OIKOS

Research

Relationships between conifer constitutive and inducible defenses against bark beetles change across levels of biological and ecological scale

Michael Howe, Charles J. Mason, Claudio Gratton, Ken Keefover-Ring, Kimberly Wallin, Alvin Yanchuk, Jun Zhu and Kenneth F. Raffa

M. Howe, C. Gratton, J. Zhu and K. F. Raffa (https://orcid.org/0000-0002-9694-0835) ✉ (kfraffa@wisc.edu), Dept of Entomology, Univ. of Wisconsin-Madison, Madison, WI 53706, USA. JZ also at: Dept of Statistics, Univ. of Wisconsin-Madison, Madison, WI, USA. – C. J. Mason, Dept of Entomology, Pennsylvania State Univ., University Park, PA, USA. – K. Keefover-Ring, Depts of Botany and Geography, Univ. of Wisconsin-Madison, Madison, WI, USA. – K. Wallin, College of Science and Mathematics, North Dakota State Univ., Fargo, ND, USA. – A. Yanchuk, Ministry of Forests, Lands, Natural Resource Operations & Rural Development, Government of British Columbia, Victoria, BC, Canada.

Oikos
129: 1093–1107, 2020
doi: 10.1111/oik.07242

Subject Editor: Johan Kotze
Editor-in-Chief: Dries Bonte
Accepted 19 April 2020

Plants resist herbivores using both constitutive and induced defenses. Limited resources and biosynthesis costs of anti-herbivore compounds may impose tradeoffs between these modalities, but the evidence is conflicting. We postulated that biological and ecological scale may influence potential tradeoffs and analyzed constitutive-induced relationships from genotypic to interspecific levels. We focused on one system, conifer phloem monoterpenes that resist bark beetle attack, to minimize variation from feeding guild or phylogeny. We examined 45 half-siblings in common gardens ($n=763$), seven populations of five species with tree-level data ($n=436$) and 29 studies of nine species with population-mean data in forests. All studies evaluated mature trees to correspond with beetle behavior in nature and employed common methods of induction and analysis. Rather than a global tradeoff between constitutive and induced defenses, relationships varied with level of biological organization. In common gardens, constitutive concentrations and inducibility (I–C) were inversely related across families and individuals. These relationships did not extend to forest populations, however, where constitutive concentrations and inducibility were positively related. Across populations and species, constitutive concentrations and inducibility were likewise positively related. There was no evidence for inducibility tradeoffs between monoterpene quantity and composition: trees showed no or positive relationships between constitutive concentrations and induced compositional changes. These results suggest environmental variability can supersede underlying physiological tradeoffs in some systems, allowing plants with greater resource pools to excel in multiple defense components. This is further supported by microsite effects on inducibility exceeding family effects in the common gardens, and by relationships between constitutive and absolute induced concentrations being positive at all levels. We present a general model for understanding how underlying physiological tradeoffs are variably manifested across scales. These results can contribute to the protection and resilience of agronomic and native ecosystems and help contextualize organismal-level allocations to ecosystem-level patterns across heterogeneous landscapes.

NORDIC SOCIETY OIKOS

www.oikosjournal.org

Keywords: allocation, monoterpenes, phytochemistry, plant defense, Scolytinae, tradeoffs

Introduction

Plant defenses against insect herbivores are often multimodal and diverse (Acevedo et al. 2015, Karban 2019). Defense components can be expressed constitutively or be induced by insect attack (Kessler and Baldwin 2008, Mithöfer and Boland 2012). Induced responses can be systemic (Schilmiller and Howe 2005) or localized within a plant (Karban and Niiho 1995), and can be elicited rapidly (Underwood 1998) or over longer time frames (Haukioja and Niemela 1979). Induced changes can include both increased concentrations of defense compounds and changes in the relative composition of compounds (Karban 2019).

Defenses can be costly (Gershenzon 1994, Zust and Agrawal 2017), so plants with access to limited energy and substrate pools must contend with a variety of tradeoffs to optimize fitness (Herms and Mattson 1992). Understanding the incidence, extent and source (i.e. genetic, environmental) of these tradeoffs can help guide breeding programs, predict pest outbreaks and optimize management strategies. For example, genetic-based tradeoffs may inform breeding programs or provide insight for across population or across species comparisons, while phenotypic tradeoffs may guide management strategies and help to develop crucial predictions of where outbreaks may occur. This knowledge can also guide strategies to build resilience into cultivated and native ecosystems faced with intensifying pest pressures due to anthropogenic change (Biedermann et al. 2019, Huang et al. 2019) by predicting what combinations of traits can likely co-occur. Understanding linkages between physiological tradeoffs and ecological patterns can also provide valuable insights into basic coevolutionary processes.

Tradeoffs can potentially arise as differential allocations to growth versus defense (Herms and Mattson 1992), resistance versus tolerance (Strauss and Agrawal 1999) or physical versus chemical defenses (Strauss et al. 2002). Differential allocation to induced versus constitutive defenses may provide a means for plants to achieve the most cost-effective deployment of antiherbivore traits (Steppuhn and Baldwin 2008, Cipollini and Heil 2010, Kempel et al. 2011). There may also be potential tradeoffs between increasing total concentrations and maximizing the diversity of defense compounds during induction (Villari et al. 2014, Karban 2019).

The literature on whether plants exhibit tradeoffs between induced versus constitutive defenses is mixed, with multiple examples of either the presence (Kempel et al. 2011, Rasmann and Agrawal 2011, Moreira et al. 2014, Villari et al. 2014) or absence (Brody and Karban 1992, Thaler and Karban 1997, Underwood and Rausher 2002, Koricheva et al. 2004) of such trends. Study systems used to evaluate these relationships have varied in the degree to which the herbivore influences plant fitness, the extent to which defenses are primarily qualitative or quantitative, and the energetic costs of the defense trait. Additionally, most studies assessing relationships between induced versus constitutive defenses have been conducted at a single level of biological organization, such as among genotypes within a species (Traw 2002, Underwood and Rausher 2002), individuals in a population (Villari et al. 2014), or various host species (Kempel et al. 2011, Rasmann and Agrawal 2011, Moreira et al. 2014), or at a single level of ecological scale, such as a glasshouse or a single or closely proximate locations in the field. However, there are many ecological processes for which biological level or ecological scale (hereafter biological scale for simplicity) strongly affects both the magnitude and direction of relationships (Levin 1992, Schneider 2006), such that microscale processes do not directly scale to macroscale patterns (Englund and Cooper 2003). Despite the ecological, evolutionary and resource management implications of potential tradeoffs between constitutive and induced plant defenses, little is known about how biological scale may affect these relationships.

We tested putative tradeoffs between plant constitutive and induced defenses by conducting a cross-scalar approach across a single multispecies model system. This approach minimizes the potentially confounding effects of variable herbivore impacts, chemical classes, forms of induction, phylogenies and plant tissues compromised. We selected a model system in which coevolved native herbivores strongly affect host plant survival in nature, related plant species employ a common defense with well-documented bioactivities, and previous studies by multiple lab groups employed standardized methods. Our model comprises interactions between Pinaceae and Scolytinae (bark beetles), with analyses ranging from intraspecific plant genotypes to inter-species. Further, we focused on monoterpenes, because these compounds: 1) exhibit high, dose-dependent repellent and toxicological activities against bark beetles and their symbionts at quantities present in vivo (Raffa and Smalley 1995, Raffa et al. 2005, Adams et al. 2011, Reid et al. 2017); 2) show concentration-dependent relationships to host survival in nature (Raffa and Berryman 1982, 1983a, Erbilgin et al. 2003, 2006, Boone et al. 2011, Zhao et al. 2011); 3) are highly inducible (Schiebe et al. 2012, Raffa et al. 2017, Celedon and Bohlmann 2019); and 4) comprise a major portion of host secondary chemistry (Raffa et al. 2017, Biedermann et al. 2019).

Bark beetles that colonize the main stems of mature trees are the greatest source of herbivore-induced mortality to conifers worldwide (Anderegg et al. 2015, Biedermann et al. 2019). Some native species undergo intermittent, landscape-scale irruptions that substantially alter forest species composition, demographic structure, carbon sequestration, economic value and successional trajectories over millions of hectares. Successful colonization is lethal to host trees due to the combined actions of beetle development within subcortical tissues, coordination of rapid pheromone-mediated mass attacks, and vectoring of moderately phytopathogenic fungi into host tissues (Safranyik and Carroll 2006, Krokene and Solheim 2007, Blomquist et al. 2010). Most tree-killing bark beetles are primarily associated with one host genus, of which they can exploit most species within their geographic range.

Adapted conifer species exploit the beetles' fungal symbionts as cues indicative of attack to elicit rapid, locally induced chemical defenses (Keeling and Bohlmann 2006, Bohlmann 2012, Keefover-Ring et al. 2016, Mason et al. 2017, Celedon and Bohlmann 2019). Phloem monoterpenes quickly rise to levels that repel or kill beetles, impede pheromone communication and inhibit symbionts (Erbilgin et al. 2003, Adams et al. 2009). Induced localized production of high quantities of monoterpenes has been shown to predict tree survival under natural conditions in *Pinus*, *Abies* and *Picea* (Schiebe et al. 2012, Biedermann et al. 2019, Huang et al. 2019). The combination of inoculation with a beetle's primary symbiotic fungus and mechanical wounding elicits responses similar to those against natural attack. Simple mechanical wounds do not elicit comparable responses. Trees further differentiate between relatively pathogenic versus nonpathogenic fungi, viable versus heat-killed fungi (Raffa and Smalley 1995), and inoculation with a beetle's fungal symbiont versus the broadly acting plant defense elicitor methyl jasmonate (Burke et al. 2017).

