Efficacy of tree defense physiology varies with bark beetle population density: a basis for positive feedback in eruptive species

Celia K. Boone, Brian H. Aukema, Jörg Bohlmann, Allan L. Carroll, and Kenneth F. Raffa

Abstract: We evaluated the ability of constitutive and inducible defenses to protect trees and restrict herbivore reproduction across the endemic, incipient (i.e., transitory), and eruptive phases of a native bark beetle species. Host defenses were major constraints when mountain pine beetle (*Dendroctonus ponderosae* Hopkins) populations were low, but inconsequential after stand-level densities surpassed a critical threshold. We annually examined all lodgepole pines (*Pinus contorta* Douglas var. *latifolia*) in six 12–18 ha stands for 3–6 years for beetle attack and establishment as beetle densities progressed through various population phases. We also assayed a suite of tree physiological and chemical attributes and related them to subsequent attacks during that year. Rapidly inducible defenses appeared more important than constitutive defenses, and total monoterpenes were more important than particular constituents. Trees that exude more resin and accumulate higher monoterpene concentrations in response to simulated attack largely escaped natural attacks when populations were low. In stands where beetles had reached incipient densities, these defenses were ineffective. Larger diameter trees had more pronounced defenses than smaller diameter trees. As populations increased, beetles selected increasingly larger, more resource-rich trees, despite their better defenses. When populations were too low for cooperative attack, beetles exploited trees weakened by lower-stem insects. Behavioral plasticity allows beetles to persist at endemic levels until conditions shift, after which positive feedbacks predominate.

Résumé : Nous avons évalué la capacité des mécanismes de défense constitutifs et induits à protéger les arbres et à limiter la reproduction des herbivores lors des phases endémique, d'émergence (c.-à-d. transitoire) et épidémique d'une espèce indigène de scolyte. Les mécanismes de défense de l'hôte constituent des contraintes majeures lorsque les populations du dendroctone du pin ponderosa (*Dendroctonus ponderosae* Hopkins) sont faibles, mais n'ont plus d'effet une fois que la densité à l'échelle du peuplement a dépassé un seuil critique. À chaque année pendant 3–6 ans, nous avons examiné tous les pins tordus (*Pinus contorta* Douglas var. *latifolia*) dans des peuplements de 12–18 ha et suivi l'atõtaque et l'établissement du scolyte à mesure que sa densité progressait en passant par les diverses phases de population. Nous avons également analysé une série d'attributs chimiques et physiologiques des arbres et nous les avons reliés aux attaques subséquentes au cours de la même année. Rapidement, les mécanismes de défense induits ont semblé plus importants que les mécanismes de défense constitutifs et les monoterpènes totaux ont paru plus importants que des constituants particuliers. Les arbres qui sécrètent plus de résine et accumulent des concentrations plus élevées de monoterpènes en réaction à une attaque simulée ont pour la plupart résisté aux attaques naturelles lorsque les populations étaient faibles. Dans les peuplements où les scolytes avaient atteint des densités qui correspondent à la phase d'émergence, ces mécanismes de défense étaient inefficaces. Les mécanismes de défense étaient plus robustes chez les arbres de fort diamètre que chez les arbres de faible diamètre. À mesure que la population augmentait, les scolytes choisissaient des arbres de plus en plus gros et riches en ressources même si leurs mécanismes de défense étaient meilleurs. Lorsque la population était trop faible pour soutenir une attaque concertée, les scolytes profitaient des arbres affaiblis par des insectes qui s'attaquent à la partie inférieure du tronc. La plasticité comportementale permet aux scolytes de se maintenir à des niveaux endémiques jusqu'à ce que les conditions changent, après quoi les feedbacks positifs prédominent.

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Introduction

Understanding mechanisms that typically constrain insect herbivore populations or, conversely, foster outbreaks is complicated by interactions among multiple drivers, thresholds separating domains of negative and positive feedback, and cross-scale interactions that amplify or buffer internal drivers (Régnière and Nealis 2007; Raffa et al. 2008). Host plant defenses are well known to affect the behaviour, survival, and reproduction of coevolved insects (Karban and Baldwin 1997). For example, an extensive literature documents strong effects of conifer monoterpenes against bark beetle adults, eggs, and associated fungi in laboratory and small-scale field assays (reviewed in Raffa et al. 2005). However, how these relationships contribute to population- and landscape-scale dynamics and their role in overall epidemiology are not well understood. A confounding factor is that the effects of host defenses at the tree level may vary with insect abundance at the stand level, a relationship that has not been tested explicitly.

Bark beetles are among the most important agents of tree mortality and ecosystem disturbance in coniferous forests. During outbreaks, they cause high mortality at a landscape scale, altering forest structure and composition, fire patterns, succession, geophysical processes, and carbon sequestration (Romme et al. 1986; Veblen et al. 1991). For example, the current outbreak of mountain pine beetle, *Dendroctonus ponderosae* Hopkins, has caused mortality to over 14 million hectares of lodgepole pine (*Pinus contorta* Douglas var. *latifolia*) forests in British Columbia alone since 1999 (Safranyik et al. 2010) and converted major vegetation types from carbon sinks to sources (Kurz et al. 2008). Despite their high reproductive potential, most eruptive bark beetle species undergo extended periods of low population density, termed the endemic phase. A combination of host availability and suitability (Aukema et al. 2006; Hicke and Jenkins 2008), favourable weather (Bentz et al. 1991; Powell and Bentz 2009), and escape from natural enemies (Turchin et al. 1999; Raffa et al. 2005) is required for populations to breach the stand- and landscape-level thresholds that separate endemic from eruptive dynamics (Raffa et al. 2008). In recent years, these outbreaks have increased in magnitude and frequency and expanded into habitats that historically have rarely or never been exposed to such disturbances (Logan et al. 2003).

Conifers possess integrated constitutive and induced mechanisms for protecting subcortical tissues, including rapid resin flow from wounds, compounds inhibitory and toxic to bark beetles and their symbionts, and hypersensitive autonecrosis that confines the invading complex. Conifer allelochemicals include monoterpenes, diterpene acids, and phenolics, of which monoterpenes are most repellant and toxic to bark beetles (Raffa et al. 2005). These compounds undergo rapid localized induction, with monoterpene concentrations at the immediate site of attack rising from relatively low levels to quantities greatly exceeding the physiological tolerance of the beetles, eggs, and fungal associates within only a few days (Raffa and Smalley 1995; Raffa et al. 2005). Additionally, resin flow from wounds created by beetle entry can physically impede attack progression, thereby allowing time for induced biosynthesis to increase the quantities and alter the composition of phytochemicals and for induced autonecrosis to confine the beetles before they make extensive progress (Raffa and Berryman 1983).

Bark beetles contend with these defenses by either avoiding vigorous trees or overcoming them through pheromone-mediated mass attacks (Wood 1972). Various types of stress reduce host resistance to bark beetles, including lightning strikes (Blanche et al. 1985), defoliation (Wallin and Raffa 2001), severe drought (Breshears et al. 2005), and root pathogens (Klepzig et al. 1991). Limiting host acceptance to stressed trees incurs less risk than entering vigorous individuals, but this strategy also has several disadvantages (Raffa 2001). Stressed trees typically are relatively rare and ephemeral, are often nutritionally suboptimal because of their relatively thin phloem, and are available to many interspecific competitors. Also, bark beetles usually can breed within a tree for only one generation, so colonization removes this sparse resource from the available pool. An alternative strategy is to overwhelm host defenses by mass-attacking vigorous trees. Bark beetles produce aggregation pheromones that exploit conifer terpenes as precursors and (or) synergists and rapidly recruit large numbers of both sexes (Wood 1982). Each beetle contributes to the depletion and exhaustion of tree defensive chemistry and physiology. The advantages to entering vigorous trees include their greater abundance, often higher nutritional quality, and reduced interspecific competitors. However, beetles may be killed or repelled if they do not attract enough recruits. Beetles determine whether to enter trees after landing (Wood 1972) based largely on their monoterpene content, with low concentrations eliciting entry and high concentrations being repellant (Wallin and Raffa 2000). These responses are plastic, as, for example, spruce beetles from eruptive populations will accept high terpene concentrations when other beetles are present, but those from endemic populations are always repelled by high concentrations (Wallin and Raffa 2004).

We evaluated the relationship between stand-level population density and the likelihood of attack success and determined which constitutive and inducible tree properties are associated with the likelihood of attack and whether the relationships between various tree properties and beetle attack vary with beetle population phase. These properties included resin flow, monoterpene content, and tree diameter, which have been shown to influence beetle performance.

