Research

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Local carbon reserves are insufficient for phloem terpene induction during drought in *Pinus edulis* in response to bark beetle-associated fungi

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Summary

- Stomatal closure during drought inhibits carbon uptake and may reduce a tree's defensive capacity. Limited carbon availability during drought may increase a tree's mortality risk, particularly if drought constrains trees' capacity to rapidly produce defenses during biotic attack.
- We parameterized a new model of conifer defense using physiological data on carbon reserves and chemical defenses before and after a simulated bark beetle attack in mature *Pinus edulis* under experimental drought. Attack was simulated using inoculations with a consistent bluestain fungus (*Ophiostoma* sp.) of *Ips confusus*, the main bark beetle colonizing this tree, to induce a defensive response.
- Trees with more carbon reserves produced more defenses but measured phloem carbon reserves only accounted for *c.* 23% of the induced defensive response. Our model predicted universal mortality if local reserves alone supported defense production, suggesting substantial remobilization and transport of stored resin or carbon reserves to the inoculation site.
- Our results show that *de novo* terpene synthesis represents only a fraction of the total measured phloem terpenes in *P. edulis* following fungal inoculation. Without direct attribution of phloem terpene concentrations to available carbon, many studies may be overestimating the scale and importance of *de novo* terpene synthesis in a tree's induced defense response.

Introduction

Conifers produce a variety of secondary metabolites for defense against herbivory (Bartwal *et al.*, 2013; Celedon & Bohlmann, 2019; Kopaczyk *et al.*, 2020). Secondary metabolites are a broad class of carbon-based molecules and, in conifers, are typically dominated by mono-, sesqui-, and diterpenes (Celedon & Bohlmann, 2019). Terpenes can be critical to successful defense against bark beetles (Smith, 1961; Manning & Reid, 2013; Erbilgin *et al.*, 2017; Roth *et al.*, 2018). When bark beetles infest tree phloem, conifers may increase the concentration and alter the phytochemical diversity of terpenes in the phloem and the volume of resin that delivers these terpenes in a process known as induction (Smith, 1961; Raffa & Smalley, 1995; Manning & Reid, 2013; Choi & Klessig, 2016; Chiu *et al.*, 2017; Kolb *et al.*, 2019; Mageroy *et al.*, 2020).

Carbon reserves provide a key substrate for the synthesis of terpenes in conifers (Wiley *et al.*, 2016; Roth *et al.*, 2018). Terpenes carry high structural and energetic costs (monoterpenes contain 10 reduced carbon atoms; Gershenzon, 1994; Celedon & Bohlmann, 2019). Trees must balance this carbon investment against requirements of respiration, osmoregulation and growth (Hartmann & Trumbore, 2016; Sapes *et al.*, 2021; Blumstein *et al.*, 2022). The supply of carbon reserves available for defense is further constrained when drought limits photosynthesis, a phenomenon known to occur in many pine species (Sala & Mencuccini, 2014; McDowell *et al.*, 2022; Peltier *et al.*, 2023; Thompson *et al.*, 2023). Because some beetle species increase attacks during drought, a critical question is whether carbon reserves are enough to fuel an induced response in trees (Raffa *et al.*, 2008; Boone *et al.*, 2011; Hart *et al.*, 2014; Marini *et al.*, 2017; McDowell *et al.*, 2019; Huang *et al.*, 2020a,b; Howe *et al.*, 2022a).

Beyond the costs of phloem terpene induction, we do not know what concentration of terpenes is required for trees to survive an attack by bark beetles. Dose–response experiments can elucidate lethal concentrations of individual chemical species required to kill particular beetle species. However, these experiments only consider a small suite of chemicals, often individually and not in biologically relevant combinations, and are conducted under highly controlled, artificial laboratory conditions (Raffa & Smalley, 1995; Zhao *et al*., 2011). An alternative approach to understanding what terpene concentrations are required to repel and defend against attacking beetles (assuming greater concentrations of terpenes imbue greater defensive capacity) is the use of mathematical equations to model conifer-bark beetle systems (Ludwig *et al*., 1978; Berryman *et al*., 1989). Past models have primarily focused on beetle and forest population dynamics (Ludwig *et al*., 1978; Berryman *et al*., 1989; Křivan *et al*., 2016; Seidl *et al*., 2016), although more recent models have incorporated the role of host resistance (Nelson & Lewis, 2008). Few studies have explicitly incorporated conifer-induced terpene concentrations into such models (Berryman *et al*., 1989; Nelson & Lewis, 2008; Huang *et al*., 2020a,b) and none have included carbon reserves as a proximal driver. Models that account for the dynamics of carbon allocation to defense with bark beetle dynamics might allow us to identify what concentration of carbon reserves and terpenes are required for conifers to survive bark beetle attacks. Such a model can be used to investigate underlying mechanisms of tree–beetle interaction and to generate new hypotheses about the role of terpene concentrations in tree resistance against beetles (Goodsman *et al*., 2017, 2018) and different attack densities. Incorporating attack density as a model parameter might further elucidate how variation in beetle density in space and time affect conifer–bark beetle interactions.

Here, we develop a theoretical model of bark beetle–conifer interactions and use a unique rainfall-exclusion experiment with mature *Pinus edulis* (Engelmann) to parameterize and test this model. Using trees that had already experienced 2 or 10 yr of experimental throughfall exclusion (drought), we conducted an inoculation experiment to simulate beetle attack-related demands on defense and carbon reserves. Just before, and 2 wk after inoculation, we measured carbon reserves (nonstructural carbohydrates; NSCs) and phloem monoterpene concentrations. Because *c*. 50% of droughted trees ultimately died from attack by bark beetles, we incorporated these data into a dynamical model to investigate the production and effectiveness of induced defense against lethal beetle attack. We investigated three questions: First, what is the minimum concentration of phloem terpenes a tree must produce to survive attack by bark beetles? Second, are carbon reserve concentrations predictive of defensive responses? And third, what is the relative role of transported vs local carbon reserves towards induced defensive responses? Compared to hydraulic failure or carbon starvation, the role of dynamics in tree defensive chemicals in mortality, and their interactions with insect pests, remains poorly understood (McDowell *et al*., 2022). Addressing these questions could improve our understanding of the linkages between carbon, water, and defense status that ultimately interact with insect pests to determine tree mortality outcomes.

Materials and Methods

Site description and experimental design

Our experiment took place in a piñon–juniper woodland *c*. 100 km south of Albuquerque, NM, USA in the Sevilleta National Wildlife Refuge and Long-Term Ecological Research site (34.386, −106.528°) and is described in full by Peltier *et al*. (2023). Briefly, we studied the response of an extreme drought, relative to average growing conditions at this site, on the physiology of the semiarid conifer, *Pinus edulis*. We established three new rainfall-exclusion plots in the winter of 2019, in addition to a control, and leveraged a preexisting long-term drought plot installed in 2010. The three new rainfall-exclusion plots simulate drought across a gradient of intensity: 45%, 75%, and 90% of total precipitation was removed year-round from each plot. A 45% long-term drought plot (herein 'Legacy') established in 2010 is identical in form to the three new plots and has been in place since installation (Pangle *et al*., 2012). Between 2010 and 2015, an outbreak of the bark beetle *Ips confusus* killed many of the mature *P. edulis* trees growing in this region. To prevent mortality in the Legacy plot, trees were sprayed with an insecticide (DragNet SFR, permethrin-based) from 2010 to 2015.

Construction of all plots followed a previously used design outlined by Pangle *et al*. (2012). Briefly, each 40 × 40 m plot was covered by large UV-resistant 0.3 cm thick polycarbonate sheets that were used to create troughs at differing coverage intensities corresponding to the precipitation removal target. Troughs were installed below the crown of mature *P. edulis* trees at spacing intervals sufficient to capture and divert 45%, 75%, or 90% of total precipitation. Within each plot, six target trees located at least 5 m from the plot edge were identified and monitored for the duration of the experiment.

In June 2022 we conducted a fungal inoculation experiment whereby all focal trees were inoculated with an ophiostomatoid-fungus (*Ophiostoma* sp.) collected from the local bark beetle (*Ips confusus*) population in 2021. This fungus is a consistent associate of this beetle (*c*. 97% prevalence) and is a newly described (Six *et al*., unpublished). Defense induction in *Pinus* typically occurs in response to beetle-associated fungi (Keefover-Ring *et al*., 2016; Denham *et al*., 2019; Kolb *et al*., 2019). Immediately before inoculation we measured photosynthesis, phloem terpene concentrations, resin flow, and comprehensively quantified carbon reserves (nonstructural carbohydrates; sugars and starch) in all tissues (needle, twig, xylem, phloem, and coarse roots). After a 2-wk induction period (described in full below) these measurements were repeated.