We currently have little understanding of whether conifers exhibit tradeoffs in their constitutive versus induced defenses against bark beetle–fungal complexes. A statistically weak positive correlation between constitutive and induced resin flow was observed during early but not late summer in a mature *Pinus taeda* planting (Lombardero et al. 2000). There was no relationship between constitutive resin canal metrics and induced terpenes or phenolics among trees in forest populations of mature *Pinus contorta* or *Pinus albicaulis* (Mason et al. 2019). Opposing increases and decreases of some phenolics, but consistent increases in monoterpene inducibility were observed during induction in forest populations of mature *Pinus sylvestris* (Villari et al. 2014). In glasshouse seedlings, relationships of total constitutive to methyl jasmonate-induced phenolics varied with species and latitudinal source (Moreira et al. 2014), and monoterpene cyclase showed opposing responses to drought in induced versus constitutive *Abies grandis* (Lewinsohn et al. 1991). Collectively, these patterns suggest an opportunity to better understand plant defense tradeoffs by employing multi-level analyses on a system characterized by intense herbivore-driven selective pressures, interactions across highly heterogeneous landscapes, and strong cross-scale processes (Raffa et al. 2008, Seidl et al. 2016, Balogh et al. 2018).

Our objective was to evaluate potential tradeoffs between constitutive and induced chemical defenses in conifer–bark beetle interactions using studies that employed common methodologies across a range of biological scales: 1) across half-siblings, 2) among individuals in common gardens; 3) among individuals in forest populations of multiple tree species; 4) across populations of a single species; 5) across populations of multiple species; and 6) across multiple tree species. We explored potential relationships from three perspectives: a) inducibility (I–C) of monoterpenes versus constitutive concentrations (i.e. the plant's allocation strategy), b) direct relationships between constitutive and induced total concentrations (i.e. the defenses an herbivore encounters) and c) total versus compositional changes (i.e. potential tradeoffs between chemical quantity and quality during induction).

Methods

Biology of model system

Adult beetles land on potential hosts, burrow into the bark and release aggregation pheromones that attract both sexes (Blomquist et al. 2010). Rapid arrival of large numbers of beetles kills the tree, thereby allowing reproduction to proceed (Raffa et al. 2008). After mating, females construct ovipositional galleries, and if colonization is successful, larvae hatch and consume phloem and symbiotic fungi (Bleiker and Six 2007). Following pupation and a teneral adult stage, beetles emerge, disperse and locate new hosts. Successful colonization is facilitated by additional symbiotic microorganisms that assist the beetles in overcoming tree defenses (DiGuistini et al. 2011, Hammerbacher et al. 2013, Wang et al. 2013).

At low concentrations, monoterpenes provide volatile cues that beetles exploit in host location and recognition (Wallin and Raffa 2000, Franklin and Grégoire 2001), and in biosynthesis and synergism of aggregation pheromones (Blomquist et al. 2010). At high doses, monoterpenes are repellent and inhibit pheromone communication (Erbilgin et al. 2003), and are toxic to beetle adults (Raffa and Smalley 1995, Reid et al. 2017), eggs (Raffa and Berryman 1983a) and symbionts (Cobb et al. 1968, Klepzig et al. 1996, Adams et al. 2011, Boone et al. 2013, Mason et al. 2015). Individual monoterpenes vary in toxicity (Raffa et al. 2005), but total concentrations exert stronger effects and are more closely related to tree survival in nature (Boone et al. 2011, Chiu et al. 2017, Reid et al. 2017). Induction also increases diterpenes that are highly fungicidal (but to date have not shown toxicity to bark beetles) (Kopper et al. 2005, Boone et al. 2011, Mason et al. 2015), and to a lesser extent phenolics (Mason et al. 2017, Raffa et al. 2017), some of which are fungicidal (Hammerbacher et al. 2013) or repellent (Faccoli and Schlyter 2007).

Experimental overview

We queried three tiers of datasets (Supplementary material Appendix 1 Table A1), a common garden study, forest trees with individual-level data, and forest trees with mean-population level data, across six levels of biological organization. The experimental structure is illustrated in Fig. 1. Each of these datasets was generated using common methodologies of induction and analysis. To evaluate potential tradeoffs from a plant allocation perspective we used the metric of inducibility (induced – constitutive) recommended by Morris et al. (2006). To evaluate the plant conditions actually encountered by the herbivore we also directly related induced to

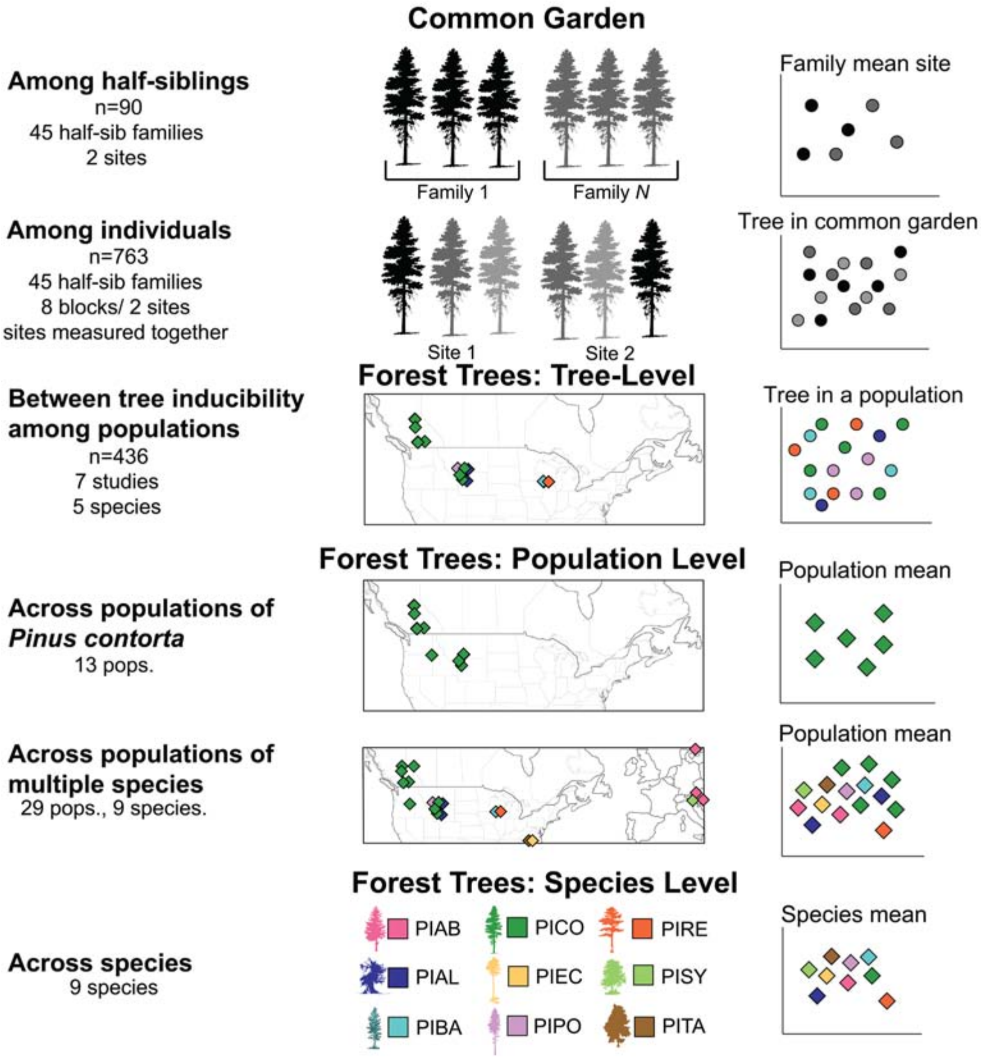

Figure 1. Experimental design for quantifying relationships between induced versus constitutive monoterpenes at six biological scales. Text on left provides experimental design and replication. Diagrams in center depict design of the common gardens and locations of populations. Species codes correspond to: PIAB = *Picea abies*; PIAL = *Pinus albicaulis*; PIBA = *Pinus banksiana*; PICO = *Pinus contorta*; PIEC = *Pinus echinata*; PIPO = *Pinus ponderosa*; PIRE = *Pinus resinosa*; PISY = *Pinus sylvestris*; and PITA = *Pinus taeda*. Mock regressions on right depict experimental units at each level. Actual regressions are in Supplementary material Appendix 2 Fig. A2. Note the first two levels represent different approaches to analyzing the same dataset from common gardens.

constitutive concentrations. Because monoterpene concentrations undergo strong localized rather than systemic changes to fungal inoculation in this system (Keefover-Ring et al. 2016, Burke et al. 2017, Mason et al. 2017), individual trees can be tested for both constitutive and induced levels without loss of independence by systemic responses, so Monte Carlo simulations are not required (Morris et al. 2006). Our cross-scalar design poses certain limitations. Specifically, the common garden study (levels 1, 2) can partition genetic and environmental sources of variation but these sources are necessarily confounded in natural populations (levels 3–6). Further, at the other end of our continuum the genetic contribution is by definition higher for the across-species study (level 6) than within-species analyses at the population and regional levels (levels 4–5).

