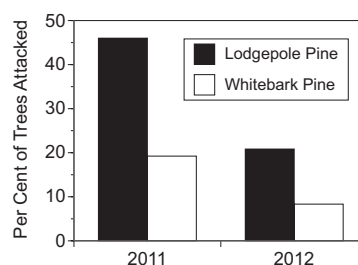
The most abundant competitor obtained in our traps was the secondary bark beetle *I. pini* (Coleoptera: Curculionidae: Scolytinae). The zero-inflated general Poisson model to predict *I. pini* as a function of species and condition (attacked vs. unattacked) was highly significant at  $P < 0.0001$ , with both species and the species  $\times$  condition interaction significant ( $\chi^2 = 13.46$ ,  $P = 0.0002$  for host species, and  $\chi^2 = 7.51$  and  $P = 0.0062$  for species  $\times$  condition). The difference-of-means test showed that *I. pini* arrival rates were significantly higher at attacked lodgepole pines than other treatments ( $P < 0.001$ ) (Fig. 5B). As with predators, arrival rates on unattacked lodgepole and whitebark pines were statistically equivalent. However, once mountain pine beetles entered trees, the combination of insect and plant volatiles emitting from attacks was more attractive in lodgepole pine, as indicated by the higher arrival rate of *I. pini* in attacked lodgepole.

We captured a total of 1,240 mountain pine beetles in the flight traps. The background pressure on lodgepole and whitebark pine trees was equivalent (Fig. S1). Additional arriving insects included at least three other genera of bark beetles, wood wasps, and parasitoids (Table S21).

## Discussion

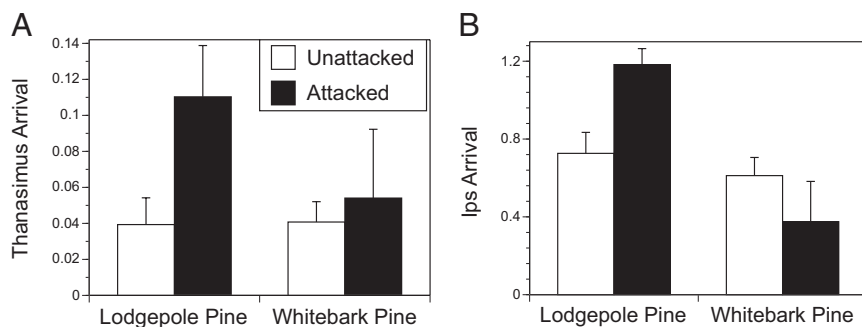
These results suggest that high-elevation pines invest relatively little energy in defense against bark beetles, an evolutionary strategy favored by less-intense selective pressure in these historically cold habitats, with selection driven primarily by competing physiological processes (32), such as tolerance to extreme conditions. Three lines of evidence support this view. First, the ability to rapidly synthesize antibiotic compounds in response to simulated attack was substantially lower in the seminaïve than historic host (Fig. 1). In previous studies, the extent of total induced monoterpene accumulation in response to challenge inoculations has been the best indicator of tree survival in the field (18, 33–35). The induced responses of at least 11 species from three conifer genera have been studied, and of these, whitebark pines appeared to show the weakest accumulation of induced toxic compounds to simulated biotic attacks (18).

Second, the chemical composition of whitebark pine phloem appears more conducive to successful attack by mountain pine beetles, especially in being more amenable to the pheromone signaling by which these insects coordinate mass attacks essential for overcoming tree defense (Fig. 2). For example, whitebark pines had higher concentrations of (–)- $\alpha$ -pinene, which stimulates the production of, and serves as a precursor for, enzymatic conversion to the aggregation pheromone *trans*-verbenol by pioneer beetles as they enter trees (25). This monoterpene also synergizes the attraction of flying beetles to the pheromones being produced by the tunneling beetles (36). Whitebark pine also has higher concentrations of myrcene, which strongly synergizes attraction of mountain pine beetle to its aggregation pheromones *exo*-brevicomin



**Fig. 4.** Preferential attack by mountain pine beetle for lodgepole pine relative to whitebark pine. Mature trees were in stands containing both tree species, and were exposed to natural attacks for 1 y.





**Fig. 5.** Arrival patterns (insects/trap) of the major predators (A) and competitors (B) of mountain pine beetles to lodgepole and whitebark pines before and after attack. Mature trees were in stands containing both tree species, and were exposed to natural attacks for 1 y.

and *trans*-verbenol (24, 37). Whitebark pines seem further conducive to mass attack because of their lower induced concentrations of 4-allylanisol, which inhibits mountain pine beetle attraction to its aggregation pheromone (26, 38). Low concentrations of this phenylpropanoid have been correlated with increased mountain pine beetle attack in ponderosa pine (27). This compound also has a direct defensive role as an inhibitor of fungal associates of bark beetles (39). In addition to these behavioral modulators, whitebark pines also contained lower concentrations of limonene, which generally has the most negative effects among conifer monoterpenes on bark beetles and their fungal symbionts in bioassays (18, 40), and has been correlated with reduced rates of attack in some field studies (33).