Fungal inoculation procedure

On 8 June 2022 we inoculated 26 trees with the bluestain fungus to simulate attack by *I. confusus*. Previous work in other pine species (Raffa & Smalley, 1995) has shown that inoculation with some beetle-associated ophiostomatoid-fungi results in rapid induction of terpene defenses. Before inoculation the fungus was purified via single spore isolations from *I. confusus* collected near our experimental site. Cultures for inoculation were grown on 2% malt agar in Petri dishes and fungal plugs were taken from the growing edges of the resulting colonies. On each tree, a majority of the woody bark cortex was removed from a *c.* 3 × 3 cm area of the bole with a chisel, leaving the phloem intact. A 1/4-inch (0.64 cm) plug of phloem was removed and replaced with a plug of the fungus in agar, after which the phloem plug was replaced on top of the fungus. To separate the effect of mechanical damage from the phloem punch and the introduced fungus (Mercado *et al.*, 2023), we separately removed a 1/2-inch phloem punch from 8 off-plot trees to quantify the effect of mechanical damage on the induced response of *P. edulis*. Simultaneously, we inoculated the same 8 trees but on different faces of the main stem with the same fungus as used in this study. The effect of mechanical damage alone on the induced response of *P. edulis* was relatively small when compared to the effect of the fungus (Supporting Information Fig. S1). Therefore, we proceeded with our experiment in the absence of a mechanical control.

Physiological measurements

We measured predawn water potential (Ψ_{pd}) using a Scholander-type pressure chamber (PMS instruments, Albany, OR, USA). Ψ_{pd} samples were collected at or 4 h before dawn, stored in plastic bags with a moist paper towel, and kept inside coolers in the shade to preserve humidity and prevent water loss before measurement (Rodriguez-Dominguez *et al.*, 2022). Net photosynthesis (A_{net}) was measured on two branches each day between 08:00 h and 11:00 h, using a Li-Cor 6800 (Lincoln, NE, USA). Branches were in partial shade, with chamber conditions as follows: PAR was set to 2000 μmol m^{-2} s^{-1}, CO_2 at 400 ppm, temperature at 25°C and humidity at 30%. All A_{net} measurements were corrected per unit projected leaf area, which was measured on scanned images of needles using the LeafArea package (Katabuchi, 2015) in R (R Core Team, 2022).

Needle, twig, phloem, xylem, and root nonstructural carbohydrate (NSC) samples were all collected on the same day. We separated needles from twigs on site, plucking each needle distally from the fascicle. This ensured that no twig tissue was also measured with needle samples. Preinoculation phloem samples were collected on the same day with a 13 mm diameter punch immediately before inoculation was conducted on each tree, all on the same day. To collect postinoculation phloem samples, we excised the necrotic tissue surrounding the inoculation site 2 wk after inoculation and split the tissue between NSC and defense measurements. To sample bole and root xylem NSC, we used a 5.15 mm diameter increment bore (Haglof, Sweden) to *c.* 30 mm depth. All NSC samples were microwaved on high for 90s within 4 h of collection and oven dried for 72 h at 65°C. In the lab, NSC was analyzed according to Landhäusser *et al.* (2018) and is fully described in Peltier *et al.* (2023).

To measure constitutive and induced phloem terpene concentrations, we collected phloem tissue samples before and 2 wk after inoculation. To minimize the effect of mechanical damage on the scale of the induced response, preinoculation phloem samples were collected from regions perpendicular and at approximately equal height to the site of fungal inoculation. To sample phloem, we first removed most of the bark cortex. We then used a 12.7 mm diameter leather punch to remove the phloem, being careful to leave the xylem undamaged, and immediately placed the samples in liquid nitrogen. After 20 wk, on 22 June 2022, the phloem tissue around the inoculation site (*c.* 4 × 8 cm) was collected for analysis of terpenes. Terpene samples were placed immediately into liquid nitrogen for transport and stored at −80°C until they could be analyzed in the lab. We then installed a plastic resin collector directly on to the phloem sampling site and collected resin after 24 h, 48 h, 72 h, 1 wk, and 2 wk, using a 14 ml falcon tube, which was then later weighed to quantify the resin mass. The mass of all falcon tubes was determined before resin flow measurements and subtracted from the final mass. Phloem terpene samples were analyzed in the lab following the protocol described by Trowbridge *et al.* (2021).

Estimating the glucose cost of phloem terpenes We estimated the maximum potential phloem terpene concentrations from local carbon reserves, using phloem carbon reserves and the total glucose cost per gram of terpene produced. We used a stoichiometric approach to determine the glucose cost of synthesis for each terpene product from its biochemical pathway, following work by De Vries *et al.* (1974) and Gershenzon (Gershenzon, 1994; Notes S1; Fig. S2). For each tree, we estimated the maximum potential defenses that could be synthesized from preinoculation phloem NSC concentrations. This approach accounts for all costs relating to carbon material, ATP, and reducing power. Our results indicate that 1 g of monoterpenes will cost a plant 3.34 g glucose (see Notes S2 for the full derivation).

To quantify the role of transported carbon reserves in phloem terpene concentrations we used allometric scaling to estimate whole-tree sugar and starch. We estimated the organ-level biomass from root-collar diameter from Grier *et al.* (1992) and used tissue-specific NSC concentrations to estimate whole-tree carbon reserves in absolute grams. Using the glucose cost of terpenes, we then calculated the maximum potential phloem terpenes that could be synthesized from this new, larger value.

Model description

Overview The process of a bark beetle attack occurs in several stages where different tree physiological processes may play a role. The first step in a bark beetle attack can be considered the pioneer phase, where a single or few beetles test a host's defensive capabilities (Blomquist *et al.*, 2010). Upon attack, pioneer beetles break through the bark cortex and are exposed to potentially toxic oleoresin that flows from specialized ducts located in the sapwood and phloem (Hood & Sala, 2015). A tree that can produce a large resin response may successfully kill pioneer beetles and avoid mass attack. Should pioneer beetles succeed in entering the tree, pheromones are released that signal to nearby beetles the presence of a viable host (Blomquist *et al.*, 2010). Once beetles mass attack, tree survival depends on both the sustained flux of resin to the site of attack and the *de novo* synthesis of phloem terpenes via induction