All included studies involved: 1) mature trees, i.e. at least 20 years old to conform with these beetles' realized niche (Maclauchlan et al. 2015) and avoid potential ontogenetic disparities (Boege and Marquis 2005, Barton and Koricheva 2010, Erbilgin and Colgan 2012); 2) standardized induction by inoculating the predominant bark beetle – vectored fungal symbiont from each system combined with a small mechanical wound to simulate beetle entry, in order to focus on host coevolved responses (Burke et al. 2017); 3) reported data of total monoterpene concentrations before and after inoculation; 4) assaying the same trees for both constitutive (intact phloem at the time of inoculation) and locally induced monoterpene concentrations sampled approximately two weeks after treatment; and 5) no more than seven fungal inoculations per tree, to omit studies whose purpose was to deplete inducible defenses by simulating mass attack (Raffa and Berryman 1983b). All chemical analyses were performed on phloem tissues within visible reaction zones by gas chromatography using closely similar standardized methods (Keefover-Ring et al. 2016, Mason et al. 2017, Raffa et al. 2017). Total monoterpenes include all identified compounds; we did not include unidentified peaks because we cannot be certain they are monoterpenes or that they have biological activities against bark beetles or their symbionts.

Levels at which relationships between constitutive and induced defenses were tested

1. Relationships across half-siblings

A common garden experiment with *P. contorta* was performed in central British Columbia, Canada, at two sites originally established by the B.C. Forest Service Research Branch (Supplementary material Appendix 1 Table A1A). The trees were planted in 1986 as two randomized complete blocks at two sites, Moffat Creek and Bowron Lake (near Prince George, BC) each with eight blocks, four-tree row plots, with 45 unique open-pollinated half-sib families. After thinning and mortality, 8–16 trees (16–26 trees total across the two sites) per family remained. Sampled trees averaged 5.8–16.7 cm in diameter at breast height (DBH). Trees were inoculated in 2006 with *Grosmannia clavigera*, the primary fungal symbiont of *Dendroctonus ponderosae*. Phloem was sampled at the time of inoculation (control) and 12 days post inoculation (induced). Control phloem removal distant from the inoculation site does not elicit stem-wide induction, so does not affect induction measures, in this system (Keefover-Ring et al. 2016, Burke et al. 2017, Mason et al. 2017). Mean estimates for total constitutive and inducibility of monoterpenes were calculated for each site by family combination (blocks were combined, as the effect of block was not significant; p-value > 0.20) and used in subsequent models. The total number of families included in analyses was 90 (45 families/site).

2. Relationships among individuals in a common garden

The common garden experiment described in the previous section was also used to examine relationships across individual trees. Sites were combined for analysis as the interaction of site and constitutive monoterpenes was not significant (p-value = 0.86). In total, 763 trees were included in the analysis (418 at Moffat Creek, 345 at Bowron Lake).

3. Relationships among individuals in forest populations

Relationships among individual trees within forest populations were examined by compiling data from previous published papers by our group that provided concentrations of monoterpenes on a per tree basis (Supplementary material Appendix 1 Table A1B). Populations of the five species used in this analysis ranged from southern Wisconsin, USA to central British Columbia, Canada. Analyses were conducted on all the populations compiled together (n = 436).

4. Relationships across populations of P. contorta

Data for monoterpene concentrations of *P. contorta* from the literature were compiled based on those published studies that met the criteria listed above and mean total monoterpene concentrations from the individuals in the common garden experiment and among forest populations (Supplementary material Appendix 1 Table A1A–C; each population had one mean value). Tree level data from Boone et al. 2011 and Ott et al. 2011 were split into two and six separate populations. Populations ranged from western Wyoming, USA to central British Columbia. Mean constitutive and induced concentrations were compared across all *P. contorta* populations (n = 13). Across population comparisons in other species were not considered because of insufficient availability of studies to serve as replicates.

5. Relationships across populations of multiple tree species

Data from the literature were compiled based on those published studies that met the criteria listed above (out of 130 published studies considered; Web of Science) and mean total monoterpene concentrations from the individuals in the common garden experiment and among forest populations (Supplementary material Appendix 1 Table A1A–C; each population had one mean value). Tree level data from Boone et al. 2011 and Ott et al. 2011 were split into two and six separate populations respectively. Populations ranged from North Carolina, USA to British Columbia in North America, and from Italy to Sweden in Europe. Mean constitutive and induced concentrations were compared across all populations. Collectively, these studies represented 29 populations and 9 species.

6. Relationships across multiple tree species

Across species comparisons were performed by summarizing the mean monoterpene response over each species (Supplementary material Appendix 1 Table A1A–C). Collectively, these studies represented nine species. This analysis used the same data sources as 5) above.

Statistical analyses

All data were analyzed in R Studio ver. 1.1.453 running R ver. 3.6.0 (<www.r-project.org>). To facilitate comparisons across scales, all data were z-score standardized by subtracting the mean from each individual data point and dividing those points by the standard deviation. Level 3 data were transformed by 0.01 (based on plotting) to include trees with no quantifiable constitutive monoterpenes (included in Raffa and Smalley 1995) and were z-score standardized within each study and species combination. Population estimates were collectively z-score standardized and used for levels 4–6. z-score standardizations were checked by plotting to ensure that standardizations did not alter the shape of the data.

For all six levels of biological organization, linear models were used to compare inducibility versus constitutive monoterpenes with a response variable of ln(inducibility) and a fixed effect of ln(constitutive), where ln(inducibility) represents ln(induced-constitutive), where constitutive and induced represent total phloem monoterpenes ($mg\,g^{-1}$) at the time of inoculation and post-inoculation, respectively. Data were centered on 0 and as a result, no intercepts were included in models. A mixed effects model (Bates et al. 2015) was used to analyze relationships among individuals in common gardens (level 2). Fixed and random effects (DBH and site nested within block respectively) were included in the model based on backward elimination with likelihood ratio tests (Kuznetsova et al. 2017). Site was excluded from the model because neither site (p-value = 0.91) nor the interaction of site and constitutive monoterpenes (p-value = 0.86) were significant. Analyses across tree species did not employ phylogenetic least squares as a correction for possible phylogenetic non-independence due to the low number of species and lack of even replication across species.

All models exploring relationships between absolute induced monoterpenes and constitutive monoterpenes were fit with the same terms as those testing inducibility versus constitutive monoterpenes for ease of comparison. Regressions and model details are provided in Supplementary material Appendix 2 Fig. A2. All assumptions for linear mixed effects models were checked by plotting Pearson residuals for mixed effects models and plotting residuals of corresponding linear models (for both mixed effects models and simple linear regressions).

Comparisons among families in the common garden experiment (level 1) were developed based on the predicted values of inducibility for each family from a mixed effects model that included family and the interaction of constitutive concentrations and family as fixed effects. All observations used to develop predictions had a fixed value for DBH (10.81, the overall mean).

Chemical richness was determined by counting the number of monoterpenes that had concentrations > 1 $mg\,g^{-1}$ phloem. Chemical diversity was computed using the Shannon–Weaver diversity index on the proportions of each monoterpene in each tree. Regressions between constitutive and induced total concentrations of monoterpenes and richness/diversity were performed on a per tree basis, i.e. each point represents the ln(constitutive) or ln(inducible) concentrations versus either the diversity index for the proportions of monoterpenes, or the richness of monoterpenes for a given tree. Data and code are available at (github).

Results

Relationships between constitutive concentrations and inducibility of total monoterpenes

Half-sibling families of *Pinus contorta* showed an overall inverse relationship between mean constitutive monoterpenes and mean inducibility (slope = −0.34, p-value < 0.001; Fig. 2; Supplementary material Appendix 2 Fig. A2), consistent with a tradeoff. There was high variability among families, with increases from constitutive to induced monoterpenes ranging from 60 to 310%. There was also substantial variation in the relationship between inducibility and constitutive concentrations, with nine half-siblings exhibiting a positive relationship and 36 exhibiting a negative relationship (Supplementary material Appendix 2 Fig. A2, panel B).

Trees in the common garden experiment underwent an average 160% increase in total monoterpenes during induction. The relationship between inducibility versus constitutive concentrations was significantly negative (slope = −0.11; p-value = 0.002; Supplementary material Appendix 2 Fig. A2). There was a positive covariate of tree diameter (slope = 0.13; p-value < 0.001), indicating larger trees had higher inducible defenses than smaller trees of the same age. Tree size did not influence the relationship of inducibility versus constitutive monoterpenes. Likewise, spatial variability affected inducibility, but not the relationship between inducibility and constitutive monoterpenes, as the best fit mixed effects model included a random intercept of block nested within site (likelihood ratio test; 25.52; p-value < 0.001). Neither site (p-value = 0.91 nor the site × constitutive interaction was significant (p-value = 0.86), so these were dropped from the

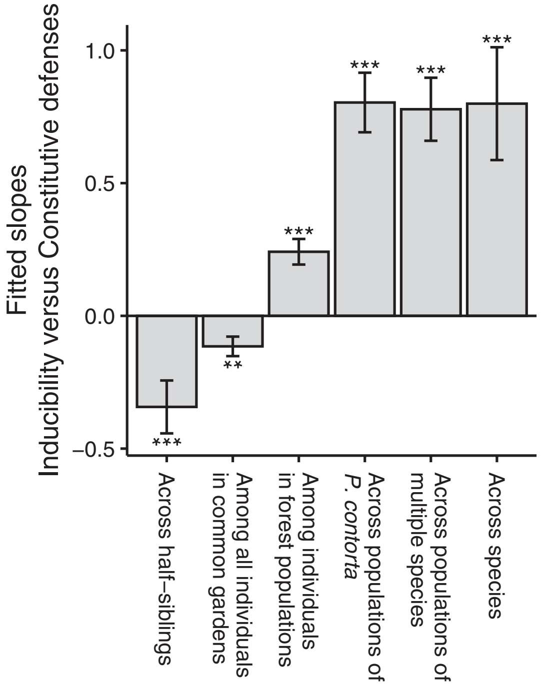

Figure 2. Relationship of inducibility (induced-constitutive) of conifer monoterpenes to total constitutive monoterpene concentrations at six levels of biological scale. Data show fitted slope estimates (± SE) from linear models relating ln(inducibility) to ln(constitutive). Asterisks denote slopes significantly different from zero (* p-values < 0.05; ** p-value 0.01–0.001; *** p-values < 0.001). Note: the degree to which genotypic and phenotypic sources of variation necessarily vary at different levels of scale. Genetic sources are best partitioned in the common garden experiment with *P. contorta* and the multispecies comparison, while phenotypic sources are best partitioned among all individuals in common gardens and among individuals in forest populations.

model. There was high variation among individuals even after accounting for block effects (conditional $R^2 = 0.03$; adjusted R^2 from linear model with block as a fixed effect = 0.08).