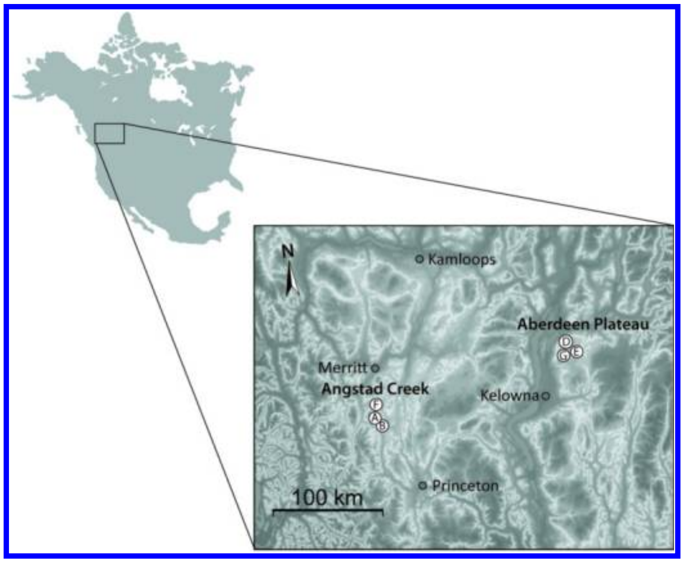
Materials and methods

Description of model system

The mountain pine beetle is the foremost biotic disturbance agent of mature pine forests in western North America (Romme et al. 1986), and intermittent eruptions by this native herbivore have occurred over large areas for as long as records are available. Adults emerge from brood trees during July and early August and disperse in search of new hosts. Females bore through the bark and cambium, construct mating chambers, and emit aggregation pheromones that attract both sexes. Larvae feed within the phloem as they tunnel and pupate. This insect has close associations with several ophiostomatoid fungi (Six 2003), of which *Grosmannia clavigera* (Robinson-Jeffery and Davidson) Zipfel, de Beer appears the most phytopathogenic based on inoculation studies (Lee et al. 2006). The precise roles of this fungus have not been resolved, but it is believed to aid beetles in overcoming tree defenses (Lee et al. 2006) and to provide nutrition for developing larvae (Six 2003). The mountain pine beetle is usually univoltine, but broods may require two years at cooler temperatures (Bentz et al. 2001). Its major host is lodgepole pine, and beetles produce larger broods in trees that have larger diameters and thicker phloem (Amman 1972).

Mountain pine beetle populations typically remain at endemic, low-density levels within a region for several decades. Following a sudden availability of susceptible hosts, as can be caused by drought, or a rapid increase in populations, as can be caused by high winter survival or immigration, or a various combination of these factors, large-scale eruptions occur in which populations exhibit different dynamics (White and Powell 1997; Safranyik and Carroll 2006).

Fig. 1. Site and stand locations in the southern interior of British Columbia, Canada.



Site establishment, progression of beetle attacks over time, and relationship of within-tree beetle success to stand-level beetle density

We randomly selected three mature lodgepole pine stands at each of two sites in southern British Columbia (Fig. 1) and monitored the development of beetle populations over 3–6 years. Candidate stands (approximately six to eight per site) were first identified from vegetation resource inventory maps (http://www.for.gov.bc.ca/hts/vridata). They were chosen for study if they (*i*) were situated within areas of historically suitable climate for mountain pine beetle (Safranyik and Carroll 2006; Safranyik et al. 2010), (*ii*) contained no detectable infestations based on annual aerial surveys (http://www.for.gov.bc.ca/hfp/health/overview/overview.htm), yet had presence verified by on-site inspection, i.e., beetles were in an endemic condition, (*iii*) were at least 10 km from recorded tree mortality caused by mountain pine beetle to minimize the potential for immigration to stands from existing infestations, and (*iv*) were separated by >1 km from other candidate stands.

Following stand selection, variable radius plots (Avery and Burkhart 2002) were systematically installed at 1 plot/ha, and mensurational characteristics were quantified. These stands averaged 15.3 ha, with 1225 trees/ha and 90.3% composition of lodgepole pine (Table 1). The presence of an endemic population in each stand was confirmed by a 100% census of lodgepole pine trees for evidence of attack (i.e., presence of boring dust in bark crevices, defensive resin exudate (pitch tubes), and (or) discolored foliage). Safranyik and Carroll (2006) defined an endemic mountain pine beetle population as one with too few beetles to successfully mass attack a healthy, large-diameter tree within a stand. These beetles primarily infest suppressed trees with impaired vigour; however, they will occasionally attempt to colonize more resistant hosts (Safranyik and Carroll 2006). Therefore, for the purposes of this investigation, we defined an endemic population as one with <5 trees/ha showing any evidence of successful or unsuccessful attack in the current year (this included successful attacks of low-vigour trees and unsuccessful attacks of healthy trees). On all attacked trees, a single small portion of bark (<80 cm^2) at 1.3 m height was removed to validate that mountain pine beetle was the attacking species and to determine if attacks were successful. Because the tree-level mountain pine beetle attacks are either successful or resisted, evidence of gallery excavation and brood production can be used as a reliable indicator of attack success (Safranyik and Carroll 2006). Two stands each were established in 2000 (A and B: Angstad Creek) and 2001 (D and E: Aberdeen Plateau), and additional stands were added in 2002 (F: Angstad Creek) and 2003 (G: Aberdeen Plateau).

During late summer (i.e., after beetle emergence and dispersal) of each year following initial stand establishment (until 2005), every tree in each stand was inspected and the number of newly attacked trees and the success or failure of the attacks were determined. Safranyik and Carroll (2006) defined an incipient population as one in which most attacked trees are in the larger diameter classes and an epidemic, i.e., eruptive, population as one in which there are large annual increases in the number of infested trees. Based on our stand censuses, we considered populations to have entered the incipient phase when more than 5 trees/ha were attacked and the eruptive phase when >20 trees/ha were attacked. By 2004, beetle populations were considered to have remained endemic (stands F and D), increased to incipient (stands A and G), or reached the eruptive phase (stands B and E).

Constitutive and induced monoterpene chemistry and resin flow

During 2005, 40 apparently healthy, nonattacked trees were sampled at each of the six stands just prior to beetle emergence and dispersal for host properties that have been associated with bark beetle performance in prior controlled laboratory or field studies. These included constitutive and induced resin flow, constitutive and induced monoterpene content, and induced autonecrosis (reviewed in Raffa et al. 2005). Trees were selected based on a stratified random sampling protocol. Trees ranging in diameter from 10 to 40 cm were assigned to six diameter classes, and the proportion of trees in each class was determined for each stand. These proportions of trees were sampled from each stand. After the flight period, beetle behaviour and performance were related to pre-attack tree physiology. The status (attacked success-

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Table 1. Mensurational characteristics (mean ± SE) and mountain pine beetle population phase for lodgepole pine stands monitored from 2000 to 2005 in the southern interior of British Columbia, Canada, 2005. Mensurational characteristics were determined at the time of plot establishment, in 2000 for stands A and B (Angstad Creek), in 2001 for stands D and E (Aberdeen Plateau), in 2002 for stand F (Angstad Creek), and in 2003 for stand G (Aberdeen Plateau). All stands were in the endemic phase at the time of plot establishment.

Population phase*	Stand	Elevation (m)	Area (ha)	Mean (SE) tree age (years)†	Mean (SE) no. trees/ha	Mean (SE) basal area (m^2/ha)	Mean (SE) DBH (cm)	Percentage lodgepole pine‡	Location	Coordinates	No. of subplots
Endemic	D	1384	13.0	119.2	1325	37.0	21.5	90.4	Angstad	49.85°N	13
				(1.97)	(153.2)	(4.1)	(0.63)		Creek	120.76°W	
	F	1204	11.9	148.9	807	30.6	28.2	80.1	Aberdeen	50.09°N	12
				(1.55)	(148.4)	(5.3)	(0.80)		Plateau	119.19°W	
	Average	1294.0	12.45	134.05	1066	33.80	24.85	85.25			
Incipient	A	1163	16.7	110.5	1265	41.5	22.8	92.5	Angstad	49.83°N	16
				(1.52)	(252.9)	(6.9)	(0.61)		Creek	120.76°W	
	G	1426	14.9	108.6	1424	49.6	22.5	94.2	Aberdeen	50.05°N	15
				(0.58)	(115.0)	(4.4)	(0.43)		Plateau	119.16°W	
	Average	1294.5	15.80	109.55	1345	45.55	22.65	93.35			
Eruptive	B	1121	17.6	116.5	1273	43.9	24.1	94.2	Angstad	49.82°N	17
				(1.18)	(190.4)	(6.7)	(0.58)		Creek	120.75°W	
	E	1346	17.9§	90.7	1257	37.6	21.2	90.6	Aberdeen	50.06°N	18
				(2.56)	(249.6)	(5.9)	(0.91)		Plateau	119.13°W	
	Average	1233.5	17.75	103.60	1265	40.75	22.65	92.40			

*Population phases were based on the conditions in 2004 (endemic, <5 attacked trees/ha; incipient, 5–15 attacked trees/ha; epidemic, >15 attacked trees/ha).

†Tree age was determined using an increment borer.

‡Species other than lodgepole pine: Douglas fir (*Pseudotsuga menziesii*), Ponderosa pine (*Pinus ponderosa*), interior spruce (*Picea* spp.), subalpine fir (*Abies lasiocarpa*).