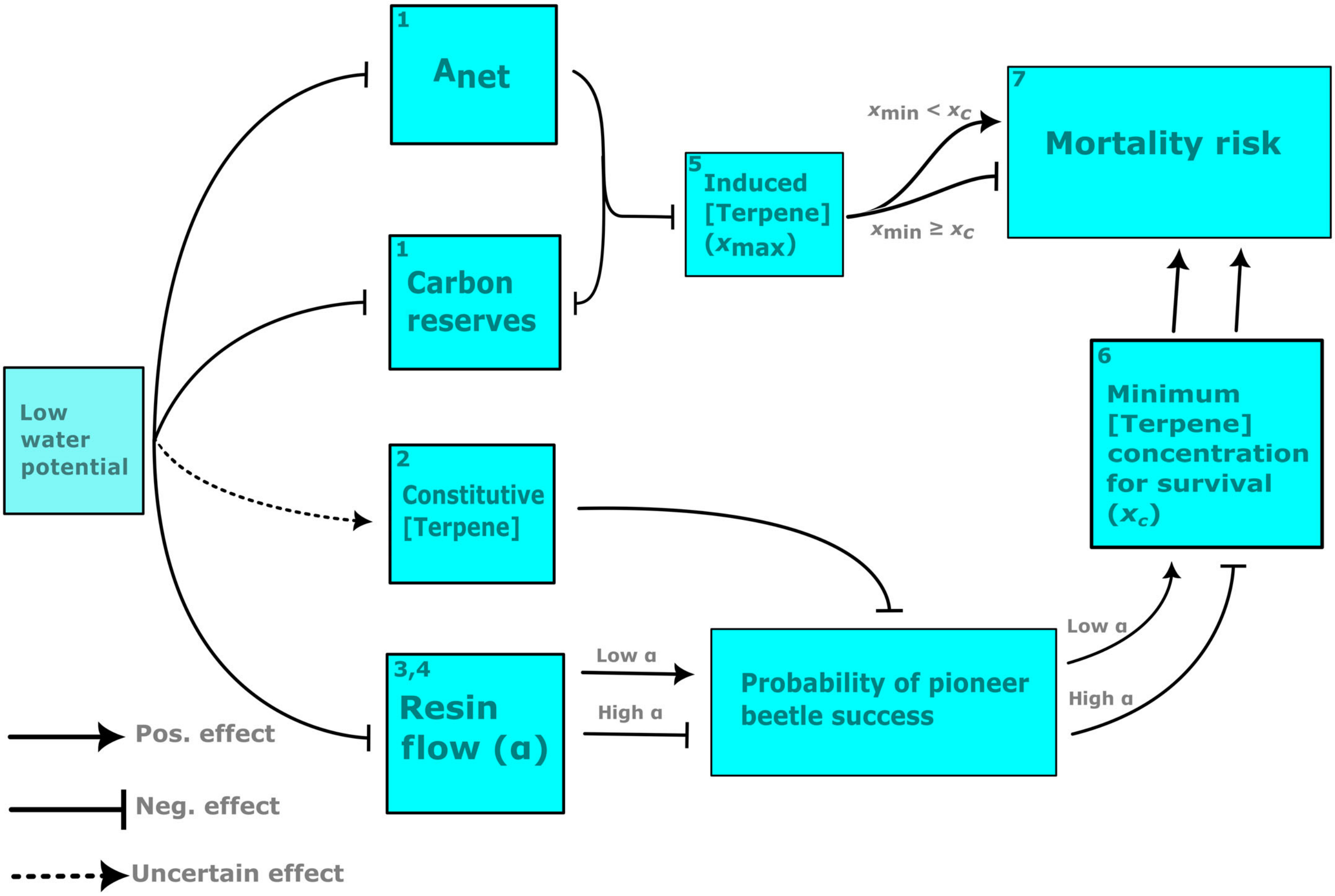

Fig. 1 How drought can predispose trees to bark beetle-induced mortality. Low Ψ_{pd} can inhibit photosynthesis, carbon reserves, and resin flow but may increase a tree's constitutive terpene concentrations. While greater constitutive terpene concentrations may reduce the probability of pioneer beetle success (Assumption 2), low-resin flow may promote their success by providing a reduced barrier to entry, thus increasing the likelihood of a mass attack and increasing a tree's mortality risk. Once a tree has failed to overcome the pioneer beetle attacks, its mortality risk is modulated by available carbon reserves which may be mobilized and used for the induction of new secondary metabolites. If carbon reserves are too low, given resin flow rates, trees will fail to meet their minimum defensive needs and thus are more likely to die from a mass attack of bark beetles. α is relative response rate, x_{max} is the maximum potential phloem terpene concentrations from carbon reserves, and x_c represents the minimum phloem terpene concentrations a tree needs to survive an attack by bark beetles. References: (1) Thompson *et al*., 2023; (2) Bryant *et al*. (1983), Herms & Mattson (1992) and Trowbridge *et al*. (2021); (3) Netherer *et al*. (2015); (4) Cabrita (2018); (5) Fig. 3; (6) Fig. 4a; (7) Fig. 7.

(though the general toxicity of these terpenes is not known for this particular bark beetle species; Smith, 1961; Manning & Reid, 2013; Chiu *et al.*, 2017; Mageroy *et al.*, 2020; Vázquez-González *et al.*, 2020). Drought can directly impact how trees respond during both the pioneer and mass attack phases of a bark beetle attack (Fig. 1).

To study whether the induced phloem terpene concentrations trees produced from carbon reserves are enough for trees to survive a mass attack by bark beetles, we model the bark beetle–conifer interaction as a bistable dynamical system. Although direct evidence that terpenes are functional defensive compounds against *I. confusus*, for which *P. edulis* is an obligate host, is not known, we use evidence from other bark beetle-conifer systems that suggests they may be effective at high concentrations (Smith, 1961; Manning & Reid, 2013; Chiu *et al.*, 2017; Mageroy *et al.*, 2020). To model how phloem terpene concentrations correspond to a tree's vulnerability to attack by bark beetles (Boone *et al.*, 2011), we use a set of ordinary differential equations:

$$\frac{dD}{dx} = \frac{A_t}{1 + t e^{\alpha x}} \qquad \text{Eqn 1}$$

$$\frac{dA}{dx} = A_b\left(1 - \frac{x}{x_c}\right) \qquad \text{Eqn 2}$$

where Eqn 1 models the change in defense concentrations as an inverse-sigmoid function, with A_t as the amount of beetles attacking the tree expressed as a percentage of the lethal beetle attack density, A_b is the size of the beetle population expressed as a percentage of the lethal beetle attack density, t is the minimum proportion of the lethal attack density necessary to induce a response, α is the induction rate relative to nearby trees (see Assumption 4, below), and x represents

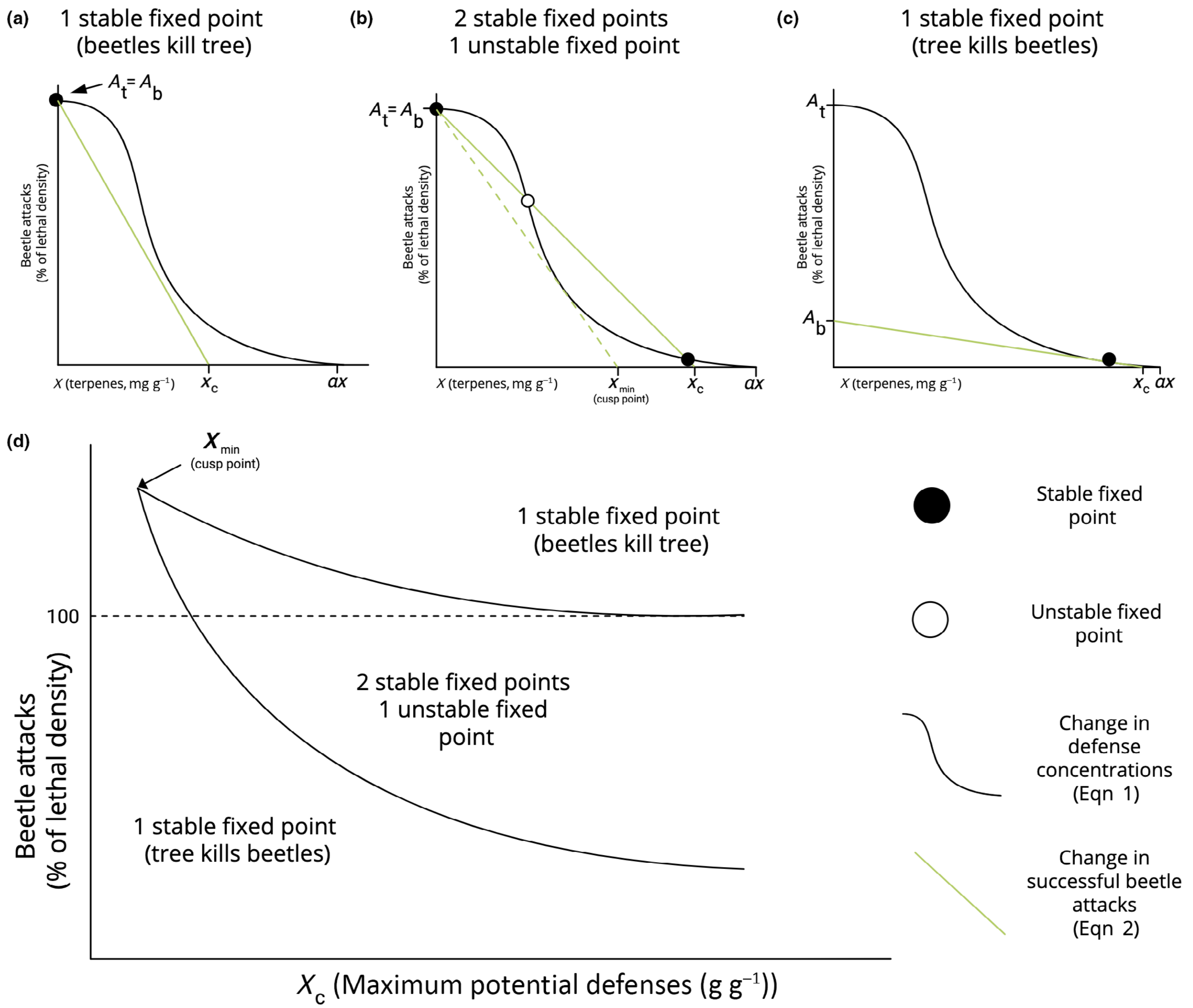

Fig. 2 Conceptual diagram of bark beetle–conifer interactions modelled as a dynamical system. Eqns 1 and 2 generate a dynamical system with three fixed points. Panels (a–c) plot Eqns 1 and 2 as functions of A_t (the lethal threshold of beetles required to kill the tree, expressed as a %), A_b the size of beetle population (expressed as a % of A_t), and carbon reserves in mg g^{-1} (x). The black curve models the change in tree defense concentrations as a function of available carbon reserves (x; Eqn 1). The green line models the change in successful beetle attacks as a function of available carbon reserves (Eqn 2). Importantly, the y-intercept of Eqn 2 is determined by A_b and the x-intercept is determined by x_c, or the maximum defense potential from carbon reserves. Holding A_b constant at A_t (so at least enough beetles are present to potentially kill the tree) and allowing x_c to vary causes the appearance and/or disappearance of the fixed points. Starting with low x_c, shown in panel (a), only a single fixed point occurs where the beetles kill the tree. As x_c increases, three fixed points occur (panel b): two stable fixed points where either the beetles kill the tree or the tree kills the beetles, and an unstable fixed point where both coexist, an impossibility thus highlighting the instability of this fixed point. As x_c decreases, the stable fixed point where the tree kills the beetles converges to the unstable fixed point of coexistence. At exactly $x_c = x_{min}$, the two fixed points merge and a cusp point appears indicating a defensive threshold below which tree survival is not possible. (d) maps all of the fixed points and their solutions as a function of x_c. Note the cusp point, x_{min}, where both curves meet.

current defense concentrations. Here, we only consider the scenario where $A_t = A_b$ such that the scenario where beetles kill the tree still exists (see Fig. 2). Eqn 2 represents the change in beetle attack density A_b as a function of current defense concentrations x relative to the maximum potential defense concentrations x_c. Our model formulation is based on the following assumptions:

(**1**) Beetle attack density decreases linearly with increasing defense concentrations (Berryman *et al.*, 1989).
(**2**) Higher concentrations of phloem terpenes result in greater tree defensive capacity (Boone *et al.*, 2011).
(**3**) Mass attack is not required to induce the synthesis of new phloem terpenes (corresponding to a very small t value in (1)).