Mature pine trees in forest populations increased total monoterpenes by 300–10 000% (median 4800%) following simulated bark beetle attack. The relationship of inducibility to constitutive monoterpenes was significantly positive (slope = 0.24; p-value < 0.001; Fig. 2). That is, trees with high constitutive monoterpene concentrations likewise underwent high induction. However, there was substantial variation, of which the best fit model explained only 5% (adjusted R^2). The relationship between inducibility and constitutive monoterpenes was positive among all five species but was only significant in *P. contorta* and *P. albicaulis* (p-values = 0.0001 and 0.02 respectively). This may be an artifact of sample size rather than between – species differences (Supplementary material Appendix 1 Table A1). Large differences in mean inducibility and mean constitutive concentrations at this level necessitated z-score standardization as the best fit mixed effects models on non-standardized data included random intercepts and slopes attributable to species, but no fixed effects.

Across populations of *P. contorta* (others lacked sufficient replication for this level; Supplementary material Appendix 1 Table A1), there was a 110% increase from constitutive to induced monoterpenes. The relationship between constitutive monoterpenes and inducibility was strongly positive (Fig. 2; slope = 0.80; p-value < 0.001) and 78% of the variability was explained by the linear regression (Adjusted R^2). Hence a population of *P. contorta* with high constitutive monoterpene concentrations is very likely to also undergo high induction. Populations did not appear to exhibit any strong geographically directional patterns in these relationships (Fig. 3).

Across various populations of all species studied (Supplementary material Appendix 1 Table A1), there was a 220% increase from constitutive to induced monoterpenes. There was a strong positive relationship between constitutive and inducibility of total monoterpene concentrations (Fig. 2; slope = 0.78; p-value < 0.001). The best fit model explained 60% of the variability (adjusted R^2). There were no apparent geographically directional patterns among populations (Fig. 3).

Across the nine North American and European species studied (Supplementary material Appendix 1 Table A1), induced responses yielded a mean 220% increase in total monoterpene concentrations. There was a strong positive relationship (Fig. 2; slope = 0.80; p-value = 0.006) between

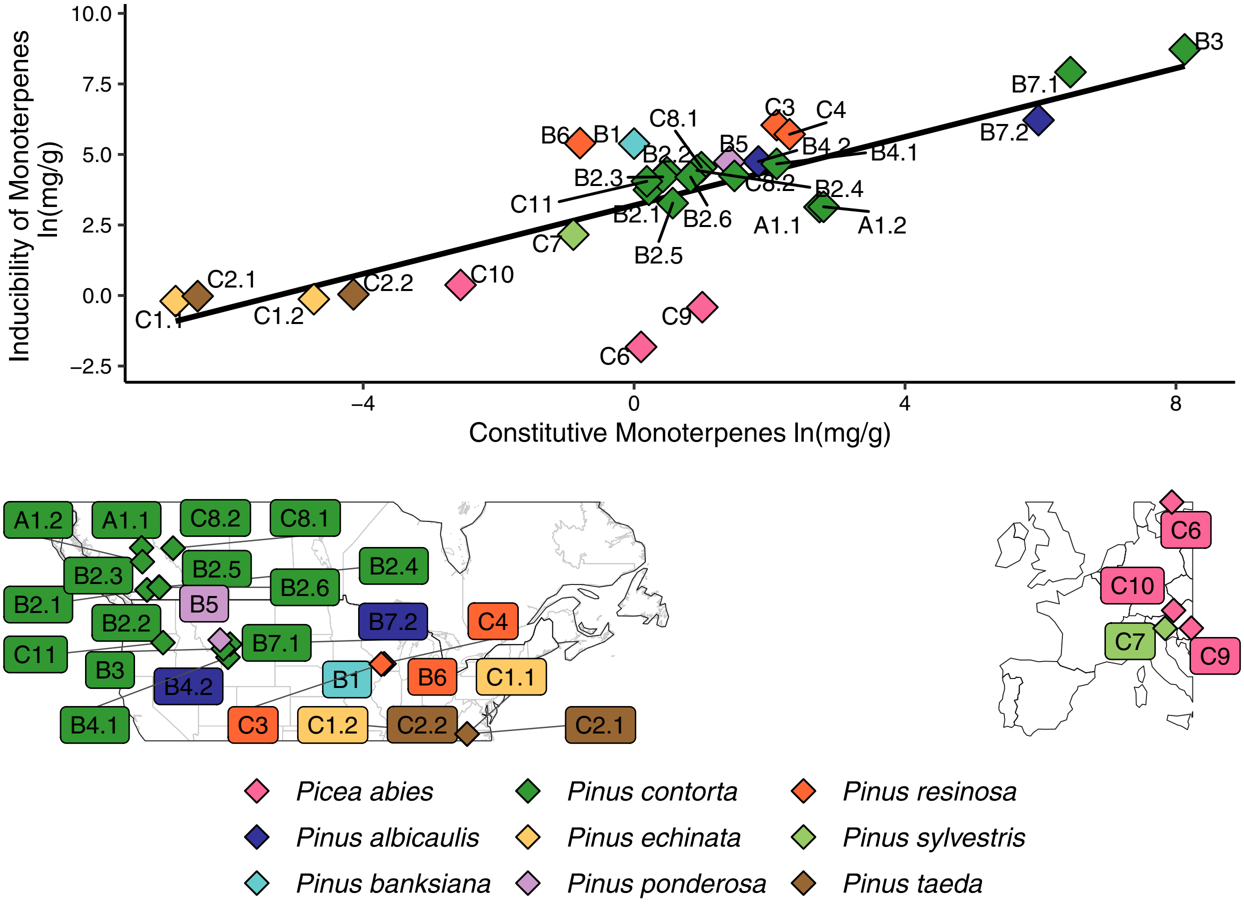

Figure 3. Relationship of inducibility to constitutive total monoterpene concentrations among populations of nine conifer species and the spatial extent of populations assayed. Population labels correspond to Supplementary material Appendix 1 Table A1. Black line represents best-fit linear regression fits.

constitutive monoterpene concentration and inducibility. Where multiple populations of single species were available, the range of inducibilities within one species commonly overlapped those of other species. The across species model explained 60% of the variability present (adjusted R^2).

Relationships between constitutive and induced absolute concentrations of total monoterpenes

Across half-siblings in the common gardens, there was no significant relationship between mean induced and constitutive monoterpenes (slope = 0.17; p-value = 0.1; Fig. 4). Overall the model explained little of the total variation present (adjusted R^2 = 0.02).

Among *P. contorta* individuals in the common gardens, there was a positive relationship between induced and constitutive total monoterpenes (slope = 0.25; p-value < 0.001; Fig. 4). There was a positive covariate of tree diameter (slope component = 0.13; p-value < 0.001), suggesting that larger trees had higher inducible defenses than smaller trees of the same age. The among tree mixed effects model explained little of the variability (conditional R^2 = 0.10) but adding spatial context to the model (adding site, block and their interaction as fixed effects) doubled the adjusted R^2 (0.20). Unlike inducibility, site (p-value < 0.001), block (p-value < 0.001), site × block interaction (p-value = 0.01) and constitutive × site × block interaction (p-value = 0.02) were all significant. This suggests that mean induced monoterpenes were significantly different between sites and among blocks, and that site and block significantly altered the relationship between induced and constitutive monoterpenes.

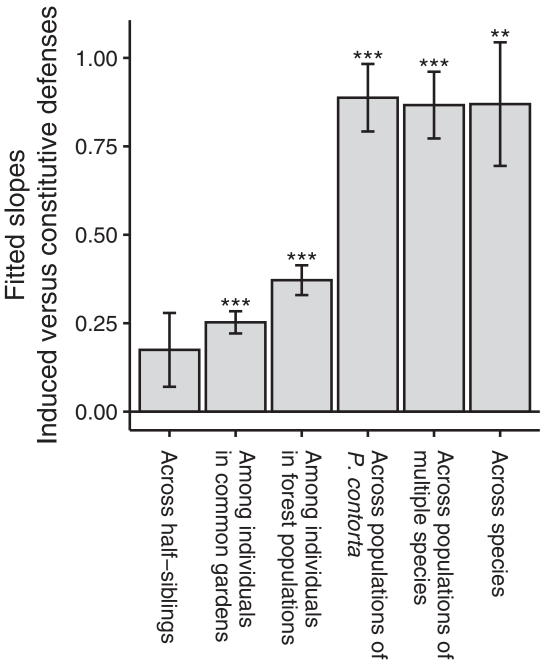

Figure 4. Relationship of induced to constitutive total monoterpene concentrations at six levels of biological scale. Data show fitted slope estimates (± SE) from linear models relating ln(induced) to ln(constitutive) for the relationship between ln(induced) and ln(constitutive). Asterisks denote slopes significantly different from zero (p-values < 0.001 ***). Note: the degree to which genotypic and phenotypic sources of variation necessarily vary at different levels of scale. Genetic sources are best partitioned in the common garden experiment with *P. contorta* and the multispecies comparison, while phenotypic sources are best partitioned among all individuals in common gardens and among individuals in forest populations.