§In 2005, a small number of previously infested trees (<3%) was removed as salvage.

fully, unsuccessfully, not attacked) of sampled trees was recorded during the subsequent stand inspections.

Constitutive resin flow was measured by boring a 1.25 cm diameter hole into the north side of the tree with an arch punch (C.S. Osborne and Co., Harrison, New Jersey) (Angstad Creek, 21–24 June; Aberdeen Plateau, 5–8 July). The resin sampler (Karsky et al. 2004) was screwed into the tree to cover the hole, and a 15 mL screw-top plastic graduated collection vial (VWR International Inc., Batavia, Illinois) was placed in the resin sampler. The collection vial was removed approximately 24 h later, the height of the resin in the vial was marked with a permanent marker, and the mass, volume, and density of the resin were calculated in the laboratory. Resin mass and volume were highly correlated (constitutive, Pearson $R^2 = 0.9783$, $P > 0.0001$; induced, Pearson $R^2 = 0.9851$, $P > 0.0001$). Of these, mass was less variable (constitutive, mass $s^2 = 0.4458$, volume $s^2 = 0.5285$; induced, mass $s^2 = 0.7608$, volume $s^2 = 1.0089$), so results of resin mass data are used throughout. A subset of 25 trees from the original 40 sampled trees in each stand was selected (based on the same stratified random sampling protocol) for chemical analysis by removing a 5 cm (length) by 2 cm (width) section of phloem from its west side with a chisel. Phloem samples were placed in a glass vial, stored in a cooler on dry ice in the field, and transferred to a –80 °C freezer in the laboratory until chemical analysis. Tools were rinsed with 70% ethanol between samples. All sampling was performed at 1.3 m height.

To evaluate a tree's inducible responses, we simulated attack by *D. ponderosae* by administering a mechanical wound combined with a single inoculation of its symbiont *G. clavigera*. This treatment elicits localized reactions chemically and morphologically similar to treatment with live beetles (Raffa and Berryman 1982) but does not cause systemically altered lesion formation or monoterpene accumulation in response to subsequent inoculations (Raffa and Smalley 1988; Wallin and Raffa 1999). Isolate *O. clavigerum* SL-Kw407 was obtained from lodgepole pine infested with *D. ponderosae* near Kamloops, B.C. (50°43′N, 120°25′W) and cultured on 20% MEA. A 1.0 cm diameter hole was bored into the east side of the tree with a core borer, and a 0.6 cm diameter core of actively growing fungus (7 to 10 days old) was inserted into the hole. The bark and phloem core was replaced and secured with laboratory tape. Fungi inoculating tools were rinsed with 70% ethanol between samples. Treatment was performed at the same time as constitutive phloem sampling, and induced traits were sampled after three weeks. The lesion that developed in response to simulated attack with *G. clavigera* was measured and sampled for induced monoterpene content as above. Induced resin flow, a 24-h collection in response to a physical wound three weeks after simulated attack, was sampled from the south side of the tree, as before.

Monoterpenes were extracted and analyzed by gas chromatography (gc) according to a modified method of Lewinsohn et al. (1993). A 1.5 mL sample of MTBE (tert-butylmethyl ether, gc grade) containing standards (monoterpene standard: 100 µg/mL isobutyl benzene; diterpene standard: 200 µg/mL dichlorodehydroabietic acid (Helix Biotechnologies, Richmond, B.C.)) was dispensed into a 2 mL glass gc vial. A 1.5 to 2.0 cm section was cut from the midsection of the frozen phloem sample with pruners and added to the vial. All utensils and equipment were rinsed with methanol between samples. Samples were shaken vigorously at moderate speed for 24 h at room temperature. In a new vial, 0.3 mL of 0.1 mol/L (pH 8.0) $(NH_4)_2CO_3$ was dispensed. The extract was removed from the initial vial with a Pasteur pipette and added to the $(NH_4)_2CO_3$ and the mixture was vortexed for approximately 20 s. This resulted in a two-layer solution with the upper portion (washed MTBE) containing the monoterpenes. Phloem samples were placed in a drying oven at 37 °C for one week. For monoterpene extraction, 0.6 mL of the original washed MTBE extract was passed over a 0.3 g silica – 0.2 g Na_2SO_4 column and immediately rinsed with 1 mL ether and collected in a new 2.0 mL gc vial. The extract was evaporated to approximately 50 to 100 µL and stored at 5 °C until analyzed by gas–liquid chromatography. All chemicals were purchased from Sigma-Aldrich unless otherwise indicated.

Monoterpene analysis was performed on an Agilent 6890 gas–liquid chromatograph with an FID using a CycloSil B column (0.25 mm i.d. × 30.0 m with 0.25 µm film) (Agilent Technologies, Santa Clara, California). The injector temperature was 300 °C, and the oven was programmed from an initial temperature of 40.0 °C (held for 3 min), then increased at a rate of 3 °C/min up to 110 °C, 10 °C/min up to 180 °C, and then 15 °C/min up to final temperature of 250 °C, at which it was held for 15 min. Flow rate was 50 mL He/min, and total run time was 53 min. The quantities of individual and total monoterpenes were calculated based on comparison of the area of the internal standard, isobutyl benzene, and the proportions of individual monoterpenes relative to the total monoterpene content were calculated. ChemStation software (Rev. A.09.01, 1990–2001) (Agilent Technologies, Santa Clara, California) was used to identify compounds and stereochemistry, based on matching retention times with pure standards.

Defensive capacity against mountain pine beetle of trees previously colonized by lower-stem insects

Pseudips mexicanus (Hopkins) is a native noneruptive bark beetle that colonizes the basal stems of trees but does not mass attack or kill them (Smith et al. 2009). It often cohabits trees with mountain pine beetle during its endemic phase (Safranyik and Carroll 2006). During the yearly censuses of each stand, the presence of *P. mexicanus* in trees was also determined. On trees where *P. mexicanus* attacks were suspected (based on the presence of boring dust much finer than that of mountain pine beetle), a small portion of the bark (<30 cm^2) was removed from the area of the stem under attack. *Pseudips mexicanus* attacks were confirmed if adult beetles were present. Trees that became infested by *P. mexicanus* during the spring of 2005 were assayed for the same constitutive and induced defensive parameters at the same times as previously described trees. As before, these test trees had not been attacked by mountain pine beetle at the time of sampling, and their physiological condition was compared with corresponding trees not previously colonized by *P. mexicanus*.

Statistical analysis

An ANCOVA model was used to assess the influence of stand-level mountain pine beetle population size, indicated by the number of attacked trees per hectare, on the proportion of entered trees that were successfully attacked. The number of attacked trees can be considered an index of beetle population size (Safranyik and Carroll 2006). A regression analysis was used to describe the relationship between beetle population size and attack success.

Preliminary graphical analysis revealed that tree physiology only changed across population phases of mountain pine beetle for one level of the covariate, i.e., trees that were entered by mountain pine beetle. Hence, we utilized ANCOVA models that restricted terms and hypothesis tests to an intercept, i.e., a constant physiological response across insect population densities for unentered trees, and a slope, permitting a changing physiological response across population densities, only for entered trees. Resin flow and total monoterpene data were log (*y*) or square root (*y*) transformed as required to meet model assumptions. Proportion of individual monoterpenes was arcsine (sqrt(*y*)) transformed for analysis. ANOVA models were used to compare constitutive monoterpene composition with induced monoterpene composition, differences in constitutive and induced responses among population phases of the mountain pine beetle, and differences in constitutive and induced responses among healthy trees and trees infested with *P. mexicanus*. Regression models were used to determine relationships between tree diameter and the number of trees entered, resin flow, and lodgepole pine monoterpene composition. All models used a mixed-effects framework incorporating site as a random factor. Where responses were not normally distributed or were categorical, generalized linear models or frequency procedures such as Fisher's exact test were used.

A nonparametric analysis was used to examine whether beetles preferentially entered certain types of trees at different population phases in relation to the suite of trees available. Total monoterpenes were determined from a sample of 24–42 trees (from the original 40 trees) prior to beetle flight, stratified across diameter classes, for each population phase (endemic, incipient, eruptive) in each of the two sites (Angstad Creek, Aberdeen Plateau). Each sample was then fit to a theoretical data distribution (e.g., exponential, Gaussian) using maximum likelihood methods, with initial distribution choice informed by visual inspection of simple histograms. Samples of size equal to the number of beetles entering the sampled trees post-hoc (n = 3–9 trees per stand per site) were drawn from the distribution reflecting the stand characteristics 999 times. On the last draw, the observed monoterpene concentrations of the trees that the beetles had entered were added to the pool. The mean of the observed data was ranked relative to the means of the randomly sampled draws. In this way, we determined which trees the beetles entered relative to a percentile distribution of all trees available in the stand. This procedure was repeated one thousand times. The bottom and top 2.5% quantiles of the final ranking of the observed data were discarded to generate a 95% confidence interval for beetle preference in each stand type and site. Data were analyzed in R (Ihaka and Gentleman 1996; R Development Core Team 2009) and SAS (SAS Institute Inc. 2003).