(**4**) Tree vulnerability is on a relative scale such that trees which are relatively slow to respond to an attack tend to be attacked first (Boone *et al*., 2011).
(**5**) Local carbon reserves are available for use in local defense synthesis (Wiley *et al*., 2016; Raffa *et al*., 2017).

From these assumptions, we analyze Eqns 1 and 2 using a linear stability analysis (Strogatz, 2018), random walk (Grinstead & Snell, 1997), and model-data comparison. These methods are designed to identify stable and unstable points of the dynamical system relevant to mortality or attack success outcomes.

Model parameterization To parameterize (1) and (2), we used induction data from the experiment described above. We fix $A_t = A_b = 1$, corresponding to 100% of the lethal attack density. This allows us to compare the dynamics of trees with unknown but potentially different lethal attack densities. We set the minimum response threshold, t, to 0.001, reflecting the low number of beetles necessary to induce a response from the host tree (Assumption 3). To establish the relative response rate parameter, α, we used resin flow data measured over a 2-wk span. We then identified the date of maximum resin flow, $r_{\max,i}$ for tree i and ordered trees from fastest to slowest, based on $r_{\max,i}$. For example, trees that reached their maximum resin flow 24 h from inoculation had $\alpha = 1$. Subsequently, all other trees were set relative to this date in hours, such that if $r_{\max,i} = 72$ h, this would correspond to $\alpha \cong 0.33$. In our study, seven trees did not produce any resin. Because α is expressed as a ratio of maximum resin flow to current resin flow ($\alpha := \frac{r_{\max,i}}{r_{\text{current},i}}$) it is undefined when resin flow is zero and thus (1) cannot be solved. We thus exclude these seven trees from further analysis. A full table of parameter definitions is included in Table 1 and alpha values for each tree is included in Table 2.

Linear stability analysis To identify the minimum concentration of phloem terpenes required for tree survival from bark beetle attack to still be possible, we use a linear stability analysis to evaluate the qualitative dynamics of (1) and (2). We are interested in several features of this system of equations but, most importantly, the fixed points. The fixed points represent steady-state solutions where the rate of change is equal to zero (Strogatz, 2018). To find these states we set (1) equal to (2) for a given set of parameters (indicating the rate of change of defense synthesis equals the rate of attack by beetles), yielding:

$$A\left(1-\frac{x}{x_c}\right) = \frac{A}{1+te^{\alpha x}} \qquad \text{Eqn 3}$$

Solutions that satisfy this equality can be summarized using a bifurcation diagram. At most, this system of equations can yield three fixed points (Fig. 2b): a stable fixed point where beetles kill the tree and defenses are low (upper left point on Fig. 2b), another stable fixed point where the tree kills the beetles and defenses are higher (lower right point on Fig. 2b), and an unstable fixed point where the beetles and tree coexist (Christiansen *et al*., 1987; Berryman *et al*., 1989). Uniqueness and existence of these fixed points is derived in Notes S2.

Table 1 Parameter definitions.

Parameter	Units	Definition
A	%	Proportion of lethal beetle attack density
t	%	Minimum proportion of lethal beetle attack density necessary to trigger a tree's induced response system
α	%	Relative response rate of tree's induced response system
x	mg g^{-1}	Total amount of a tree's induced defenses
$x_{\max}$	mg g^{-1}	Maximum potential amount of a tree's induced defenses calculated from total nonstructural carbohydrates
x_c	mg g^{-1}	Minimum amount of defenses a tree needs to produce to survive
σ	Unitless	Variance term for the random walk

Table 2 Relative response rate (α), number of mature *Pinus edulis* trees with the corresponding response rate (N), cusp point (x_c), number of observed dead trees in each category, and number of predicted dead trees.

α	N	x_c	Observed	Predicted
0	11	NA	NA	NA
0.071	5	141.919	2	4
0.143	4	71	1	2
1	10	10	1	0

Our focus is on identifying the minimum concentration of phloem terpenes required for tree survival from bark beetle attack to still be possible. In the context of (4), we define a vulnerable tree as any individual whose maximum defense potential, x_c, is below some critical threshold, $x_{\min}$. To find $x_{\min}$, consider Eqn 2, which models the beetle dynamics and is the only equation containing the parameter x_c. If x_c increases (corresponding to greater carbon reserves), the unstable fixed point (open circle on Fig. 2a–c) approaches the stable fixed point where beetles kill the tree and eventually both disappear, leaving only the stable fixed point of tree survival (Fig. 2c). Thus, having a greater maximum defense potential from carbon reserves for a given response rate implies trees can better defend themselves against attacking beetles. As x_c decreases, the opposite occurs, and the unstable fixed point now approaches the stable fixed point where the tree kills the beetles (Fig. 2a). x_c occurs when these two points merge at the cusp point (Fig. 2b dashed green line & Fig. 2d). For each tree, we analytically derive x_c (Notes S2) and compare whether observed and maximum potential defenses are above or below this threshold.

Estimating mortality risk Eqns 1–3 are deterministic representations of bark beetle–conifer dynamics that are subject to some level of stochasticity (Sharma *et al*., 2015; Yuan *et al*., 2022). In other words, just because a tree is weak and vulnerable does not mean that it will always be attacked by beetles nor does it mean that conditions cannot change allowing trees to recover (but see Anderegg *et al*., 2015). To model these stochastic elements and thus explore how environmental perturbations might impact the mortality risk of conifers, we simulate two random walks on the bifurcation diagram (Fig. 2d) generated in Eqn 3 and evaluate how a tree's mortality risk changes as parameter sets vary.

A random walk is a special case of a Markov chain describing the movement of a random variable, X, according to its current location plus random noise:

$$X_{t+1} = X_t + N\left(\mu, \sigma^2\right) \qquad \text{Eqn 4}$$

where X_t is the current location of X, X_{t+1} denotes the realized observation of X at the next time point, and $N(\mu, \sigma^2)$ is normally distributed step size with mean $\mu = 0$ and variance $\sigma^2 = 10$ or 100. In a low-variance world ($\sigma^2 = 10$), trees are highly sensitive to initial conditions suggesting their physiological status at attack initiation is a major predictor of mortality. In a high-variance world (variance $\sigma^2 = 100$), trees can move further from their starting location, reflecting a highly stochastic environment or highly responsive tree. Under each scheme, and for each tree-level parameterization, we ran 25 individual random walks for 1000 time-steps each (Fig. S3).

To test if there is an interaction between time to maximum resin flow (α; i.e. how quickly a tree responds to attack) and the minimum phloem terpene concentration required for trees to survive an attack (x_c). We simulated 1000 measurements of defense concentrations incremented by 0.1 from 0.1 to 1, representing potential α values (resulting in 10 000 simulated measurements). For each α, we estimated the cusp point (x_{min} where bistability still occurs; Notes S2) and classified each measurement as either above or below that threshold (dead or alive). This simulation was replicated 1000 times.

Model-data comparison By August of 2022, 43% of all trees at the site were killed by bark beetles. This provided a unique opportunity to test our dynamical model against data. First, using the x_c values derived in (2) we classified each tree as vulnerable or not vulnerable using α (relative response rate), x_c (maximum defense potential from preinoculation carbon reserves), and measured defense concentrations. We tested this classification to evaluate how well this model predicts whether trees live or die. Finally, we compared the proportion of dead trees in our plots to what our model predicts under a small-variance and large-variance random walk to evaluate whether the mortality we observed exceeded what could be predicted under random noise.