Relationships between constitutive and induced monoterpene concentrations were positive among individual trees in forest populations (slope = 0.37; p-value < 0.001; Fig. 4). However, there was high variability (adjusted R^2 = 0.15). There was a positive relationship between constitutive and induced monoterpenes for all species, but this was only significant for *P. cortorta* (slope = 0.34; p-value < 0.001) and *P. albicaulis* (slope = 0.59; p-value < 0.001). As with inducibility versus constitutive analyses, the lack of significant trends for other species may reflect smaller sample sizes.

Across populations of *P. contorta* (Supplementary material Appendix 1 Table A1), relationships between induced versus constitutive concentrations were positive (slope = 0.88; p-value < 0.001; Fig. 4). That is those populations with high constitutive concentrations of monoterpenes likewise had high induced concentrations. The model explained 86% of the variability.

Across all populations of all species, the relationship of induced versus constitutive monoterpene concentrations was significantly positive (slope = 0.87; p-value < 0.001; Fig. 4). The model explained 74% of the variability.

Across species, the relationship of induced versus constitutive concentrations was significantly positive (slope = 0.87; p-value = 0.001). The model explained 73% of the variability.

Relationships between monoterpene concentrations and changes in composition

All tree species underwent some compositional changes during induction. There were multiple cases of compounds present in only trace amounts increasing to appreciable quantities (> 1 mg g^{-1}) in induced tissue, and multiple cases of individual compounds increasing or decreasing in their relative proportion of the total monoterpene fraction, but no cases of new monoterpenes appearing in induced tissues. In the common gardens experiment, the number of compounds that increased from trace levels to at least 1 mg g^{-1} was positively related to total concentrations of both constitutive and induced monoterpenes (p-value = 0.002), and these relationships varied by half-sibling family (p-value < 0.001). The increase in the number of such compounds was negatively related to total constitutive concentrations (p-value < 0.001), positively related to total induced (p-value < 0.001) concentrations, and positively related to inducibility (slope = 0.34; p-value < 0.001).

In forest trees, the number of compounds with concentrations of at least 1 mg g^{-1} was positively related to both constitutive and induced total concentrations (p-value < 0.001), and varied among species (ANOVA: p-value < 0.001).

The change in the number of compounds with concentrations of at least 1 $\mathrm{mg\,g^{-1}}$ was negatively related (p-value < 0.001) to total constitutive and induced monoterpenes, but not to inducibility (p-value > 0.05).

Induction altered phloem monoterpene diversity indices in all tree species (Table 1). These included both increases and decreases in Shannon–Wiener index values. Overall, there were no consistent relationships between monoterpene diversity and total constitutive monoterpenes, induced monoterpenes, or inducibility. Likewise, there were no consistent relationships between induced changes in monoterpene diversity and total constitutive monoterpenes, induced monoterpenes, or inducibility. The exception was *P. contorta* forest populations, where trees with the highest total monoterpenes had less diverse compositions (slope $= -0.02$, p-value $= 0.02$), and inducibility and changes in diversity were positively related.

Discussion

These results indicate that biological scale can influence both the incidence and direction of relationships between constitutive and induced plant defenses. Negative relationships, i.e. tradeoffs, that appear when genotype and location are controlled do not necessarily extend to phenotypes growing in natural communities across heterogeneous landscapes. For example, there were overall negative relationships between constitutive and inducible monoterpenes among half-siblings and individuals in common gardens. Yet trees within forest populations showed positive relationships between constitutive and inducible monoterpenes and there were likewise strong positive relationships across intraspecific populations, interspecific populations and across conifer species. The overlapping inducibilities among host species is particularly relevant for nonspecialist herbivores that can behaviorally select the least well-defended individuals among various sympatric species.

The role of scale has important implications to our understanding of plant defense systems, how herbivores interact with plant defenses in nature, and how these related processes do not necessarily operate in concert (Mithöfer and Boland 2012). From a plant physiology perspective, various life history strategies may generate internal tradeoffs between allocation to constitutive and induced defenses (Kempel et al. 2011, Moreira et al. 2014, Villari et al. 2014). Yet from the herbivore perspective, encountering a host plant in the field with weak constitutive defenses can be a reliable predictor that it also has weak induced defenses, at least in some systems. In conifers, straightforward relationships between constitutive and induced phloem monoterpenes were always positive. Positive correlations between constitutive and induced defenses may be particularly informative for herbivore species that actively search for stressed individuals distributed across heterogeneous landscapes.

Host spatial context strongly affected both mean constitutive and mean induced monoterpenes, but not necessarily their relationship. For example, in common gardens, the effect of family was less than that of microsite. There was likewise substantial variability in the mean constitutive and

Table 1. Compositional changes in monoterpenes in conifer phloem during induced response to simulated bark beetle attack, and their relationships to total concentrations at three levels of biological scale. (A) Changes in the richness†, relative proportions and diversity‡ of compounds during induction; (B) relationship of changes in monoterpene diversity to total concentrations during induction. Values represent means (± SEM). H denotes diversity index values for constitutive, induced; ΔH denotes change in diversity index; brackets denote concentrations; C represents constitutive diversity (H_c) or concentration ([C]); I represents induced; and I–C represents inducibility. Asterisks denote significant changes in diversity (1-sample T-test) and slopes significantly different from zero below 0.05 (*), 0.01 (**) and 0.001 (***); n.s. denotes non-significant slopes.

Phloem tissue	Common garden	Among trees in forest populations			
	Pinus contorta	*P. contorta*	*P. albicaulis*	*P. ponderosa*	*P. resinosa*
(A) Changes in phloem monoterpene composition during induction					
Monoterpene richness					
Constitutive	3.45 (0.05)	4.67 (0.15)	6.7 (0.34)	1.58 (0.39)	0 (0)
Induced	5.58 (0.06)	5.18 (0.17)	9.9 (0.26)	7.8 (0.31)	8.5 (0.35)
% of compounds with a higher proportion in constitutive or induced tissue					
Constitutive	3	5	2	9	1
Induced	2	7	4	5	0
Monoterpene diversity					
Constitutive	1.2 (0.01)	1.25 (0.02)	1.55 (0.02)	1.93 (0.03)	1.01 (0.03)
Induced	1.28 (0)***	1.19 (0.02)***	1.61 (0.02)***	1.53 (0.04)***	1.13 (0.03)
(B) Relationships between total monoterpene concentration and diversity of monoterpenes					
H_C versus [C]	n.s.	−0.02*	n.s.	n.s.	n.s.
H_I versus [I]	n.s.	−0.03**	n.s.	n.s.	n.s.
H_I versus [I–C]	n.s.	−0.02*	n.s.	n.s.	n.s.
ΔH versus [C]	n.s.	n.s.	n.s.	n.s.	n.s.
ΔH versus [I]	n.s.	n.s.	n.s.	n.s.	n.s.
ΔH versus [I–C]	n.s.	0.02*	n.s.	n.s.	n.s.

† Quantity > 1 $\mathrm{mg\,g^{-1}}$ phloem.

‡ Shannon–Wiener diversity index.

mean inducibility of monoterpenes among forest populations (Fig. 3). The variability observed among even closely situated tree populations (Fig. 3; Supplementary material Appendix 1 Table A1) suggest important fine-scale drivers, but the extent to which these are genetic or environmental is unknown. Site factors such as elevation (Rasmann et al. 2014), ectomycorrhizal fungi (Kanekar et al. 2018) and nutrient availability (Sampedro et al. 2011, Mason et al. 2019) are known to affect defense expression in conifers.

The positive relationships between constitutive and induced total monoterpenes in forest trees raises the question of where key tradeoffs may lie, as plants possess finite resources to support various defense modalities and other functions (Coley et al. 1985). Tradeoffs could conceivably occur between monoterpene quantity and quality, or between conifers' major metabolic defense pools. However, we did not observe evidence for the former, and relationships between terpenes and phenolics are either nonsignificant or positive in *P. contorta*, *P. albicaulis* and *P. resinosa* (Mason et al. 2017, Raffa et al. 2017). Tradeoffs could likewise occur between chemical and anatomical defenses, but there were no significant relationships between resin duct metrics and either terpenoids or phenolics in *P. contorta* and *P. albicaulis* (Mason et al. 2019). Tradeoffs can occur between defense traits and growth (Herms and Mattson 1992), but the evidence in this system is mixed and scale-dependent. In a common garden study, mortality was higher in slower-growing *P. ponderosa* prior to a *D. ponderosae* outbreak but higher in fast-growing trees during the outbreak (de la Mata et al. 2017). However, the authors cautioned that without tree defense data this does not unambiguously demonstrate a growth–defense tradeoff. Moreover, resin ducts and resin flow were positively related to *P. ponderosa* growth rate in their sites (Hood and Sala 2015). Resin ducts were likewise positively related to *P. ponderosa* growth rate in a separate study (Kane and Kolb 2010). In even-aged *P. contorta* stands, constitutive and induced monoterpenes, and resin flow, were all positively related to tree size, and chemical and anatomical defenses were likewise positively related to size in *P. albicaulis* (Mason et al. 2019). In nature, trees with slow or declining growth are typically the most likely to be killed by bark beetles (Raffa and Berryman 1982, Waring and Pitman 1985, Bleiker et al. 2005, Knapp et al. 2013, Williams et al. 2018). This is partly because higher-scale drivers such as drought impair both defenses against bark beetles and growth (Huang et al. 2019).