Multivariate analyses were conducted on subsets of trees selected on the basis of hierarchical clusters (Ward method) (JMP 8) (SAS Institute Inc. 2009) and subjected to multidimensional scaling (MDS) using Euclidean distance analysis and one-way analysis of similarities (ANOSIM (Primer 6)) (Clarke 1993). Percentage contribution of the proportion of individual monoterpenes to the Euclidean distance between samples was determined using SIMPER (Primer 6) (Clarke 1993). Raw data are used for graphical representation unless otherwise indicated.

Results

Relationship of within-tree mountain pine beetle success to stand-level beetle density

Initial mountain pine beetle populations were at endemic levels in all stands, ranging from 0.42 to 1.82 attacked trees/ha. Over the subsequent time course, some populations persisted in the endemic phase, while others increased to incipient and eruptive phases.

Attack success was strongly affected by beetle population size ($F_{[1,21]} = 10.27$, $P = 0.0043$). The relationship between mountain pine beetle population size and attack success was best described as a single, three-parameter exponential rise to a maximum of nearly 1.0 (Fig. 2). The positive relationship between beetle success at the within-tree level and local population size was consistent across all stands and years. For example, between-stand stand variation did not affect the proportion of entered trees that was successfully attacked over the full time course ($F_{[5,21]} = 0.856$, $P = 0.53$). Thus, individual stands showed dramatic ranges of beetle success, with beetle density as the primary driver. Likewise, the trend was not driven by variably favourable years. That is, within individual years, the proportion of attacks that were successful consistently rose with stand-level beetle densities (Fig. 2). In all stands during the periods in which populations were at endemic levels, the mean (±SE) proportion of attacked trees that were successfully attacked was low: 0.31 ± 0.046 (n = 18). However, once populations increased beyond the endemic phase, attack success increased rapidly: incipient = 0.59 ± 0.029 (n = 4); eruptive = 0.84 ± 0.035 (n = 6) (Fig. 2). We also modeled stand-level beetle population density versus beetle success with the relationship forced through zero and yielded the same general curve (Supplementary Fig. S1).[1]

The proportions of trees sampled for physiological and biochemical properties in 2005 that were entered, and the proportions of the entered trees in which the attacks were successful, are shown in Supplementary Fig. S2.[1] As with the full data set, the likelihood of beetle success within individual trees varied strongly with local beetle abundance.

Differences between constitutive and rapidly induced phloem physiology of lodgepole pine

Lodgepole pine responded to simulated attack by *D. ponderosae – G. clavigera* complexes by increasing resin flow by 1.3 times (constitutive, 0.45 ± 0.04 g/day, n = 279; in-

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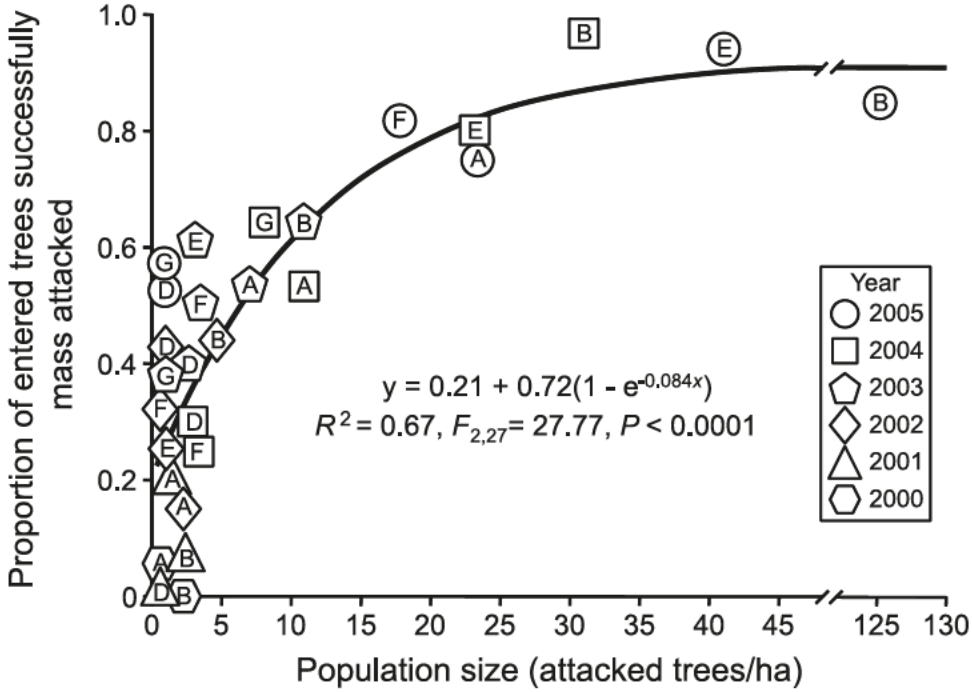
[1] Supplementary results consisting of graphs and tables describing the proportion of constitutive and induced individual monoterpenes and the accompanying univariate, multivariate, and nonparametric analysis of mountain pine beetle behaviour during different population phases are available with this article on the journal Web site (nrcresearchpress.com/cjfr).

Fig. 2. Relationship between mountain pine beetle population size, as indicated by the total number of attacked trees per hectare, and the proportion of attacked trees that were successfully mass attacked. Beetle populations were monitored by 100% census in six stands ranging from 13–18 ha for 3–6 years in southern British Columbia.



duced, 0.60 ± 0.05 g/day, $n = 269$; $F_{[1,545]} = 7.61$, $P = 0.0060$). Likewise, total local monoterpene concentration increased by 35.0 times (constitutive, 2.52 ± 0.21 mg/g phloem, $n = 199$; induced, 87.97 ± 5.88 mg/g phloem, $n = 206$; $F_{[1,402]} = 1145.14$, $P < 0.0001$).

Simulated attack by mountain pine beetle elicited compositional changes in the relative proportions of some monoterpenes (Fig. 3; Supplementary Table S1).[1] Proportions of β-pinene (1.5×), 3-carene (1.5×), unidentified compound 1 (1.1X), limonene (3.1×), and β-phellandrene (1.2×) increased, whereas those of α-thujene (1.6×), camphene (1.9×), terpinene (1.3×), and unidentified compound 2 (4.0×) decreased. Proportions of α-pinene, myrcene, and terpinolene did not change. A one-way ANOSIM of 133 trees selected on the basis of multivariate hierarchical cluster analysis (Ward method) indicated significant differences between Euclidean distances of constitutive and induced chemistry ($P = 0.001$) (Supplementary Fig. S3).[1] β-phellandrene (56.2%), 3-carene (26.4%), and β-pinene (12.4%) were the largest contributors to the average squared distance.

Differences between entered and non-entered lodgepole pines during various population phases of mountain pine beetle

All relationships between tree defense and beetle behaviour are based on within-year performances, i.e., tree physiology just prior to beetle flight and beetle attacks shortly thereafter, in 2005.

Constitutive resin and monoterpenes

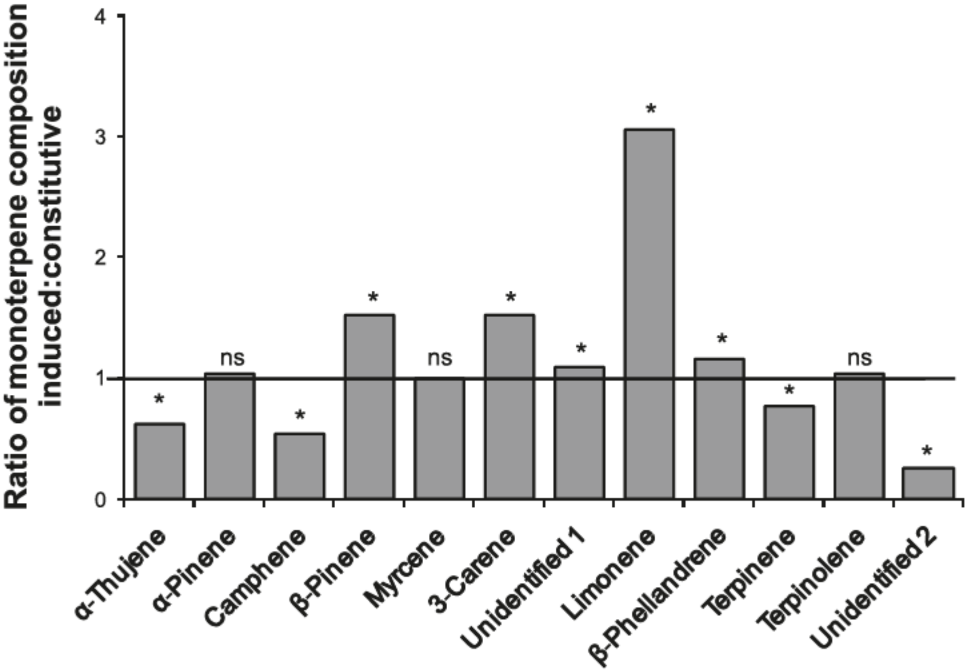
Constitutive resin flow varied between entered trees ($n = 41$) and non-entered trees ($n = 238$) ($F_{[1,275]} = 4.40$, $P = 0.0454$) (Fig. 4*a*). However, both the extent and the direction of this relationship differed among beetle population densities. At endemic densities, beetles preferentially attacked trees with lower (1.9×) constitutive resin flow rates (Fig. 4*a*, left). However, in stands with incipient population densities, beetles entered trees without regard to constitutive resin flow rates (Fig. 4*a*, center). In stands with eruptive beetle population densities, beetles preferentially attacked trees with higher (1.8×) constitutive resin flow rate (Fig. 4*a*, right). The density (mass/volume) of constitutive resin did not differ between entered and non-entered trees ($F_{[1,275]} = 2.66$, $P = 0.1044$).