Results

The carbon cost of defenses

Following our derived glucose costs of monoterpenes, across all trees, the average concentration of phloem terpenes that could have been synthesized from measured phloem carbon reserves alone was just 9.1 mg g^{-1} (±0.1 mg g^{-1}). This was significantly less than the average measured phloem terpene concentrations (41 mg g^{-1}, ±5.1, *t*-test, $P < 0.05$, $t = 7.026$, df = 47). That is, trees produced an order of magnitude more phloem terpenes than expected if local carbon reserves were the sole source of carbon (Fig. 3). Using whole-tree carbon reserves for comparison, the maximum concentration of phloem terpenes that could be produced on average was 48.8 mg g^{-1} (±12.65 mg g^{-1}). Notwithstanding the challenges of phloem transport during drought (Sevanto, 2014), the multiple demands for carbon reserves that would further reduce this number (Dietze *et al.*, 2014; Sapes *et al.*, 2021), or the fact that we saw little change in carbon reserves during the experiment (Fig. S5), the disparity between observed and predicted phloem terpenes appears small if trees can use all of their carbon reserves (Fig. S6). Put another way, the observed phloem terpene concentrations measured after the inoculation would have required *c.* 90% of our estimate of whole-tree carbon reserves.

Trees with slower response rates are more likely to die

On average, the trees in our study took *c.* 4 d (±1 d) to reach maximum resin flow (Table 2). The relative response rate $\left(\alpha = \frac{\text{No. days to max resin flow of fastest tree}}{\text{No. days to max resin flow of current tree}}\right)$ of individual trees, however, varied considerably. 38% of the trees in our study reached maximum resin flow rates within 24 h ($\alpha = 1$), 15% of trees reached maximum resin flow rates within 7 d ($\alpha = 0.143$), and 19% of trees reached maximum resin flow rates in 2 wk ($\alpha = 0.071$). 27% of trees produced no resin and thus could not be used to parameterize our model because the solution for α would be undefined (i.e. dividing by zero; $n = 7$).

We expected that trees that responded more quickly to initial attack by bark beetles by exuding a lot of resin quickly would require lower concentrations of phloem terpenes to survive an attack (Assumption 2). This would occur because resin is often the first defense a tree has against bark beetles, and a tree's capacity to physically repel beetles with resin should reduce the likelihood that beetles enter the tree. In our model, the cusp point (x_c), or minimum phloem terpene concentration where tree survival is still possible (see Fig. 2), increased exponentially as relative response rate decreased (Fig. S4). Trees that maximized resin flow within 24 h of inoculation ($\alpha = 1$) could still survive with extremely low-phloem terpene concentrations ($x_c = 10$ mg g^{-1} DW). Below 141.919 mg g^{-1} of phloem terpenes, the slowest trees to maximize resin flow ($\alpha = 0.071$, 14 d) became vulnerable to bark beetle attack (Fig. 4b). Our model predicted that among the fastest trees to maximize resin flow in response to inoculation (i.e. $\alpha = 1$; 24 h), all had sufficient defenses to avoid mortality (Fig. 5c). Two of the four trees that took 7 d to reach maximum resin flow rates ($\alpha = 0.143$; 10% of the 19 trees analyzed here) had observed phloem terpene concentrations 97.4% and 63.1% below the lethal threshold (71 mg g^{-1}), respectively. Among the five trees that took 14 d to reach maximum resin flow rates (26% of trees), four had observed phloem terpene concentrations 69% (±9%) below the lethal threshold of 141.919 mg g^{-1}. Among the trees in our study for which the model could parameterized, we predicted that 42% of trees would be vulnerable to attack ($n = 8$). In other words, these eight trees had maximum phloem terpene concentrations that were on average 150% (±38%) below the minimum they would need to survive if 100% of the lethal density of bark beetles attacked. Thus, our model predicts a mortality rate that is just 1% lower than what we observed in our study (predicted 42% vs observed 43%, respectively).

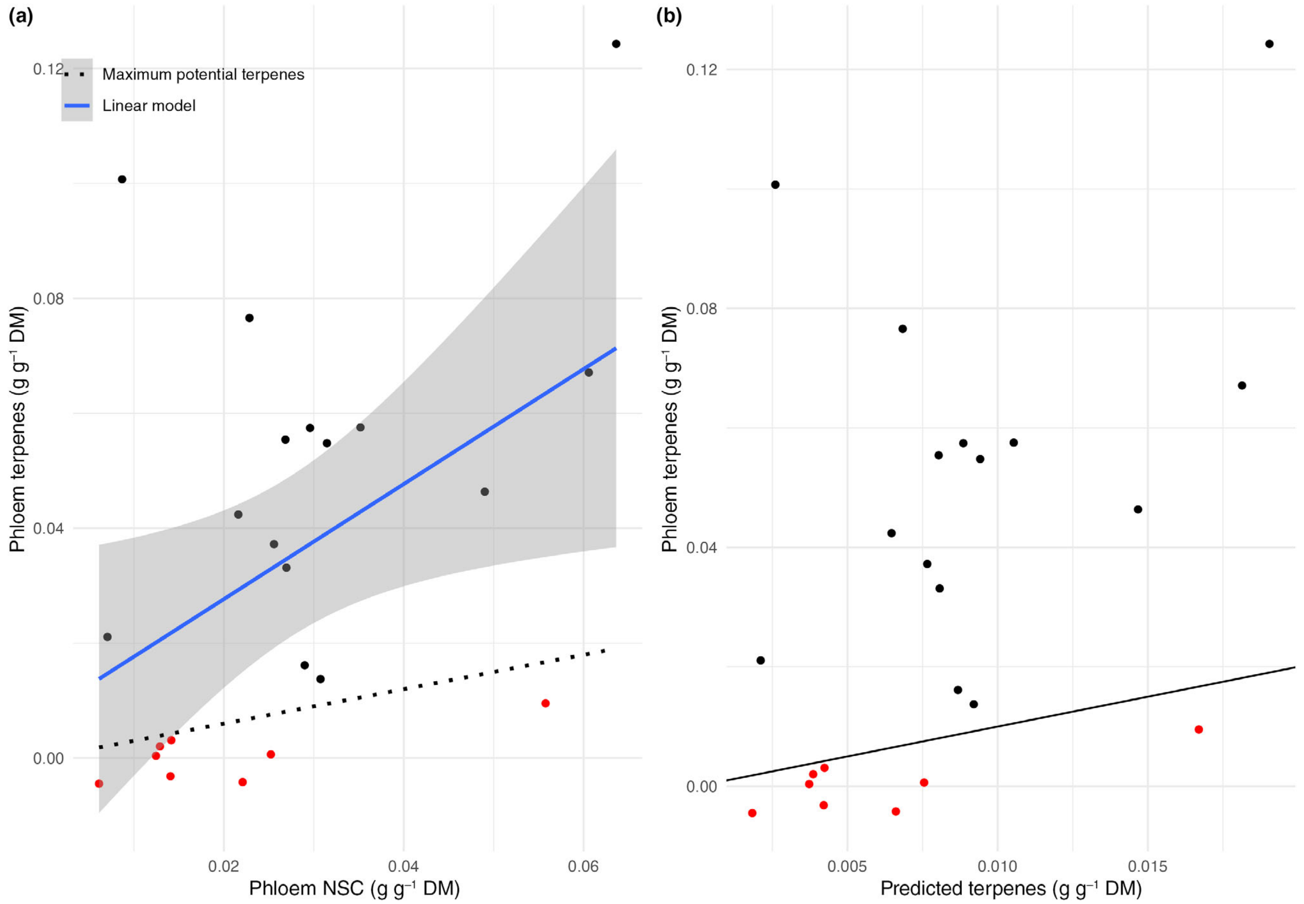

Fig. 3 Stored nonstructural carbohydrates are not enough to synthesize induced defenses. Using phloem total NSC (sugar + starch), we calculated the maximum potential monoterpenes each mature *Pinus edulis* tree could produce. We assume that stomata are closed and thus all energetic needs are met from carbon reserves. For all but 8 trees (red circles in a, b), the observed defense concentrations exceeded what could be produced from NSCs alone. Here, points represent individual trees ($n = 26$). In (a), black and red points represent individual trees used in the regression analysis (solid blue line). The dotted black line in (a) is a continuous estimate of maximum defense potential from carbon reserves. In (b), the maximum potential terpenes from NSC (x-axis) are plotted against the observed terpenes (y-axis). The black line in (b) represents the 1 : 1 line. As with (a), 18 out of 26 trees synthesized more terpenes than NSC alone could produce. This discrepancy may be due to the relatively local effects of the inoculation, suggesting trees may have transported resources from elsewhere in the bole to the site of inoculation. The shaded region in (a) indicates the 95% confidence interval of the regression line.

Among the 19 trees examined in the model, we predicted 11 would survive and eight would die. Our model only misclassified three trees as dead (Fig. 6), each in a different drought intensity (45%, 75% and 90%). This corresponds to a 68% correct classification rate (we observed 13 dead and six living trees of the 19 considered in this model; Fig. 6). Only one tree that died in our experiment was classified as not vulnerable, thus most of the misclassification was in trees with low defenses that did not die. This is not surprising since this model only predicts vulnerability and not mortality and does not account for beetle aggregation behavior.