Rather than indicating an absence of tradeoffs, our results illustrate the value of distinguishing between plants' allocation strategies and the overall resources their environment provides, i.e. between budgeting and gross income. For example, monoterpenes in *Pinus* are directly related to availability of phloem carbohydrates, N and Ca (Goodsman et al. 2013, Cook et al. 2015, Raffa et al. 2017, Roth et al. 2018), and diterpenes are only inversely related to growth under conditions of low K (Sampedro et al. 2011). Thus, trees located in microsites that provide more light, nutrients and moisture can grow faster, produce more constitutive and induced defense chemicals and construct more anatomical defenses than trees in deficient sites. As in human economies, the rich can allot more to every function and still get richer. We propose that tradeoffs between chemical classes, defense modalities and other processes, while important intra-organismal processes, may not necessarily be manifested in or scale up to ecosystem-level patterns due to high spatial variability of key resources. A major need, therefore, is to better integrate plant allocation processes with environmental heterogeneity, and to better understand how their interactions drive herbivore abundance and plant performance across landscapes.

Changes in the relative proportions of monoterpenes during induction appear to complement rather than substitute for increased total quantities. All tree species showed some proportionate alterations and increases in the number of abundant defense compounds, but which compounds underwent which transformations and the overall changes in diversity varied with species. This array of permutations is consistent with the 'moving target' hypothesis by which complexity and variability of bioactive compounds in itself confers added defense (Zangerl and Berenbaum 1993, Karban 2019, López-Goldar et al. 2019). Variable relationships between concentration and composition can have important ecological ramifications. In the conifer-bark beetle example, monoterpene toxicity to the herbivore and its symbionts is largely determined by total concentration (Chiu et al. 2017, Reid et al. 2017), whereas behaviors such as attraction, repellency and pheromone signaling show strong compound-specific responses (Wood 1982, Zhang 2001, Burke and Carroll 2016).

The extent to which biological scale influences tradeoffs between constitutive and induced defenses in various systems likely varies with specific features of each plant–herbivore interaction. We propose a conceptual model (Fig. 5) to integrate this diversity into a more manageable framework, help reconcile the highly variable results in the literature, and suggest future lines of research. This model suggests that lower scale processes, such as biosynthesis costs and relative heritability of defense traits drive the extent to which genetically programmed constitutive – induced allocations are selected for and phenotypically expressed. Higher scale processes such as ecosystem heterogeneity and plant tolerance to common ranges of the herbivore's population density, affect the slope of these relationships. At the organismal level, tradeoffs are stronger where biosynthetic and maintenance costs of defense traits are high, and trait efficacy is strongly quantitative (Orrock et al. 2015). For example, monoterpenes are highly dose-dependent (Raffa et al. 2005) and costly to synthesize and store (Gershenzon 1994). Multifunctionality, such as protection from UV radiation and drought, (Neilson et al. 2013), or complementary activities of constitutive and induced defense traits (Raffa et al. 2017) reduce costs. Tradeoffs will likely be lower in systems where resource quality strongly affects defense compared to systems showing strong genetic control. For example, conifer monoterpene concentrations are strongly influenced by overall resource quality

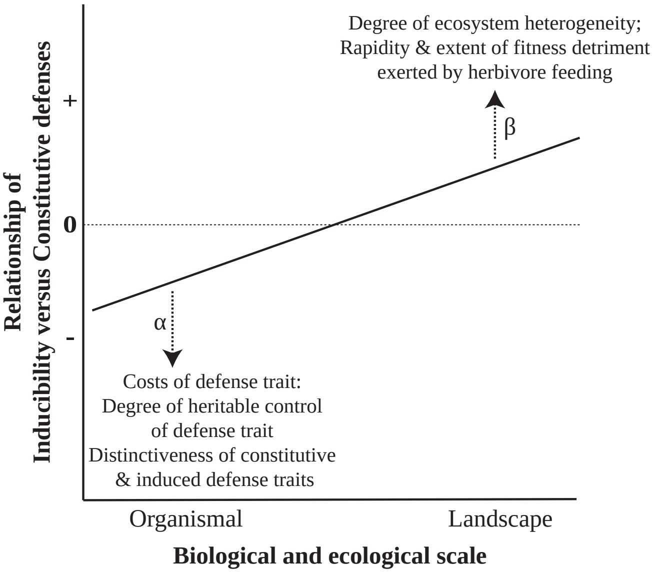

Figure 5. Conceptual framework for integrating biological scale into relationships between constitutive and induced plant defenses. Organismal-level processes (α) such as biochemical costs, distinctions between constitutive and induced defense traits, and relative heritability of defense traits, predominantly affect each plant–herbivore system's y-intercept. These can pull the y-intercept downward, generating greater tradeoffs. Ecosystem-scale processes (β) such as the relative heterogeneity of the landscape in which the plant and herbivore interact, and the tolerable time lag before herbivore feeding reduces host fitness, more strongly affect the slope. In the example indicated for conifer–bark beetle interactions, uneven availability of resources allow some plants to be superior in multiple traits, and the brief period after initial herbivore contact that determines host survival versus death favors complementarity between defense modalities. In other systems there may be no (slope = 0 along y = 0), or relatively consistent (slope = 0 along −y) tradeoffs. Note: the regression line does not rotate about any point (i.e. the entire line can rise or lower in addition to changing slope), nor need it be linear (as drawn here for simplicity).

(Raffa et al. 2005, Huang et al. 2019), whereas more consistent tradeoffs between constitutive and induced defenses were observed within (but not across) *Asclepias* species, whose cardenolide defenses show high genotypic variation (Rasmann and Agrawal 2011, Züst et al. 2015, Hastings and Agrawal 2019). The extent to which higher scales affect relationships may be further modified by the stochasticity versus directionality of resource availability (Rasmann and Agrawal 2011, Moreira et al. 2014, Hahn and Maron 2016), fitness costs exerted by herbivore populations (Orrock et al. 2015), and degree of historical exposure (Clark et al. 2010, 2014, Kempel et al. 2011, Burke et al. 2017, Raffa et al. 2017).

There are several limitations to our analyses. First, we do not have genotypic data at the level of 'trees in forest populations', so we cannot partition genetic, environmental or gene by environment effects on total monoterpenes or relationships between constitutive and induced concentrations. Second, different tree species occupy different geographic ranges, so our analyses could not control for environment while still assaying the mature trees these herbivores utilize in nature. Third, because we analyzed mature trees in situ we cannot account for different degrees of relatedness among trees of different populations and species, which can influence between-group comparisons (Agrawal and Weber 2015, López-Goldar et al. 2018). Conifer populations arise by a diversity of means such as wind dispersal, bird dispersal and human planting, yielding a range of variabilities. Finally, our between-species analysis was not designed to capture broad phylogenetic trends. Future studies that integrate advancing molecular methods for genetically profiling trees in situ (Six et al. 2018), quantitation of traits validated to contribute to defense (Celedon and Bohlmann 2019), and environmental measures proven to influence those traits (Trowbridge et al. 2019) across multiple scales can help address these limitations.

Implications of this work extend from our basic understanding of trophic interactions to resource management and plant protection. For example, a cross-scalar framework can help guide development and deployment of optimal combinations of defense traits via genetic and cultural means, to improve the resistance and resilience of valuable ecosystems. It can also improve the ability of population models to predict pest outbreaks by identifying when and where it is essential to assay both constitutive and induced defenses to gauge susceptibility. These results also indicate how underlying plant allocation strategies do not necessarily scale up to the chemical environment with which herbivore populations are actually confronted in nature, thereby refining our ability to incorporate ecological feedbacks into evolutionary theory.

Conclusions

- Half-siblings of lodgepole pine showed the most pronounced tradeoffs between constitutive concentrations and inducibility of monoterpenes among all scales.
- Among lodgepole pine individuals there was a tradeoff between constitutive concentrations and inducibility of monoterpenes. Microsite variability in the form of block effects superseded half-sibling effects.
- Overall, among individuals from forest populations there was a positive correlation between constitutive concentrations and inducibility of monoterpenes. Multiple species exhibited significant positive correlations. None showed negative relationships.
- Populations of mature lodgepole pine trees in forests showed positive correlations between constitutive concentrations and inducibility of monoterpenes.
- Conifer species showed positive correlations between their constitutive concentrations and inducibility of monoterpenes in forests. There were likewise positive correlations between constitutive concentrations and inducibility of in forest populations across all tree species.
- Relations between absolute constitutive and induced monoterpenes were always positive, regardless of biological level.

- Induced responses of all conifer species included both increases in total concentrations and changes in proportionate composition of monoterpenes. There were no tradeoffs between these components of induction.
- The most parsimonious mechanism for the above patterns, plus the positive relationships between monoterpene levels and trees size, is that environmental heterogeneity can supersede underlying physiological allocation processes such that trees on the most superior sites can excel in multiple defense traits.

Data availability statement

Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.nk98sfq7t> (Howe et al. 2020).

Acknowledgements – This work was supported by the University of Wisconsin – Madison College of Agricultural and Life Sciences, Graduate School and Vilas-Sorenson Professorship. We thank John Orrock, Dept. Integrative Biology and Richard Lindroth, Dept. Entomology, University of Wisconsin-Madison for critical presubmittal reviews.
Author contributions – All authors contributed to writing the manuscript, the project was conceived by KFR, CJM and MH, authors AY, CJM, KW, KFR and KKR each provided portions of the data, CJM and MH conducted data curation, and MH and JZ performed data analysis.