The total quantities of constitutive monoterpenes in phloem extracts did not affect whether mountain pine beetles entered trees ($F_{[1,195]} = 0.5006$, $P = 0.4801$), and this result did not vary with the beetle's population density ($F_{[1,195]} = 1.1538$, $P = 0.2841$) (Fig. 4*b*). However, there were several differences in the proportions of individual monoterpenes between entered ($n = 33$) and non-entered trees ($n = 166$) (Supplementary Table S2),[1] and these relationships differed among population phases (Supplementary Fig. S4).[1] The constitutive proportions of α-pinene, β-pinene, and unidentified compound 2 were higher in trees that beetles had attacked during its endemic population phase. However, each of these was lower in entered than non-entered trees during the eruptive phase. Proportions of α-pinene and β-pinene were also higher in entered than not-entered trees during the incipient phase.

Induced resin and monoterpenes

Induced host chemistry and physiology, elicited locally in response to simulated attack, were more strongly related to subsequent natural attacks than were constitutive properties (Fig. 5). These relationships likewise differed both quantita-

Fig. 3. Altered monoterpenes composition during induced reaction of lodgepole pine trees. Ratio of the proportion of induced to constitutive individual monoterpenes (asterisk (*) indicates significance at $P < 0.001$; ns indicates no significance at $P > 0.05$).



tively and qualitatively among population phases. Induced resin flow varied between trees that were subsequently entered (n = 40) or not entered (n = 229) by natural populations ($F_{[1,265]} = 4.60$, $P = 0.0330$). Induced resin flow in response to simulated attack was higher (2.0×) in trees that were subsequently not entered at endemic beetle population densities (Fig. 5*a*, left). At incipient population densities, there were no differences between the induced resin flow of entered versus non-entered trees (Fig. 5*a*, center). At eruptive population densities, beetles entered trees with higher (1.6×) induced resin flow (Fig. 5*a*, right). The density of induced resin did not differ between entered and non-entered trees ($F_{[1,275]} = 1.29$, $P = 0.2572$).

Similar to induced resin flow, the total quantity of induced monoterpenes in response to simulated attack varied between trees that were subsequently entered (n = 32) or not entered (n = 174) by mountain pine beetle ($F_{[1,202]} = 11.84$, $P = 0.0007$) (Fig. 5*b*). This relationship varied with beetle population phase. The total quantity of induced monoterpenes was higher (1.8×) in trees that beetles avoided, when populations were in the endemic phase. However, there were no differences in induced monoterpene concentrations between entered and non-entered trees during the incipient phase. Beetles preferentially entered trees with high induced monoterpenes (2.1×) during the eruptive phase.

Relationships of individual monoterpene concentrations to simulated beetle attack likewise varied with beetle population densities (Supplementary Fig. S5 and Table S3).[1] The proportion of induced β-pinene was higher in entered (n = 32) than non-entered (n = 174) trees during the endemic and incipient phases ($F_{[1,202]} = 4.06$, $P = 0.0452$). However, there was an opposite trend in eruptive stands. The relationship between the proportion of induced myrcene and beetle attack likewise changed between the endemic and eruptive phases; myrcene content did not differ between entered and non-entered trees at endemic or incipient population densities but was higher in entered trees at eruptive densities ($F_{[1,202]} = 4.62$, $P = 0.0328$). The proportion of induced terpinene was also higher in trees that were subsequently entered than not entered at eruptive population densities, but not at endemic or incipient levels ($F_{[1,202]} = 4.14$, $P = 0.0432$). Multivariate analyses did not reveal additional consistent groupings of monoterpene constituents between non-entered and entered trees in any population phase.

Lesion development in response to simulated attack was not related to subsequent attack at any population phase (Fig. 5*c*).

Tree diameter

Overall, the mean diameter of entered trees was 1.4 times larger than that of non-entered trees ($F_{[1,275]} = 65.94$, $P < 0.0001$). However, this relationship varied with population phase, with an increasing preference for larger trees as stand-level beetle populations increased. The ratio of entered to non-entered tree diameters was 1.17 in endemic stands, 1.30 in incipient stands, and 1.58 in eruptive stands (Fig. 6).

Although larger trees were preferred by the mountain pine beetle, they were also better defended. Constitutive resin flow ($F_{[1,276]} = 11.42$, $P = 0.0008$) and total monoterpenes ($F_{[1,196]} = 6.20$, $P = 0.0137$) increased with tree diameter (Table 2). Induced resin flow ($F_{[1,266]} = 36.97$, $P < 0.0001$) and total induced monoterpenes in response to simulated attack ($F_{[1,203]} = 4.67$, $P = 0.0320$) likewise increased with tree diameter. Lesion length did not vary with tree diameter ($F_{[1,192]} = 1.34$, $P = 0.2482$). Some relative proportions of individual monoterpenes also varied with diameter. Among individual constitutive monoterpenes, the proportions of myrcene ($F_{[1,196]} = 9.73$, $P = 0.0021$), unidentified compound 1 ($F_{[1,196]} = 4.54$, $P = 0.0341$), and β-phellandrene ($F_{[1,196]} = 9.73$, $P = 0.0021$) increased with

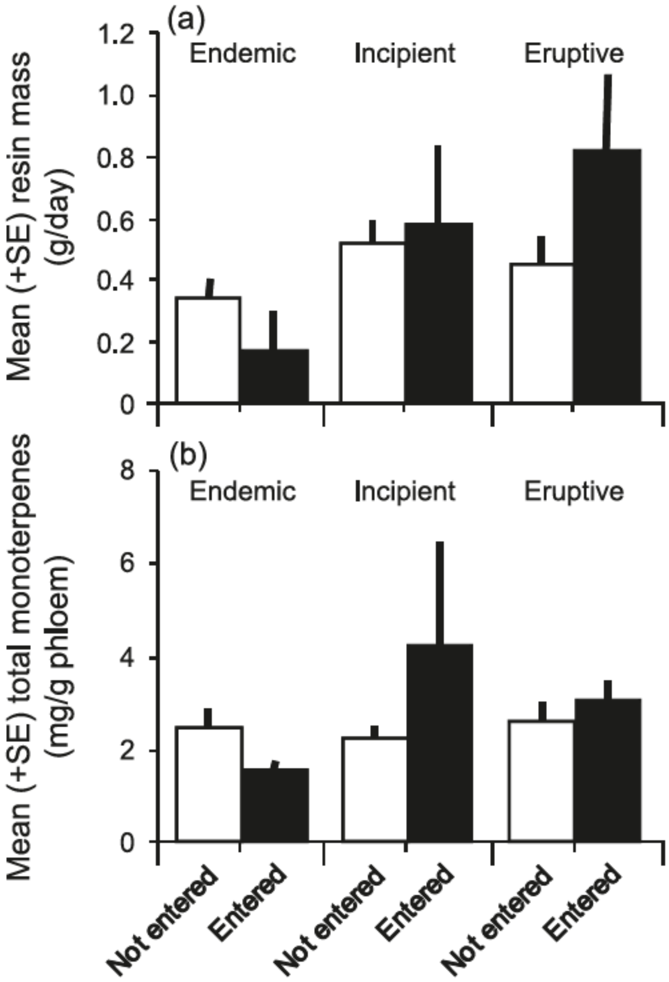


Fig. 4. Constitutive resin flow and monoterpene content of lodgepole pine phloem in trees not entered versus trees entered by the mountain pine beetle during the beetle's various population phases: (*a*) resin flow (g/day) (entered, $F_{[1,275]} = 4.0418$, $P = 0.0454$; entered × phase, $F_{[1,275]} = 5.5798$, $P = 0.0189$); and (*b*) total monoterpenes (mg/g dry mass phloem) (entered, $F_{[1,195]} = 0.5006$, $P = 0.4801$; entered × phase, $F_{[1,195]} = 1.1538$, $P = 0.2841$). All relationships are based on within-year tree and beetle performances, 2005.