Mortality risk is greater in a low-variance world

We found that trees in the small-variance simulation ($\sigma = 10$) were more likely to become vulnerable to attack by bark beetles, compared to those in the large-variance simulation ($\sigma = 100$; Fig. 5c). This was most strongly pronounced in trees that took 2 wk to reach maximum resin flow (i.e. when $\alpha = 0.071$), where the mortality risk was 14% in the small-variance simulation and < 1% in the large-variance simulation. However, as trees approached maximum resin flow rates within 24 h ($\alpha = 1$) the mortality risk decreased to a minimum of 0.9% when $\sigma = 10$. Interestingly, mortality risk (or probability that a tree becomes vulnerable to attack by bark beetles; see Fig. 2) increased in the large-variance simulation ($\sigma = 100$) to 1.9% when trees were faster to respond. Thus, when perturbations are sufficiently large, having a fast response rate is not necessarily enough to entirely avoid mortality risk. That said, these probabilities of mortality are all very low (< 10%) and thus if the driver of mortality risk is simply random noise, trees are unlikely to be successfully attacked by bark beetles.

Defense amount and rate describe mortality risk

Our simulation of 10 000 defense measurements highlights the potential tradeoff between how quickly a tree responds to attack relative to nearby trees (α) and its ability to *de novo* synthesize (or

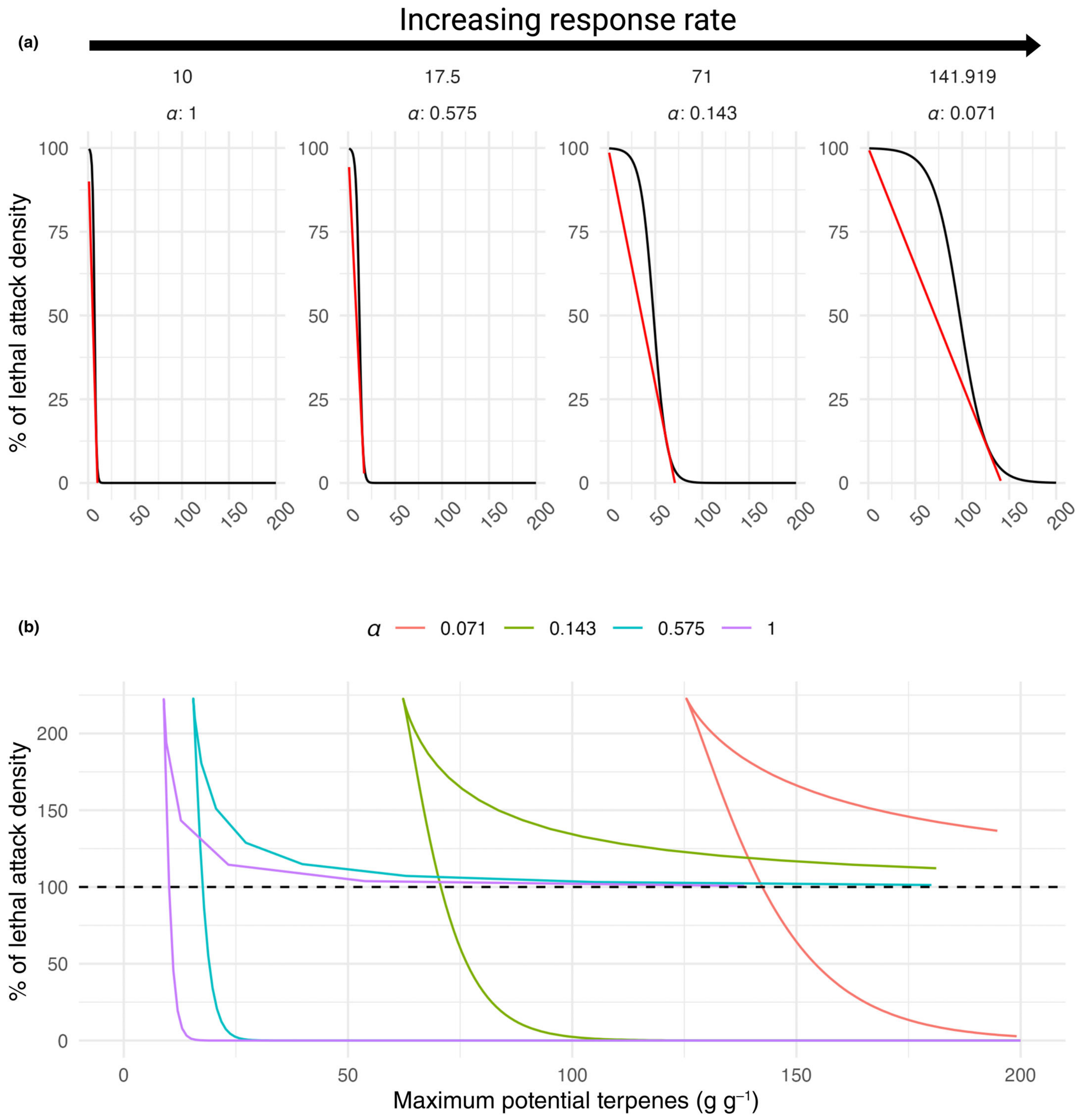

Fig. 4 Slow trees are more vulnerable than fast trees to attack by bark beetles. A plots Eqns 1 and 2 for different parameter values of α (relative response rate) and x_c (maximum potential terpenes at the cusp point) values derived from our experiment with *Pinus edulis*. x_c (tangent of Eqn 2, red line, on Eqn 1, black line) are shown on the diagonals, indicating the minimum concentration of phloem terpenes required for the stable state of tree survival to still be possible. (b) shows bifurcation diagrams for each value of α (relative induction rate). As α decreases (trees respond slower to attack), the location of the cusp point moves further to the right. This corresponds to a higher minimum defense threshold before trees enter the vulnerable state, or the last point when the fixed point of tree survival still exists. Intuitively, this implies that slow-responding trees must mount larger defensive responses to survive attack by bark beetles.

translocate) phloem terpenes (x_c). For trees that produced maximum defenses below 10 mg g^{-1}, the mortality risk was 100% whenever the relative response rate was greater than 24 h ($\alpha < 0.9$). Yet, even when trees responded within 24 h ($\alpha = 1$), mortality risk still exceeded 50% as long as phloem terpene concentrations were at or below 10 mg g^{-1} (Fig. 7). With phloem