References

Acevedo, F. E. et al. 2015. Cues from chewing insects – the intersection of DAMPs, HAMPs, MAMPs and effectors. – Curr. Opin. Plant Biol. 26: 80–86.

Adams, A. S. et al. 2009. Effects of symbiotic bacteria and tree chemistry on the growth and reproduction of bark beetle fungal symbionts. – Can. J. For. Res. 39: 1133–1147.

Adams, A. S. et al. 2011. Responses of bark beetle-associated bacteria to host monoterpenes and their relationship to insect life histories. – J. Chem. Ecol. 37: 808–817.

Agrawal, A. A. and Weber, M. G. 2015. On the study of plant defence and herbivory using comparative approaches : how important are secondary plant compounds. – Ecol. Lett. 18: 985–991.

Agrawal, A. A. and Hastings, A. P. 2019. Tradeoffs constrain the evolution of an inducible defense within but not between plant species. – Ecology 100: e02857.

Anderegg, W. R. L. L. et al. 2015. Tree mortality from drought, insects and their interactions in a changing climate. – New Phytol. 208: 674–683.

Balogh, S. L. et al. 2018. Single-generation effects on terpenoid defenses in lodgepole pine populations following mountain pine beetle infestation. – PLoS One 13: 1–22.

Barton, K. E. and Koricheva, J. 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. – Am. Nat. 175: 481–493.

Bates, D. et al. 2015. Fitting linear mixed-effects models using lme4. – J. Stat. Softw. 45: 361–373.

Biedermann, P. H. W. et al. 2019. Bark beetle population dynamics in the Anthropocene: challenges and solutions. – Trends Ecol. Evol. 34: 914–924.

Bleiker, K. P. and Six, D. L. 2007. Dietary benefits of fungal associates to an eruptive herbivore: potential implications of multiple associates on host population dynamics. – Environ. Entomol. 36: 1384–1396.

Bleiker, K. P. et al. 2005. Resistance of fast – and slow-growing subalpine fir to pheromone – induced attack by western balsam bark beetle (Coleoptera: Scolytinae). – Agric. For. Entomol. 7: 237–244.

Blomquist, G. J. et al. 2010. Pheromone production in bark beetles. – Insect Biochem. Mol. Biol. 40: 699–712.

Boege, K. and Marquis, R. J. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. – Trends Ecol. Evol. 20: 441–448.

Bohlmann, J. 2012. Pine terpenoid defences in the mountain pine beetle epidemic and in other conifer pest interactions: specialized enemies are eating holes into a diverse, dynamic and durable defence system. – Tree Physiol. 32: 943–945.

Boone, C. K. et al. 2011. Efficacy of tree defense physiology varies with bark beetle population density: a basis for positive feedback in eruptive species. – Can. J. For. Res. 41: 1174–1188.

Boone, C. K. et al. 2013. Bacteria associated with a tree-killing insect reduce concentrations of plant defense compounds. – J. Chem. Ecol. 39: 1003–1006.

Brody, A. K. and Karban, R. 1992. Lack of a tradeoff between constitutive and induced defenses among varieties of cotton. – Oikos 65: 301–306.

Burke, J. L. and Carroll, A. L. 2016. The influence of variation in host tree monoterpene composition on secondary attraction by an invasive bark beetle: implications for range expansion and potential host shift by the mountain pine beetle. – For. Ecol. Manage. 359: 59–64.

Burke, J. L. et al. 2017. Consequences of distributional asymmetry in a warming environment: invasion of novel forests by the mountain pine beetle. – Ecosphere 8: e01778.

Celedon, J. M. and Bohlmann, J. 2019. Oleoresin defenses in conifers: chemical diversity, terpene synthases, limitations of oleoresin defense under climate change. – New Phytol. 224: 1444–1463.

Chiu, C. C. et al. 2017. Toxicity of pine monoterpenes to mountain pine beetle. – Sci. Rep. 7: 6–13.

Cipollini, D. and Heil, M. 2010. Costs and benefits of induced resistance to herbivores and pathogens in plants. – CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 5: 1–25.

Clark, E. L. et al. 2010. Differences in the constitutive terpene profile of lodgepole pine across a geographical range in British Columbia, and correlation with historical attack by mountain pine beetle. – Can. Entomol. 142: 557–573.

Clark, E. L. et al. 2014. Comparison of lodgepole and jack pine resin chemistry: implications for range expansion by the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae). – PeerJ 2: e240.

Cobb, F. et al. 1968. Inhibitory effcts of volatile oleoresin components on *Fomes annosus* and four *Ceratocystis* species. – Phytopathology 58: 1327–1335.

Coley, P. D. et al. 1985. Resource availability and plant antiherbivore defenses. – Science 230: 895–899.

Cook, S. P. et al. 2015. Changes in a primary resistance parameter of lodgepole pine to bark beetle attack one year following fertilization and thinning. – Forests 6: 280–292.

de la Mata, R. et al. 2017. Insect outbreak shifts the direction of selection from fast to slow growth rates in the long-lived conifer *Pinus ponderosa*. – Proc. Natl Acad. Sci. USA 114: 7391–7396.

DiGuistini, S. et al. 2011. Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont *Grosmannia clavigera*, a lodgepole pine pathogen. – Proc. Natl Acad. Sci. USA 108: 2504–2509.

Englund, G. and Cooper, S. D. 2003. Scale effects and extrapolation in ecological experiments. – Adv. Ecol. Res. 33: 161–213.

Erbilgin, N. and Colgan, L. J. 2012. Differential effects of plant ontogeny and damage type on phloem and foliage monoterpenes in jack pine (*Pinus banksiana*). – Tree Physiol. 32: 946–957.

Erbilgin, N. et al. 2003. Effect of varying monoterpene concentrations on the response of *Ips pini* (Coleoptera: Scolytidae) to its aggregation pheromone: implications for pest management and ecology of bark beetles. – Agric. For. Entomol. 5: 269–274.

Erbilgin, N. et al. 2006. Exogenous application of methyl jasmonate elicits defenses in Norway spruce (*Picea abies*) and reduces host colonization by the bark beetle *Ips typographus*. – Oecologia 148: 426–436.

Faccoli, M. and Schlyter, F. 2007. Conifer phenolic resistance markers are bark beetle antifeedant semiochemicals. – Agric. For. Entomol. 9: 237–245.

Franklin, A. J. and Grégoire, J. C. 2001. Dose-dependent response and preliminary observations on attraction range of *Ips typographus* to pheromones at low release rates. – J. Chem. Ecol. 27: 2425–2435.

Gershenzon, J. 1994. Metabolic costs of terpenoid accumulation in higher plants. – J. Chem. Ecol. 20: 1281–1328.

Goodsman, D. W. et al. 2013. Variation in carbon availability, defense chemistry and susceptibility to fungal invasion along the stems of mature trees. – New Phytol. 197: 586–594.

Hahn, P. G. and Maron, J. L. 2016. Review a framework for predicting intraspecific variation in plant defense. – Trends Ecol. Evol. 31: 646–656.

Hammerbacher, a. et al. 2013. A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce. – Plant Physiol. 162: 1324–1336.

Haukioja, E. and Niemela, P. 1979. Birch leaves as a resource for herbivores: seasonal occurrence of increased resistance in foliage after mechanical damage of adjacent leaves. – Oecologia 39: 151–159.

Herms, D. A. and Mattson, W. 1992. The dilemma of plants: to grow of defend. – Q. Rev. Biol. 67: 1689–1699.

Hood, S. and Sala, A. 2015. Ponderosa pine resin defenses and growth: metrics matter. – Tree Physiol. 35: 1223–1235.

Howe, M. et al. 2020. Data from: Relationships between conifer constitutive and inducible defenses against bark beetles change across levels of biological and ecological scale. – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.nk98sfq7t>.

Huang, J. et al. 2019. Tree defence and bark beetles in a drying world: carbon partitioning, functioning and modelling. – New Phytol. 225: 26–36.

Kane, J. M. and Kolb, T. E. 2010. Importance of resin ducts in reducing ponderosa pine mortality from bark beetle attack. – Oecologia 164: 601–609.

Kanekar, S. S. et al. 2018. Ectomycorrhizal fungal species differentially affect the induced defensive chemistry of lodgepole pine. – Oecologia 188: 395–404.

Karban, R. 2019. The ecology and evolution of induced responses to herbivory and how plants perceive risk. – Ecol. Entomol. 25: 339–347.

Karban, R. and Niiho, C. 1995. Induced resistance and susceptibility to herbivory: plant memory and altered plant development. – Ecology 76: 1220–1225.

Keefover-Ring, K. et al. 2016. Rapid induction of multiple terpenoid groups by ponderosa pine in response to bark beetle-associated fungi. – J. Chem. Ecol. 42: 1–12.

Keeling, C. I. and Bohlmann, J. 2006. Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. – New Phytol. 170: 657–675.

Kempel, A. et al. 2011. Tradeoffs associated with constitutive and induced plant resistance against herbivory. – Proc. Natl Acad. Sci. USA 108: 5685–5689.

Kessler, A. and Baldwin, I. T. 2008. Defensive function of herbivore-induced plant volatile emissions in nature. – Science 291: 2141–2144.

Klepzig, K. D. et al. 1996. Combined chemical defenses against an insect–fungal complex. – J. Chem. Ecol. 22: 1367–1388.

Knapp, P. A. et al. 2013. Mountain pine beetle selectivity in old-growth ponderosa pine forests, Montana, USA. – Ecol. Evol. 3: 1141–1148.