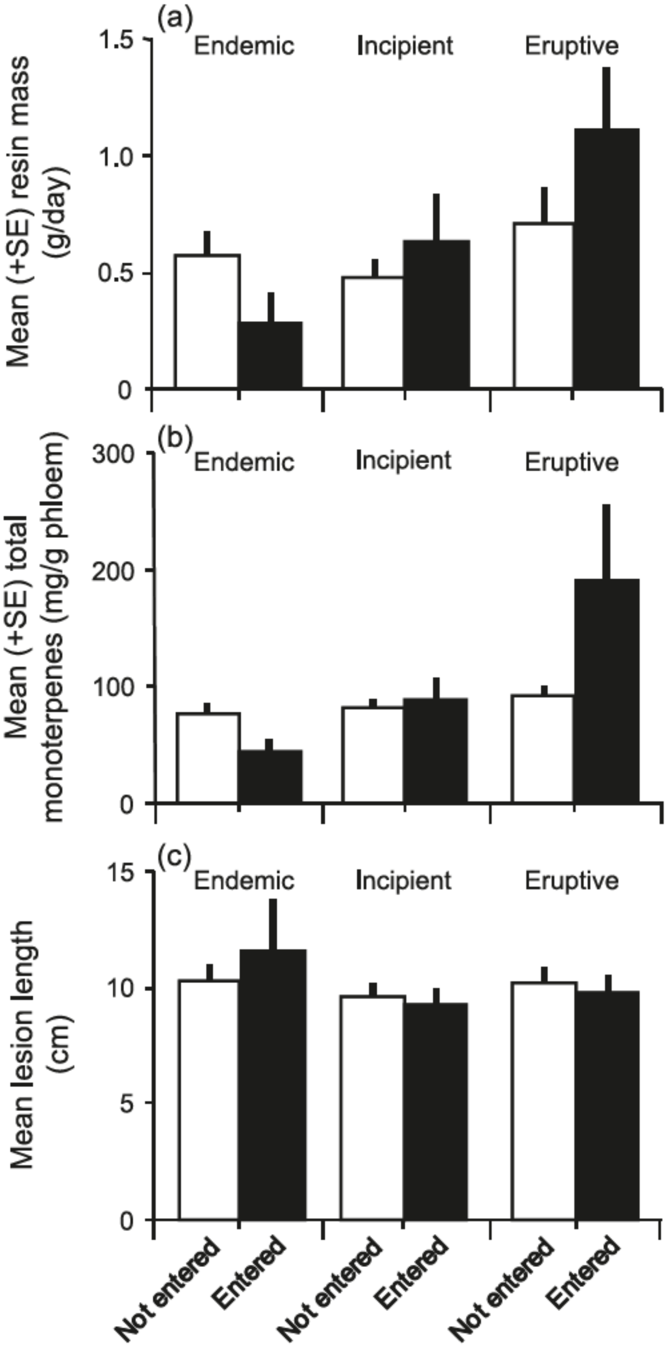


Fig. 5. Locally induced physiological characteristics, in response to simulated mountain pine beetle attack, of lodgepole pine trees subsequently not entered versus those entered by the mountain pine beetle during the beetle's various population phases: (*a*) resin flow (g/day) (entered, $F_{[1,265]} = 4.5954$, $P = 0.0330$; entered × phase, $F_{[1,265]} = 7.6731$, $P = 0.0006$); (*b*) total monoterpenes (mg/g dry mass phloem) (entered, $F_{[1,202]} = 11.8421$, $P = 0.0007$; entered × phase, $F_{[1,202]} = 17.8881$, $P < 0.0001$); and (*c*) lesion length (cm) in response to simulated attack with *G. clavigera* (entered, $F_{[1,191]} = 0.2368$, $P = 0.6271$; entered × phase, $F_{[1,191]} = 0.0728$, $P = 0.7876$). All relationships are based on within-year tree and beetle performances, 2005.

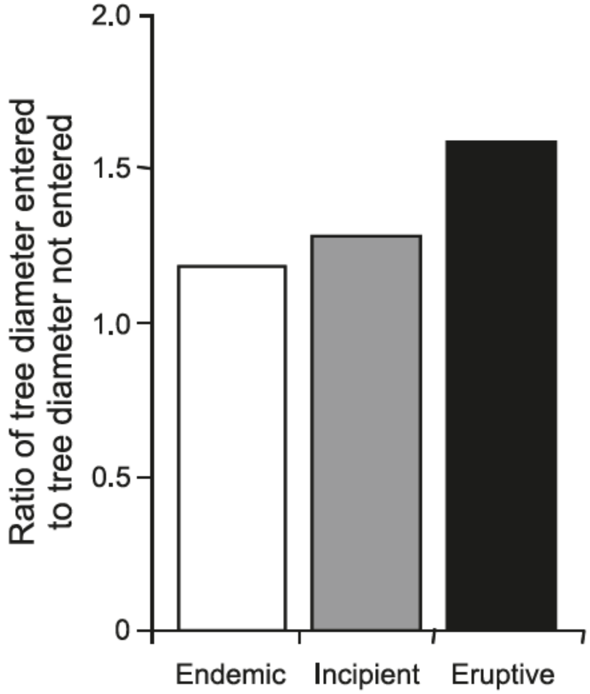
tree diameter, whereas constitutive α-thujene ($F_{]1,196[} = 8.53$, $P = 0.0039$) and unidentified compound 2 ($F_{[1,196]} = 7.45$, $P = 0.0069$) decreased (Supplementary Table S4*a*).[1] The proportion of induced myrcene ($F_{[1,203]} = 8.18$, $P = 0.0047$) increased, whereas the proportion of limonene ($F_{[1,203]} = 7.95$, $P = 0.0052$) and unidentified compound 2 ($F_{[1,203]} = 5.48$, $P = 0.0205$) decreased, with increasing diameter (Supplementary Table S4*b*).[1]

Stand-level responses to population transitions of mountain pine beetle

Physiological traits of trees remaining at various beetle population phases

As the beetle population progressed from its endemic through eruptive phases, it removed trees with certain physiological properties from the forest. The remaining trees in stands where the beetle had reached incipient or eruptive

Fig. 6. Ratio of tree sizes (diameter (cm) at 1.3 m height) of lodgepole pine trees entered and not entered by the mountain pine beetle during its different population phases: entered, $F_{[1,274]} = 0.0688$, $P = 0.9452$; entered × phase, $F_{[1,274]} = 7.8447$, $P = 0.0055$).



populations produced more constitutive resin than those in endemic stands ($F_{[2,276]} = 4.18$, $P = 0.0162$) (Fig. 7). Total constitutive monoterpenes were also highest in eruptive stands ($F_{[2,196]} = 4.05$, $P = 0.0455$) (Fig. 7). The proportion of myrcene was higher in trees in stands where populations were eruptive than endemic ($F_{[1,196]} = 7.18$, $P = 0.0080$) or incipient ($F_{[1,196]} = 4.50$, $P = 0.0352$), and unidentified compound 2 was lower in stands where populations were eruptive than endemic ($F_{[1,196]} = 7.91$, $P = 0.0054$) or incipient ($F_{[1,196]} = 3.72$, $P = 0.06$) (Supplementary Fig. S6 and Table S5).[1] The density of constitutive resin also differed among population phases ($F_{[2,276]} = 4.29$, $P = 0.0393$), with the highest values in eruptive stands.

Induced physiology also differed among stands of varying beetle population densities. Induced resin flow ($F_{[2,266]} = 3.99$, $P = 0.0197$) and total monoterpenes ($F_{[2,203]} = 5.75$, $P = 0.0037$) of trees that were still alive after populations grew were higher in stands with eruptive than endemic (1.5×) or incipient (1.6×) populations (Fig. 7). The density of induced resin was highest in eruptive stands ($F_{[2,266]} = 4.10$, $P = 0.0440$). The proportion of induced myrcene was 1.2× lower in trees in endemic than eruptive stands ($F_{[1,203]} = 6.05$, $P = 0.0148$) (Supplementary Fig. S6 and Table S5).[1]

Behavioural plasticity of beetles through various population phases

As a result of both changes in beetle behaviour through population phases (Figs. 4, 5, not entered vs. entered) and increases in average tree resistance among the remaining host population following removal of less defended trees (Fig. 7, endemic, incipient, eruptive), beetles attacked increasingly vigorous trees as their populations rose. The average resin flow of entered trees was 0.18 g/day in stands with endemic beetle populations, 0.58 g/day in stands in which populations had reached incipient levels, and 0.82 g/day in stands with eruptive populations ($F_{[1,276]} = 5.58$, $P = 0.0189$). As with constitutive properties, induced resin flow to simulated attack among subsequently entered trees increased as the beetle population rose from the endemic (0.28 g/day) to incipient (0.63 g/day) to eruptive phases (1.11 g/day) ($F_{[1,266]} = 7.67$, $P = 0.0060$) (Fig. 5*a*). Likewise, induced monoterpene concentration to simulated attack among subsequently entered trees increased as the population rose from endemic (43.56 mg/g phloem) to incipient (89.10 mg/g phloem) to eruptive phases (190.98 mg/g phloem) ($F_{[1,203]} = 17.89$, $P < 0.0001$) (Fig. 5*b*).