Finally, whitebark pines exude relatively less resin when entered than lodgepole pines, and its supply is more readily depleted by mass attacks (Fig. 3). High resin flow can delay beetle progress while induced defenses are being activated, thus reducing the likelihood of beetles being able to initiate aggregation, and has been associated with reduced levels of attack incidence (19). This overall low investment in three components of defense—induced biosynthesis, relative composition of bioactive compounds, and resin flow—is even more striking considering that long-lived plants usually have the greatest allocation to defense (32). These findings lend strong support to the hypothesis that mountain pine beetles will encounter less-defended hosts as they expand their geographic range to higher elevations and latitudes (41, 42). These distinctions between highly adapted and seminaïve hosts also offer insights into the coevolutionary significance of plant secondary chemistry (43–45). In particular, plants' abilities to initiate actively induced defenses, and likewise to interfere with specific insect strategies for overcoming those defenses, appear particularly important and indicative of historic selection pressures.

In contrast to what might be predicted from the trees' chemical and physiological defense capabilities, we did not detect a preference by mountain pine beetle for whitebark pine. Rather, in mixed stands, beetles were more likely to attack lodgepole than whitebark pines. It appears that during this initial phase of heightened, more continuous contact between this herbivore and a relatively naïve tree, mountain pine beetle appears more behaviorally attuned to its historic host. This finding is not entirely unexpected, as previous studies have similarly illustrated that we cannot simply assume host-seeking insects prefer plants that are optimally suited for their brood. Rather, host selection, species associations, and host-preference switching are dynamic and complex processes that operate across both space and time (46), involve multiple genetically and environmentally determined traits, require simultaneous adjustments by different life-stages, and encompass a broad suite of interacting chemoreceptors, neurological pathways, and conditioning experiences (47, 48). It is also conceivable that associational cues between entry into whitebark pine and subsequent overwintering mortality could contribute to preference for lodgepole pine when available, but Langor et al. (49) found no evidence of host-based assortative mating within sites. Thus, whitebark pines in lower elevation, mixed composition stands may gain partial escape, at least for now, from mountain pine beetle's relatively higher

orientation toward lodgepole pine. However, the equally high rates of entry into either host species under no-choice conditions, and the increased rate of natural attacks in sites where lodgepole pine is less abundant, suggest that whitebark pines at higher elevations may be at greater risk. This complexity in spatial context and local insect adaptation may help explain why previous studies comparing relative attack rates have generated conflicting results (50). If warming temperatures sustain continuous interaction, the lower defensive ability of whitebark pine would presumably select for behavioral shifts in relative preference, which is known to have a substantial genetic component in bark beetles (30).

The historic role of lodgepole pine as the primary host of mountain pine beetle may have influenced the behavioral responses of its major predators and competitors as well. In unattacked trees in mixed stands, equal numbers of *T. dubius* and *I. pini* were caught in unbaited flight traps suspended from whitebark and lodgepole pines. Once trees were attacked, however, these predators and competitors arrived in greater numbers at lodgepole than whitebark pines. This finding suggests that the chemistry of lodgepole pine combines with mountain pine beetle pheromones to generate odor plumes more attractive to these natural enemies than does that of whitebark pine. This theory raises the possibility that mountain pine beetles acquire both partially predator-free and defense-free (51) space when colonizing host species and habitats with lesser evolutionary history.

Collectively, these results suggest that under high-temperature regimes beetles may more easily transition from endemic to eruptive levels in whitebark than lodgepole pine stands. Once killed, whitebark pines support greater brood production than lodgepole pine in the absence of cold temperatures, as indicated by both laboratory rearing (52) and field caging/emergence (53) experiments. Furthermore, the size of emerging beetles and proportion of females is either equivalent or larger among mountain pine beetles that developed in whitebark pine, probably because of a thicker-phloem effect (52, 53). The consequences of high mortality to this keystone species (14) could be far-reaching and long-lasting given the low resilience typically associated with such slow growing, late-reproducing plants. Unfortunately, management techniques developed for lodgepole pine (e.g., early harvesting, thinning, pesticides), are poorly suited for high-elevation systems because of a variety of operational, environmental, and economic factors.