Kopper, B. J. et al. 2005. Effects of diterpene acids on components of a conifer bark beetle–fungal interaction: tolerance by *Ips pini* and sensitivity by its associate *Ophiostoma ips*. – Environ. Entomol. 34: 486–493.

Koricheva, J. et al. 2004. Meta-analysis of tradeoffs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? – Am. Nat. 163: E64–E75.

Krokene, P. and Solheim, H. 2007. Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. – Phytopathology 88: 39–44.

Kuznetsova, A. et al. 2017. lmerTest package: tests in linear mixed effects models. – J. Stat. Softw. 82: 1–26.

Levin, S. A. 1992. The problem of pattern and scale in ecology. – Ecology 73: 1943–1967.

Lewinsohn, E. et al. 1991. Defense mechanisms of conifers: differences in constitutive and wound-induced monoterpene biosynthesis among species. – Plant Physiol. 96: 44–49.

Lombardero, M. J. et al. 2000. Environmental effects on constitutive and inducible resin defences of *Pinus taeda*. – Ecol. Lett. 3: 329–339.

López-Goldar, X. et al. 2018. Inducibility of plant secondary metabolites in the stem predicts genetic variation in resistance against a key insect herbivore in maritime pine. – Front. Plant Sci. 9: 1–14.

López-Goldar, X. et al. 2019. Genetic variation in the constitutive defensive metabolome and its inducibility are geographically structured and largely determined by demographic processes in maritime pine. – J. Ecol. 107: 2464–2477.

Maclauchlan, L. E. et al. 2015. Impacts and susceptibility of young pine stands to the mountain pine beetle, *Dendroctonus ponderosae*, in British Columbia. – J. Ecosyst. Manage. 15: 1–18.

Mason, C. J. et al. 2015. Contrasting patterns of diterpene acid induction by red pine and white spruce to simulated bark beetle attack, and interspecific differences in sensitivity among fungal associates. – J. Chem. Ecol. 41: 524–532.

Mason, C. J. et al. 2017. Spatial and temporal components of induced plant responses in the context of herbivore life history and impact on host. – Funct. Ecol. 31: 2034–2050.

Mason, C. J. et al. 2019. Anatomical defences against bark beetles relate to degree of historical exposure between species and are allocated independently of chemical defences within trees. – Plant Cell Environ. 42: 633–646.

Mithöfer, A. and Boland, W. 2012. Plant defense against herbivores: chemical aspects. – Annu. Rev. Plant Biol. 63: 431–450.

Moreira, X. et al. 2014. Tradeoffs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. – Ecol. Lett. 17: 537–546.

Morris, W. F. et al. 2006. On testing for a tradeoff between constitutive and induced resistance. – Oikos 112: 102–110.

Neilson, E. H. et al. 2013. Plant chemical defense : at what cost ? – Trends Plant Sci. 18: 250–258.

Orrock, J. L. et al. 2015. Error management in plant allocation to herbivore defense. – Trends Ecol. Evol. 30: 441–445.

Ott, D. S. et al. 2011. Genetic variation of lodgepole pine, *Pinus contorta var. latifolia*, chemical and physical defenses that affect mountain pine beetle, *Dendroctonus ponderosae*, attack and tree mortality. – J. Chem. Ecol. 37:1002–1012.

Raffa, K. F. and Berryman, A. A. 1982. Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated microorganisms. – Environ. Entomol. 11: 486–492.

Raffa, K. F. and Berryman, A. A. 1983a. The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). – Ecol. Monogr. 53: 27–49.

Raffa, K. F. and Berryman, A. A. 1983b. Physiological aspects of lodgepole pine wound responses to a fungal symbiont of the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae). – Can. Entomol. 115: 723–734.

Raffa, K. F. and Smalley, E. B. 1995. Interaction of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle–fungal complexes. – Oecologia 102: 285–295.

Raffa, K. F. et al. 2005. Interactions among conifer terpenoids and bark beetles across multiple levels of scale: an attempt to understand links between population patterns and physiological processes. – Recent Adv. Phytocehm. 39: 80–118.

Raffa, K. F. et al. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. – Bioscience 58: 501.

Raffa, K. F. et al. 2017. Defence syndromes in lodgepole – whitebark pine ecosystems relate to degree of historical exposure to mountain pine beetles. – Plant. Cell Environ. 40: 1791–1806.

Rasmann, S. and Agrawal, A. A. 2011. Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. – Ecol. Lett. 14: 476–483.

Rasmann, S. et al. 2014. Differential allocation and deployment of direct and indirect defences by *Vicia sepium* along elevation gradients. – J. Ecol. 102: 930–938.

Reid, M. L. et al. 2017. Toxicity of monoterpene structure, diversity and concentration to mountain pine beetles, *Dendroctonus ponderosae*: beetle traits matter more. – J. Chem. Ecol. 43: 1–24.

Roth, M. et al. 2018. Successful colonization of lodgepole pine trees by mountain pine beetle increased monoterpene production and exhausted carbohydrate reserves. – J. Chem. Ecol. 44: 209–214.

Safranyik, L. and Carroll, A. L. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. – In: The mountain pine beetle: a synthesis of its biology, management and impacts on lodgepole pine. Natural Resources Canada, Candian Forest Service, pp. 3–66.

Sampedro, L. et al. 2011. Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. – J. Ecol. 99: 818–827.

Schiebe, C. et al. 2012. Inducibility of chemical defenses in Norway spruce bark is correlated with unsuccessful mass attacks by the spruce bark beetle. – Oecologia 170: 183–198.

Schilmiller, A. L. and Howe, G. A. 2005. Systemic signaling in the wound response. – Curr. Opin. Plant Biol. 8: 369–377.

Schneider, D. C. 2006. The rise of the concept of scale in ecology. – Bioscience 51: 545.

Seidl, R. et al. 2016. Small beetle, large-scale drivers: how regional and landscape factors affect outbreaks of the European spruce bark beetle. – J. Appl. Ecol. 53: 530–540.

Six, D. L. et al. 2018. Are survivors different? Genetic-based selection of trees by mountain pine beetle during a climate change-driven outbreak in a high-elevation pine forest. – Front. Plant Sci. 9: 1–11.

Steppuhn, A. and Baldwin, I. T. 2008. Induced defenses and the cost-benefit paradigm. – In: Induced plant resistance to herbivory. Springer, pp. 61–83.

Strauss, S. Y. and Agrawal, A. A. 1999. The ecology and evolution of plant tolerance to herbivory. – Trends Ecol. Evol. 14: 179–185.

Strauss, S. Y. et al. 2002. Direct and ecological costs of resistance to herbivory. – Trends Ecol. Evol. 17: 278–285.

Thaler, J. S. and Karban, R. 1997. A phylogenetic reconstruction of constitutive and induced resistance in *Gossypium*. – Am. Nat. 149: 1139–1146.

Traw, M. B. 2002. Is induction response negatively correlated with constitutive resistance in black mustard? – Evolution 56: 2196–2205.

Trowbridge, A. M. et al. 2019. Drought supersedes warming in determining volatile and tissue defenses of piñon pine (*Pinus edulis*). – Environ. Res. Lett. 14: 0655006.

Underwood, N. C. 1998. The timing of induced resistance and induced susceptibility in the soybean-Mexican bean beetle system. – Oecologia 114: 376–381.

Underwood, N. and Rausher, M. 2002. Comparing the consequences of induced and constitutive plant resistance for herbivore population dynamics. – Am. Nat. 160: 20–30.

Villari, C. et al. 2014. Testing phenotypic tradeoffs in the chemical defence strategy of Scots pine under growth-limiting field conditions. – Tree Physiol. 34: 919–930.

Wallin, K. F. and Raffa, K. F. 2000. Influences of host chemicals and internal physiology on the multiple steps of postlanding host acceptance behavior of *Ips pini* (Coleoptera: Scolytidae). – Environ. Entomol. 29: 442–453.

Wang, Y. et al. 2013. A specialized ABC efflux transporter GcABC-G1 confers monoterpene resistance to *Grosmannia clavigera*, a bark beetle-associated fungal pathogen of pine trees. – New Phytol. 197: 886–898.

Waring, R. H. and Pitman, G. 1985. Modifying lodgepole pine stands to change susceptibility to mountain pine beetle attack. – Ecology 66: 889–897.

Williams, H. et al. 2018. Subwatershed-level lodgepole pine attributes associated with a mountain pine beetle outbreak. – Forests 9: 552.

Wood, D. L. 1982. The role of pheromones, kairomones and allomones in the host selction and colonization behavior of bark beetles. – Annu. Rev. Entomol. 27: 411–446.

Zangerl, A. R. and Berenbaum, M. R. 1993. Plant chemistry, insect adaptations to plant chemistry and host plant utilization patterns. – Ecology 74: 47–54.

Zhang, Q. 2001. Olfactory recognition and behavioural avoidance of angiosperm non-host volatiles by conifer bark beetles. – Agric. For. Entomol. 6: 1–19.

Zhao, T. et al. 2011. Induced terpene accumulation in Norway spruce inhibits bark beetle colonization in a dose-dependent manner. – PLoS One 6(10): e26649.

Supplementary material (available online as Appendix oik-07242 at <www.oikosjournal.org/appendix/wlb-07242>). Appendix 1–2.

Zust, T. and Agrawal, A. A. 2017. Tradeoffs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis tradeoff: a fitness-imposed constraint on two competing functions, resulting in patterns of negative association between the functions. – Annu. Rev. Plant Biol. 68: 513–534.

Züst, T. et al. 2015. Growth – defense tradeoffs for two major anti-herbivore traits of the common milkweed *Asclepias syriaca*. – Oikos 124: 1404–1415.