Relationship of defensive capacity against mountain pine beetle to prior colonization by lower stem beetles

Overall, infestation of basal stems by *P. mexicanus* was associated with reduced defenses against mountain pine beetle. Constitutive resin mass was 3.8 times higher in trees not infested with *P. mexicanus* than infested trees ($F_{[1,276]} = 17.08$, $P < 0.0001$) (Fig. 8*a*). Total constitutive monoterpenes did not differ (Fig. 8*b*). Induced resin flow was equivalent (Fig. 8*c*), and total induced monoterpenes ($F_{[1,203]} = 25.76$, $P < 0.0001$) (Fig. 8*d*) were 2.0 times lower in trees infested with *P. mexicanus*. There were likewise some minor differences in the proportions of particular monoterpenes between trees not infested versus infested with *P. mexicanus* (Supplementary Fig. S7 and Table S7).[1] As with the other comparisons, lesion size showed no relationship ($F_{[1,192]} = 0.5471$, $P = 0.4604$). We did not see any evidence of systemic defenses induced by prior infestation by *P. mexicanus*. That is, physiological measurements at the time of "constitutive sampling" were equivalent or reduced between trees infested or not infested by *P. mexicanus*.

Discussion

These results indicate that the role of tree defense in constraining mountain pine beetle reproductive success and the efficacy of plant defense mechanisms in repelling attack can range from crucial to inconsequential depending on beetle population density. Specifically, the same host traits that determine which trees are attacked or unattacked at low stand-level beetle populations may be unimportant or even have an opposite relationship at high densities. When beetle populations were at endemic levels, trees with higher concentrations of induced monoterpenes in response to simulated attack and higher constitutive and induced resin flow are less likely to be attacked. However, these relationships are lost as beetle populations progress to the incipient phase and are reversed during the eruptive phase. Such density-dependent influences on the efficacy of host defenses may partially explain why there has not been more abundant evidence that plant resistance plays an important role in population dynamics of coadapted herbivores.

A suite of physiological characteristics, rather than just a single trait, contributes to the defenses that can favour the survival of individual trees and likewise constrain beetle populations. However, certain properties appear to be especially important, particularly those involving induced reactions.

Table 2. Regression of tree size (cm) (diameter at 1.3 m height) and physiological characteristics of lodgepole pine trees. All data were collected in 2005.

			Intercept			Slope			
Dependant variable	*n*	df	Estimate	*F*	*P*	Estimate	*F*	*P*	R^2
Constitutive resin flow (g/day)	279	276	0.0966	0.7569	0.3862	0.0181	11.4244	0.0008	0.0365
Constitutive total monoterpenes (mg/g dry mass phloem)	199	196	–0.0238	0.0529	0.8200	0.0124	6.2001	0.0137	0.0255
Induced resin flow (g/day)	269	266	–0.0938	0.6724	0.4119	0.0332	36.9664	<0.0001	0.1188
Induced total monoterpenes (mg/g dry mass phloem)	206	203	1.4778	121.882	<0.0001	0.0139	4.6656	0.0320	0.0176
Lesion length (cm)	195	192	8.7696	61.3089	<0.0001	0.0619	1.34	0.2482	0.0018

The strongest predictor of tree defense (at least when populations were not high enough to negate it) is the localized accumulation of monoterpenes in response to simulated attack. In addition, constitutive and especially induced resin flow were higher in trees that were not attacked than in those that were, when beetle population densities were in the endemic phase. High resin flow and volatile emission can interfere with pheromone emission by an entering beetle, thus preventing it from attracting the recruits needed to overcome host tree defenses, or even flush it from its entry site (Raffa 2001; Erbilgin et al. 2003, 2006). The density of this resin did not provide significant information with regard to host resistance, as its mass and volume were highly correlated. Lesion length was not related to attack frequency, suggesting that the qualitative and quantitative allelochemical content of the necrotic region, or perhaps the rate of lesion formation (Wallin and Raffa 2001), is more important than its final size.

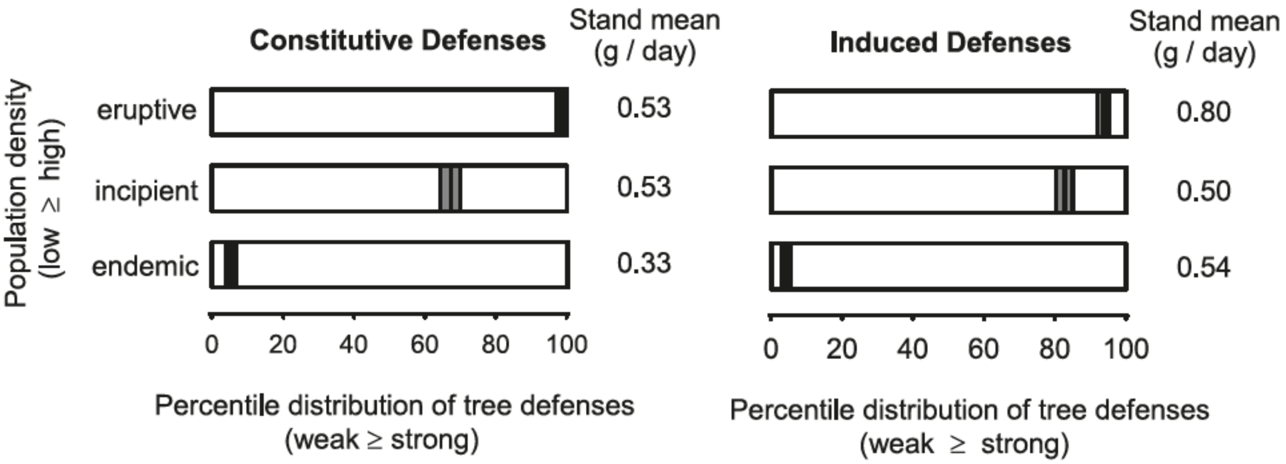
While biologically active, monoterpenes can be energetically expensive to synthesize and store (Keeling and Bohlmann 2006). Thus, employing both constitutive and induced defenses is optimal for resisting tree-killing bark beetles. In addition to the total quantity of monoterpenes, some individual compounds may be particularly important. For example, the concentration of induced limonene was always higher in non-attacked than attacked trees (Supplementary Fig. S5),[1] and this compound is one of the most highly inducible (Supplementary Table S1)[1] and biologically active against bark beetle – fungal complexes (Raffa 2001; Raffa et al. 2005). Trees selected at endemic and incipient populations were also higher in constitutive α-pinene (Supplementary Fig. S4),[1] which this beetle exploits as a precursor for pheromone biosynthesis (Wood 1982). As with total monoterpenes, this relationship was lost during the eruptive phase. Of these compounds, limonene occurs in conifers almost entirely in the (–) form, but α-pinene occurs in both forms, so potential variation in this monoterpene's role with chirality cannot be excluded.

The interaction of tree defense physiology with beetle population density provides some insight into how a threshold separating endemic from eruptive dynamics can be breached. The selective culling of weakened trees by endemic populations and the requirement that colonized trees be killed for beetles to reproduce presents host-searching adults with a shifting tradeoff. That is, the surviving host population is increasingly well defended, even as the rising beetle population is better able to overcome them. Under most circumstances, a slight increase in beetle numbers is probably insufficient to breach this threshold, or unfavourable weather may cause high mortality resulting in a return to low populations. In the event of a large population increase, however, this threshold can be surpassed. Such rapid increases are more likely if stressors are widespread, e.g., region-wide drought, defoliation, or disturbance, stands are homogeneous, immigration is substantial, or increased temperatures reduce winter mortality or shorten development time (Logan et al. 2003). The role of temperature is of particular concern in that most projections of global climate change predict increasing variance in addition to increasing means (Solomon et al. 2007). Our results suggest that climatic episodes that release beetle populations such as warm temperatures (Bentz et al. 2001) or drought (Breshears et al. 2005) could then be followed by self-sustaining dynamics, even after the initial releasor subsides (Raffa et al. 2008).