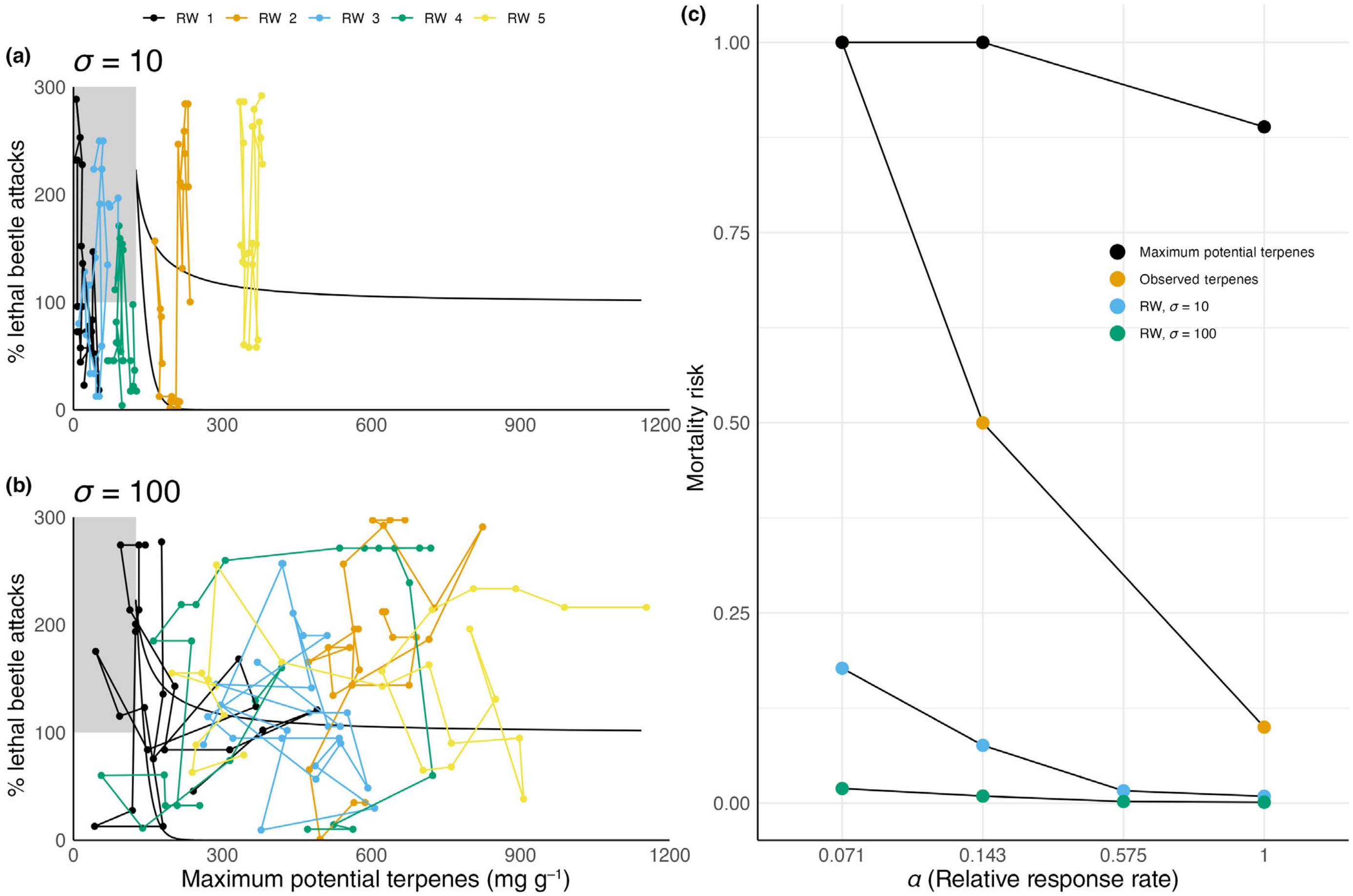

Fig. 5 A trees vulnerability depends on tree physiological status before attack. (a, b) show five representative samples of the random walk approach (each shown in a different color) used to simulate stochasticity in our dynamical system. In (a), trees show high sensitivity to initial conditions, such that the points in the random walk don't move far from the starting point. In (b), trees move widely across the sample space but seldom fall beyond the cusp point avoiding the region of high vulnerability (shaded grey region) to bark beetles. (c) plots the stationary probabilities that trees fall below the cusp point and are thus vulnerable. Our model suggests that the observed proportion of trees that are vulnerable to bark beetles far exceeds what we would expect under random noise. σ indicates the SD.

terpene concentrations increasing slightly to 17.5 mg g^{-1}, mortality risk fell from 100% to just 25% when tree resin flow rates were maximized within 40 h ($\alpha = 0.6$) and 0 if trees maximum resin flow rates were reached within 34 h ($\alpha \geq 0.7$). This pattern continued when phloem terpene concentrations were at 71 mg g^{-1}, with a drop in mortality risk from 100% to 0% as long as maximum resin flow rates occurred within the first 240 h from initial attack ($\alpha > 0.1$). When defense concentrations were, on average, 141.919 mg g^{-1}, the mortality risk was almost surely 0 (Fig. 7).

Discussion

If local carbon reserves were insufficient to support the induced phloem terpene concentrations we observed, then why were measured defense concentrations so high? Because we quantified the total induced phloem terpenes as the difference between the pre- and postinoculation terpene concentrations, we cannot attribute this discrepancy to elevated local constitutive terpenes. It is likely that trees in our study imported defenses or carbon reserves from elsewhere in the bole to the site of inoculation. Phloem terpenes are synthesized in specialized epithelial cells that form resin ducts that span the sapwood and phloem of conifers (Ro & Bohlmann, 2006). These resin ducts also are a form of long-term storage of terpenes which are the dominant chemical found in conifer resin (Vázquez-González *et al.*, 2021, 2022). Thus, a single phloem punch on the bole of a tree could cause the influx of stored resin and terpenes to that site, such that terpenes sampled at that site represent much more than local production (Table 3).

The observation that induced phloem terpene concentrations in response to a fungal inoculation far exceeded what local carbon reserves could support suggests inoculation studies may be overestimating the concentration of defenses that are newly synthesized (Fig. 3). Ultimately, only a maximum of *c.* 13% (±9.9%) of the measured phloem terpene concentrations could be attributed to local carbon reserves. Thus, trees become more vulnerable to bark beetle attack during drought because carbon reserves cannot support new terpene synthesis. However, even the maximum

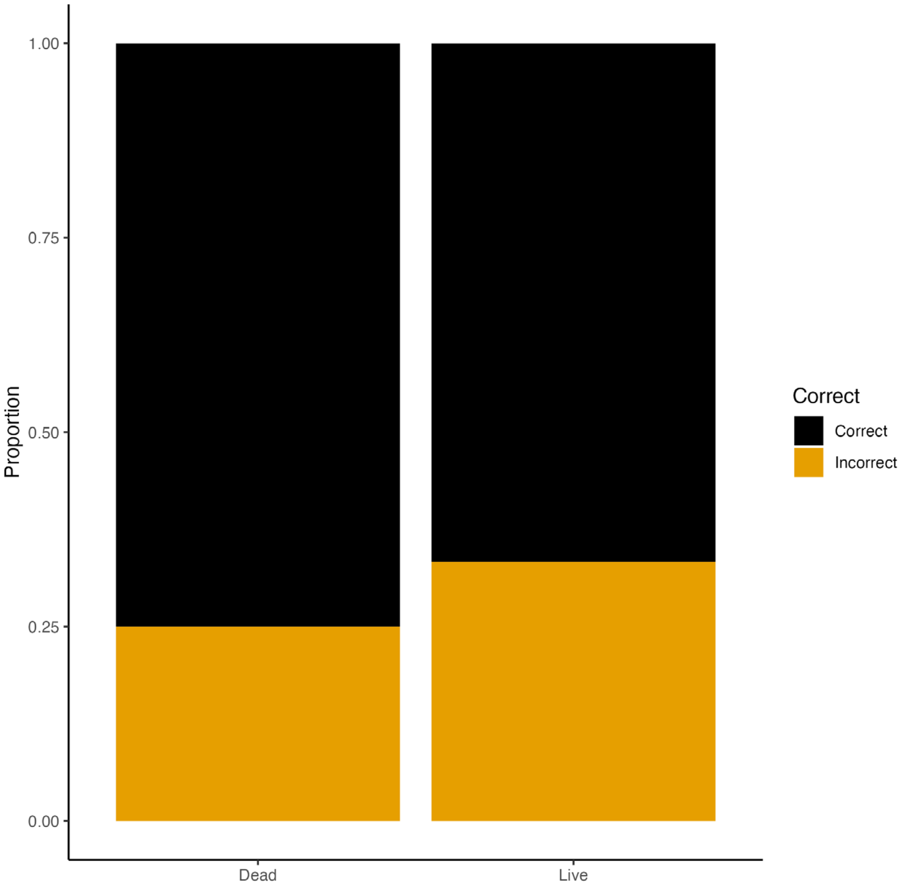

Fig. 6 The bistable model of bark beetle–conifer interactions predicts which trees are killed ($n = 4$) with high accuracy. Mature individuals of *Pinus edulis* were classified as alive or dead based on whether observed phloem terpene concentrations exceeded, or were below, the cusp point. We then compared these results to whether trees were alive or dead in the ecosystem drought experiments. Bars represent the proportion of total observed in each class that were correctly classified (black) and incorrectly classified (orange) as either alive or dead.

defense potential here is likely larger than what trees can actually synthesize *de novo*, as carbon must be allocated to multiple sinks and defenses may include more expensive (C-intensive) compounds. Trees have numerous carbon demands that would simultaneously deplete available reserves, such as maintenance respiration. Trees also produce sesquiterpenes (15 C atoms), diterpenes (20 C atoms), and phenolics, and partitioning among these metabolites is not clear. Partitioning carbon reserves among these groups would yield lower estimates of a tree's defensive potential. Greater phytochemical diversity could, in turn, increase the effectiveness of a tree's defenses (Richards *et al*., 2016) and avoid autoxicity from having too much of a single compound (though evidence of this in pines is restricted to seedlings; see Fernandez *et al*., 2008). Inoculation experiments are popular for measuring induced responses in conifers during drought (Keefover-Ring *et al*., 2016; Cale *et al*., 2017; Kolb *et al*., 2019; Nagel *et al*., 2022). Without simultaneous measurements of carbon reserves and terpene costs, studies that use point measurements at a single inoculation site could be overestimating the scale of *de novo* terpene synthesis in many species. This may be caused by several issues. First, a mass attack by bark beetles occurs over the entire surface area of a tree stem, meaning that our measurement of observed phloem terpenes would be diluted because less resin would be available at a single attack location on the bole. Thus, the actual defensive capacity of trees may be significantly less than what our experiment and model suggest. Second, because we grew fungus on agar plates before inoculation, the fungus was likely introduced at a greater concentration and level of activity than may be normally introduced by beetles alone. As a result, it is possible that trees yielded a larger induced response at the site of inoculation. Tuning the concentration of fungus introduced via inoculation to the concentration carried by beetles may yield more precise estimates of the induced response of conifers.