Searching for sources of genetic resistance on a continental scale could provide a proactive approach, but would be logistically challenging and expensive if unfocused. Our results suggest that such efforts could be expedited by concentrating on induced responses to controlled biotic induction, and composition of host volatiles relating to beetle communication (Figs. 1 and 2). Additional work should include other chemical groups that can influence bark beetles and their symbionts, such as diterpene acids and phenolics (16, 18). Lodgepole pines are known to have some genotypic variation in traits associated with susceptibility to mountain pine beetle (54). The high interdemec variation commonly seen in island- and mountaintop-inhabiting species (55) suggests that isolated whitebark pine forests might harbor greater heterogeneity than the more continuously distributed

lodgepole pine. For example, whitebark pines in California separated by only 250 km had fourfold differences in myrcene composition (40) compared with our Wyoming sites 1,000 km away, where myrcene concentrations were intermediate of the California populations. In addition, some instances of resisted attacks on whitebark pine have been reported (14).

These results provide a mechanistic link between two major environmental threats, transport of invasive species and climate-driven range expansions, in that lack of coevolved defense is an important driver of each (41, 42, 51). Mountain pine beetle has also expanded its northern range, where it is attacking lodgepole pines in historically unexposed areas, and has spread eastward to breach the geophysical barrier of the Rocky Mountains to attack hybrid lodgepole-jack pine, *Pinus banksiana*, in Alberta, Canada (7, 56). Previous dispersal events likely deposited small numbers of beetles in these habitats, but populations quickly collapsed because of Allee effects. In contrast, the established range of this irruptive insect now connects it through contiguous host habitat with the pine biomes of north-central and eastern North America for the first time (7). As with whitebark pine, we lack sufficient information to predict the dynamics of these new plant-herbivore and tritrophic relationships (2, 7) and their ecophysiological consequences. Like high-elevation whitebark pines, however, all three native pines in midwestern North America (red, jack, white) have higher concentrations of  $\alpha$ -pinene than do lodgepole pines and at least two appear to have less 4-allylanisole (18, 33, 57), which could potentially increase susceptibility. There are likely parallels between this and other systems, and comparative studies would help delineate how changing temperature and precipitation will influence new host-herbivore associations. Such comparative approaches could be especially fruitful where they incorporate multiple drivers known to affect herbivore success, and integrate cross-scale interactions.

## Methods

**Tree-Defense Chemistry and Physiology.** Tree physiology experiments were conducted in four stands of mixed lodgepole/whitebark pine composition, to avoid site-related sources of between-species variation. These stands were located in the Bridger-Teton National Forest, WY (two stands separated by 0.83 km at Togwotee Pass, 43.83°N, 110.18°W and two stands separated by 0.65 km at Elkhart Park, 43.0°N, 109.76°W). DBH was recorded for all trees, and all trees were cored twice at breast height to determine age. Fifteen healthy, unattacked trees each of lodgepole and whitebark pine were sampled at one of the Togwotee Pass stands in 2009, and 10 each of the two species were sampled at Togwotee Pass in 2011. Forty-two lodgepole pines and 40 whitebark pines were sampled at Elkhart Park in 2011, yielding a total sample size of 67 lodgepole and 65 whitebark pine trees across both years.

A 5-cm-long strip of phloem was removed with a razor blade from each tree, sealed in a plastic tube, and frozen at  $-20^{\circ}\text{C}$  until analysis. These served as the constitutive samples. We elicited induced responses by applying challenge inoculations that mimicked the combined entry of the mountain pine beetle and its major fungal symbiont, *G. clavigera* (33, 34). The fungus was isolated from mountain pine beetle larvae that were rolled onto malt extract agar. We applied the fungus to the xylem/phloem interface using sterile techniques, and the 2-cm bark-phloem plug was immediately replaced to seal the site of introduction. The resulting induced reaction zone was removed with a razor blade after 1 wk, and stored as above.

Monoterpenes were extracted from finely chopped constitutive and induced phloem samples during 24 h of agitation in 1 mL of hexane, as described previously (33, 34). The solution was filtered with glass wool. The vials were rinsed twice with 250  $\mu\text{L}$  of hexane, yielding a final volume of 1.5 mL monoterpene-hexane solution. One microliter of 0.1% isobutylbenzene was added to each sample as an internal standard. The remaining phloem was dried at  $25^{\circ}\text{C}$  for 1 wk and weighed. Samples were analyzed using a Shimadzu 17 gas chromatograph with an Agilent Technologies, J&W cyclodex column (30-m long, 0.25-mm internal diameter, and 0.20- $\mu\text{m}$  film thickness) with helium as the carrier gas. Each analysis began at an initial temperature of  $60^{\circ}\text{C}$  for 10 min, followed by an increase in  $5^{\circ}\text{C}$  per minute until  $160^{\circ}\text{C}$ . Injector and detector (FID) temperatures were at  $220^{\circ}\text{C}$ . Thirty monoterpene standards of those commonly found in pines were acquired from Sigma-Aldrich and analyzed as described above. We compared the retention times for these

standards with the peaks on the chromatogram produced after the run of each sample. Weights of each monoterpene were calculated by integrating the area under the curve of the monoterpene and the internal standard, and multiplied this value by the density of the internal standard.