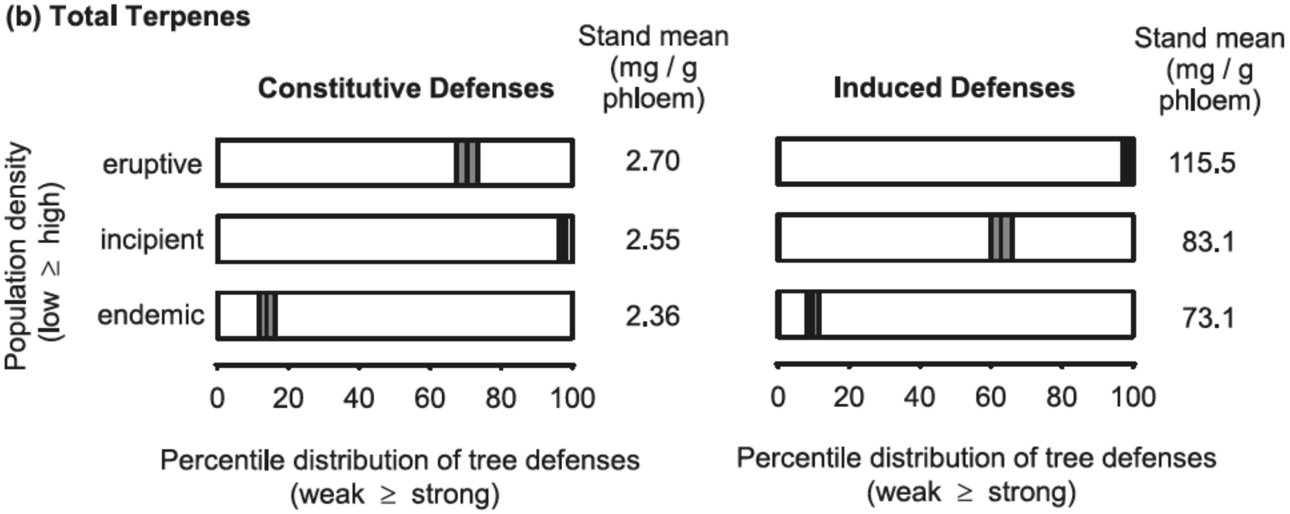
The tradeoff between the defensive capabilities and nutritional quality of host trees adds a third component to how local beetle populations may surpass an eruptive threshold. Large diameter trees are better defended, having higher constitutive and induced resin flow and higher induced monoterpenes (Table 2). This result is somewhat surprising because outbreaks are often associated with large diameter trees (Safranyik and Carroll 2006). The relationship in Fig. 6 helps explain this paradox. As beetle populations increase, they show an increasing preference for large diameter trees, which can support more larvae if the host can be successfully attacked (Amman 1972). Hence, the well-founded relationship between tree diameter and mountain pine beetle outbreaks does not appear to arise from large-diameter trees being more susceptible, but rather from high populations being able to overcome the resistance of and, therefore, achieve higher replacement rates in large diameter trees. The mechanism that drives this behavioural switch is unknown. It is known that mountain pine beetles have a visual preference for large silhouettes that can lead to higher landing rates on larger trees but that they often do not enter them if their chemistry is deemed unsuitable after landing (Hynum and Berryman 1980). It is also known that host entry by bark beetles is mediated by monoterpenes in a dose-dependent fashion and that these behaviours are mediated via gene–environment interactions (Wallin and Raffa 2000). For example, spruce beetles from eruptive populations are more likely to enter media with high monoterpene concentrations when other beetles are present, but individuals from endemic populations make monoterpene-dependent decisions that are not influenced by the presence of other beetles

Fig. 7. Preferences exhibited by mountain pine beetle during different population phases for entry into lodgepole pines of varying constitutive (left) and induced (right) (*a*) resin flow and (*b*) total monoterpenes. The mean defensive capability of the trees that the beetles selected (measured post-hoc from $n = 9–13$ trees in each stand) was ranked relative to the means of 999 samples of random trees drawn from the rest of the stand (by pulling samples of size $n = 9–13$ from a theoretical distribution fit by maximum likelihood to empirical data of $n = 52–81$ trees stratified across diameter classes; for details, see Supplementary Table S6). The shaded portions represent 95% confidence limits about the median of the rankings, based on repeating this procedure 1000 times. Shaded portions to the left of the spectrum represent beetles selecting trees that are less well defended than what would be expected by random chance; to the right, more. Values beside bars indicate stand mean for that physiological parameter. Resin flow (g/day): constitutive ($F_{[2,276]} = 5.4586$, $P = 0.0162$); induced ($F_{[2,266]} = 6.4335$, $P = 0.0118$). Monoterpene content (mg/g dry mass phloem): constitutive ($F_{[2,196]} = 4.0499$, $P = 0.0455$); induced ($F_{[2,203]} = 11.7090$, $P = 0.0008$).

(a) Resin Flow



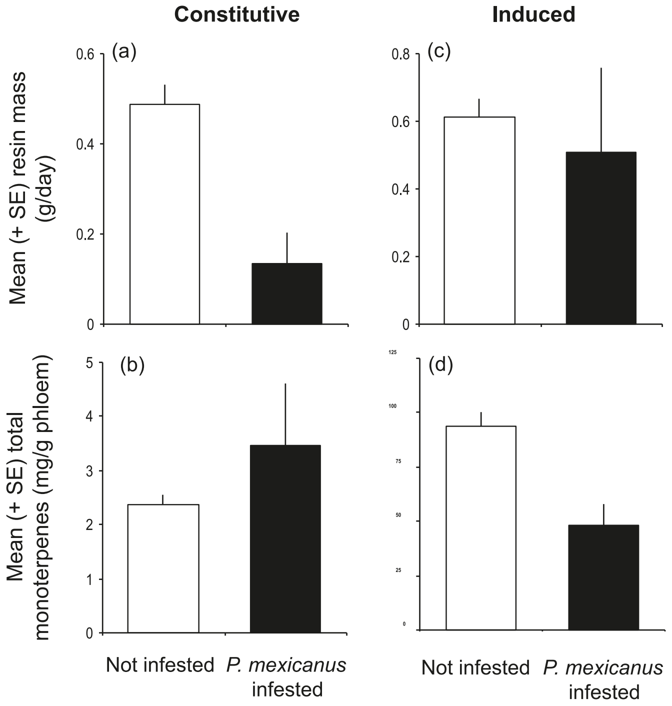
(b) Total Terpenes



(Wallin and Raffa 2004). Thus we speculate that there are higher rates of abandonment on large-diameter trees during endemic than eruptive conditions, which could generate our observed pattern. However, explicit testing is needed to test this hypothesis.

These results also provide insight into how populations of tree-killing bark beetles that rely on mass attack to overcome host defenses can persist during lengthy endemic periods. In this system, trees colonized by lower-stem insects provide a reservoir of hosts with compromised defenses (Figs. 8*a*, 8*d*). We cannot separate whether prior infestation reduces tree defenses or both species independently colonize the same trees. However, predisposition by *P. mexicanus*, at least to some extent, seems the more likely explanation. First, if the association were independent, the sequence of *D. ponderosae* being followed by *P. mexicanus* would occur as frequently as the converse, but the latter was observed in all cases (Smith et al. 2011). This cannot be attributed to the later flight period of *D. ponderosae*, because most external stresses such as drought occur after *P. mexicanus*'s activity period and before *D. ponderosae*'s, rather than during the dormancy period after *D. ponderosae*'s. Also, several seasons sometimes transpire between the two colonization events (Smith et al. 2011). Second, trees of equivalent physiological condition are more likely to be colonized by *D. ponderosae* if they harbour *P. mexicanus*, and *D. ponderosae* reproduce more in such trees (Smith et al. 2011). Third, results from related systems show strong evidence of predisposition to tree-killing bark beetles by root and lower-stem beetles (Klepzig et al. 1991).

Fig. 8. Physiological characteristics of lodgepole pine trees not infested versus infested by *Pseudips mexicanus*: (*a*) constitutive resin mass (g/day) ($F_{[1,276]} = 17.0829$, $P < 0.0001$); (*b*) constitutive total monoterpenes (mg/g dry mass phloem) ($F_{[1,196]} = 0.7815$, $P = 0.3778$); (*c*) induced resin mass (g/day) ($F_{[1,266]} = 5.2866$, $P = 0.0223$); and (*d*) induced total monoterpenes (mg/g dry mass phloem) ($F_{[1,203]} = 33.6618$, $P < 0.0001$).



Similar relationships between tree defensive ability and herbivore density seem likely to prevail across those species of bark beetles that undergo landscape-scale eruptions. In contrast, we predict that most other species will show more consistent relationships among host defense, population density, and incidence of attack and that this manifold can provide a mechanistic basis for separating the dynamics of underlying life history strategies. We also predict similar relationships for other groups of eruptive species that engage in cooperative exploitation of their host. Cooperative behaviour appears to be particularly prevalent among eruptive species and likely shapes both plant–herbivore and herbivore–enemy relationships differentially across population phases (Maron et al. 2001). Where possible, direct measures of both herbivore population density and plant defense physiology should be included. For example, the broad range of attack success in endemic populations (Fig. 2) was likely influenced by our use of an indirect measure of population density, number of attacked trees, and interyear variation in environmental quality for both beetles and trees. Additionally, improved quantification of stand-related differences in susceptibility, which were beyond the objectives of our study, could improve understanding of endemic–eruptive transitions in population dynamics.

Identifying how the effects of plant defense on herbivore success vary with population density could improve our abilities to relate key processes across multiple scales, integrate pattern and process, and characterize impacts of insects on plant fitness and ecosystem function.

The population-dependent efficacy of plant defense likewise has implications to natural resource management where land use requires that insects be kept below damaging populations or range expansions driven by climate change threaten new hosts or habitats (Logan et al. 2003). Enhancing resistance through cultural practices, for example, seems likely to help prevent eruptions from occurring, but unlikely to halt them. Conversely, the low importance of tree resistance during outbreaks would be misinterpreted if extrapolated to suggest that it is not an important constraint against eruption onsets. Historically, the preponderance of research on forest herbivores has been done on eruptive populations for reasons of funding, experimental practicality, and concern. Our results highlight the need for additional attention during their endemic, incipient–transitory, and posteruptive phases.

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