Total monoterpenes for each sample were calculated in milligrams per gram of dry phloem using the weight for each sample. Total monoterpene retrieval differed between years because of increased volatilization in 2011 as consequence of longer transport distances from the field sites. Raw total monoterpene data were standardized between years using annual minimum-maximum values, and the resulting proportions were arcsin square-root transformed to achieve normality. We applied a GLM in SAS v9.3 to test for differences in total monoterpenes as a function of species, treatment, and their interaction. We included tree diameter, site, and year as covariates. Age was strongly correlated with tree diameter by site and species, and was therefore not included in the analysis because of missing observations.

In 2011, we examined the pitch tubes from 20 naturally attacked lodgepole pines and 11 naturally attacked whitebark pines, located at Elkhart Park. We photographed all pitch tubes up to a height of 1.82 m with a ruler as a reference in the photograph, and calculated their areas using measurement tools in the open-source software ImageJ v1.46. We compared the resin flow from lodgepole and whitebark pines by species, treatment (attacked vs. mass attacked), and their interaction, with tree diameter as a covariate using the GLM procedure in SAS v9.3.

**Mountain Pine Beetle Host Plant Preference.** We evaluated the relative preference of mountain pine beetle for lodgepole vs. whitebark pines through a combination of field and laboratory studies. The field studies were conducted in 2011 and 2012. In 2011, we evaluated natural attack patterns in mixed stands at Elkhart Park and Togwotee Pass. In 2012, we established transects that extended from stands with a high component of lodgepole pine, through more evenly distributed stands, to nearly pure whitebark stands near Big Sandy Lake, also in Bridger-Teton National Forest ( $42.74^{\circ}\text{N}$ ,  $109.19^{\circ}\text{W}$ ). We examined for signs of attack from 20 to 21 June to 17–19 October in 2011, and 1–2 July to 11–12 September in 2012. Each tree was visited weekly through July 29 (2011) and August 15 (2012) and then again at the end of the season. Attack status (unattacked, attacked, mass attack) was recorded at each visit. We also recorded whether the needles on each tree had turned red as evidence of tree mortality. We tallied the species and diameter of all living and dead trees within a 7.98-m radius plot (200  $\text{m}^2$ ) around each study tree, to provide information on the spatial context of host availability. Total sample size was 102 trees in 2011 ( $n = 50$  lodgepole and  $n = 52$  whitebark pines) and 72 trees in 2012 ( $n = 24$  lodgepole and  $n = 48$  whitebark pines).

We tested responses of adult females (the host-selecting sex) to lodgepole and whitebark pines under no-choice conditions. We removed disks of bark plus phloem from 10 apparently healthy trees of each species at 1.3-m height using a 14-cm circular drill bit. The samples were immediately placed in plastic bags and stored in an ice chest. The samples were then placed in 15-cm Petri dishes with the phloem surface immersed in a layer of melted paraffin wax to minimize desiccation. Five female beetles were added to each Petri dish, and allowed 3 d to either reject or tunnel into the bark disk at room temperature. After 3 d we measured the distance tunneled by those females that entered the bark. Mountain pine beetles for this assay were collected using 12-funnel flight traps, baited with myrcene, *exo*-brevicomin, and *trans*-verbenol. The number of beetles that entered each disk was averaged, and the averages per tree species were compared using a pairwise *t* test.

**Natural Enemy Responses.** We sampled populations of mountain pine beetles and associated insects at the 102 trees in the 2011 field experiment using unbaited flight traps. One 12-unit multiple funnel trap was suspended from each tree at 2-m above the ground and less than 1 m from the tree. Trap contents were collected weekly from 20 to 21 June until 8–9 August, and again on 17–19 October, for a total of 670 sample collections. At each sampling period, the tree's condition relative to mountain pine beetle attack was noted. Insects were preserved in vials and ethanol, and returned to the laboratory for identification. Because of the large number of zero counts by taxon in the insect trap data, we used zero-inflated Poisson regression using the GENMOD procedure in SAS v9.3 to test natural enemy abundances as a function of host species and attack status (attack vs. unattacked).

**ACKNOWLEDGMENTS.** We thank the National Park Service and University of Wyoming-National Park Service Research Center for site access and lodging; Megan Fork, Katherine Molter, Christopher Foelker, Melissa Greulich, Andrew Long, John Muller, Christopher Pennings, Hannah Manninen, Hannah Hubanks, Ryan Sword, and Austin Pethan for field assistance; and Claudio

Gratton and three anonymous reviewers for critical review. This work was supported by National Science Foundation Grant DEB-0816541 (to K.F.R.

and P.A.T.) and by the University of Wisconsin College of Agricultural and Life Sciences.

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