An important limitation of our approach is the inability to parameterize this specific model for the seven trees that did not response to fungal inoculation. While we do not know the reason why these trees lacked a response, it is possible that our inoculation of a single site on the bole was not sufficient to induce a response. This suggests that Assumption 3, that the minimum beetle attack density to trigger defense induction is small, may be incorrect. While experimentally determining this threshold parameter by varying the number of inoculations on a tree may be possible, the fact that some of our trees did respond to a single inoculation suggests this parameter is dynamic and potentially tree-specific. It is also possible that these seven trees did yield resin but simply took longer than our 2-wk measurement period was able to capture. Assuming this is the case, we conducted a sensitivity analysis on these seven trees by altering their time to maximum resin flow beyond the 2-wk period. We tested four alternatives: trees took either 3, 4, 5, or 6 wk to maximize resin flow. We re-ran our model under these conditions and tested the

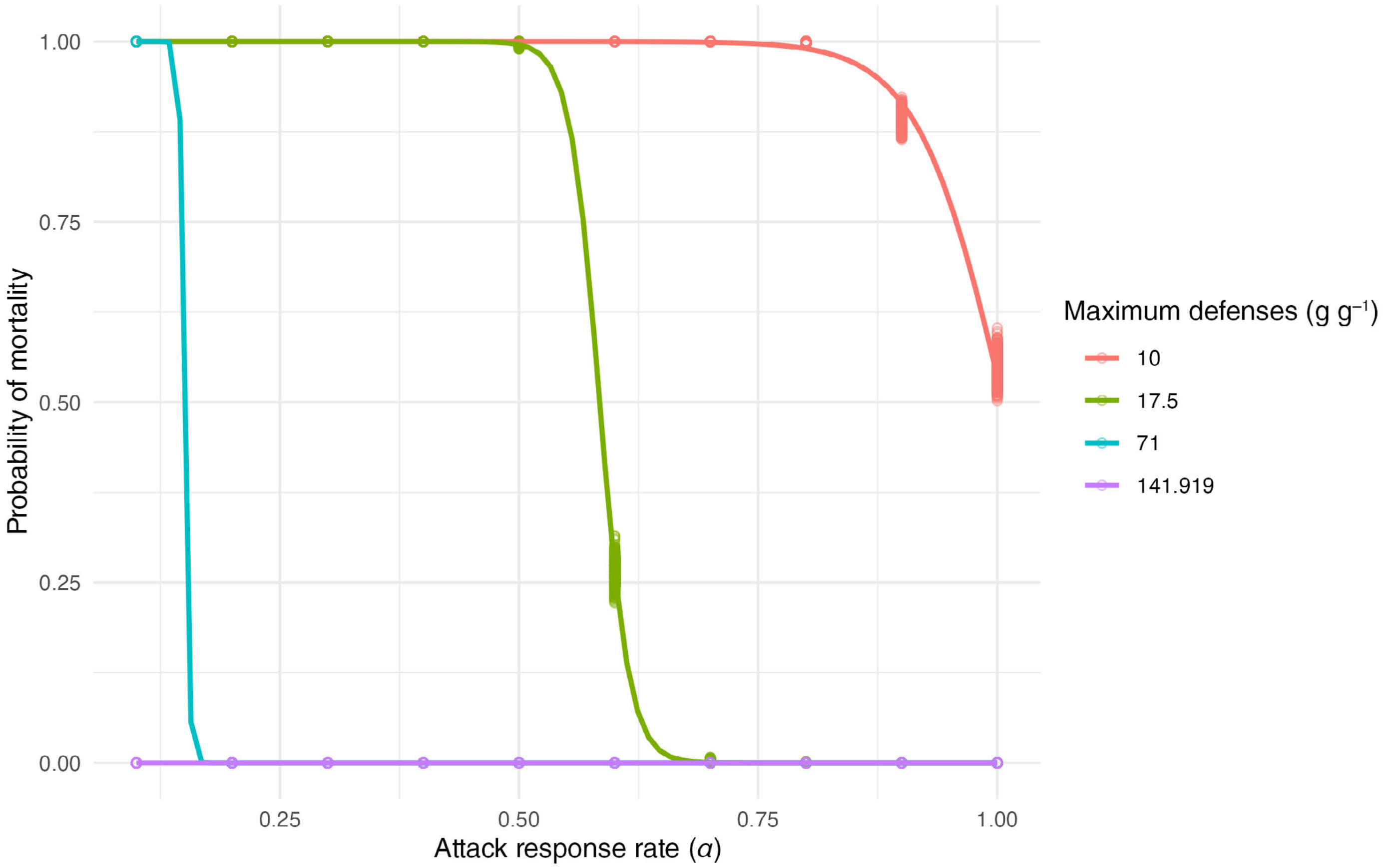

Fig. 7 How quickly trees respond to attack is important to their survival. Across a range of α values we simulate the probability of vulnerability given a tree's maximum defense potential. For trees with the highest defenses, vulnerable is almost surely avoided. Yet, as tree maximum defense potential approaches the minimum, the probability of mortality never falls below 50% even for the fastest trees. Points represent ensemble means of 1000 simulations. Lines are logistic regressions.

general predictive ability of our model. Under all four alternative scenarios, our model proved to be robust with a correct classification rate of 68% under each scenario (Fig. S7). Of these seven trees, two trees survived the outbreak of *I. confusus* that followed our experiment. One of these trees was exposed to drought for more than a decade (Peltier *et al.*, 2023) and the other was exposed to a 75% decrease in precipitation. While the exact cause of these trees' survival is not known, it is possible that severe drought contributed to nutrient limitation in these trees (Houle *et al.*, 2016; Hevia *et al.*, 2019; Gonzalez de Andres *et al.*, 2022), making them inhospitable to bark beetle larvae (Six & Elser, 2019). Future work should investigate the impacts of drought on host tree – bark beetle stoichiometries.

Our results further emphasize the limit of local carbon reserves as the sole energy source for induced production of terpenes in conifers (Christiansen *et al.*, 1987; Berg *et al.*, 2006; Hart *et al.*, 2014; Kolb *et al.*, 2019). If trees were only able to use their stored carbon reserves to mount a defensive response, mortality is highly likely (Fig. 5c). This is consistent with many landscape-scale studies that have shown a link between regional drought and bark beetle outbreaks (Hart *et al.*, 2014; Seidl *et al.*, 2016). Our model's capacity to accurately reflect these dynamics and correctly predict mortality (Fig. 6) likely comes from incorporating both induced and constitutive defenses, which can play critical roles in mediating bark beetle attacks in conifers (Gaylord *et al.*, 2013; Hood & Sala, 2015; Kolb *et al.*, 2019; Nagel *et al.*, 2022). However, the mortality rates predicted by our model and observed in our study were well above what could be predicted if beetles were attacking and killing trees simply by random chance (Fig. 5). This highlights the importance of interactions among multiple co-mechanisms of drought mortality (e.g. hydraulic failure, carbon starvation, and biotic attack; McDowell *et al.*, 2022) in modulating the vulnerability of conifers to secondary-aggressive bark beetles.

Finally, we link our concept of relative response rate (α) to phloem terpenes and how quickly a tree responds to attack (Fig. 7). We suggest this could be useful in rapid field assays of individual tree resilience to future bark beetle attack as resin flow is a commonly assessed physiological indicator (Gaylord *et al.*, 2013; Hood & Sala, 2015). Trees producing resin more quickly may compensate for lower terpene concentrations by repelling pioneer beetles, possibly limiting the likelihood of a mass attack. Slow resin flow means trees must generate a larger induced response to survive. This highlights known tradeoffs between investment in constitutive vs induced defenses (Sampedro *et al.*, 2011; Moreira *et al.*, 2014; but see Runyon

Table 3 Observed resin flow rates are sufficient to transport the observed phloem terpenes.

ID	Photosynthesis (μmol m^{-2} s^{-1})	Ψ_{pd}	Observed flow rate (g h^{-1})	Minimum necessary flow rate (g h^{-1})
121	0.002	−3.65	0.077	0.0002
122	−0.001	−3.40	0.003	0.0000002
131	0.172	−2.65	0.088	0.00004
134	0.009	−2.70	0.015	0.0000001
137	0.022	−2.75	0.045	0.00005
138	0.013	−2.00	0.008	0.000007
139	0.049	−2.75	0.015	0.000019
152	0.021	−2.25	0.059	0.00004
153	0.012	−3.10	0.082	0.000006
154	−0.003	−3.00	0.004	−0.0000001
155	−0.006	−2.80	0.066	0.000013
1611	−0.007	−2.70	0.002	0.000003
164	−0.006	−2.80	0.025	0.000006
166	0.008	−3.00	0.046	0.0000006
168	−0.011	−2.75	0.084	0.00004
169	0.391	−2.70	0.043	0.00003
175	0.002	−2.90	0.008	0.000009
176	0.002	−2.60	0.116	0.00004

In all trees, net photosynthetic rates were effectively 0. Predawn water potential was below the phloem turgor loss threshold of −2 MPa (Sevanto *et al*., 2014). The observed resin flow in g h^{-1} is orders of magnitude greater than the minimum necessary flow rate to explain the observed phloem terpenes that could not be explained by carbon reserves alone.

et al., 2022; Fig. S7). Trees constitutively producing large quantities of secondary metabolites may have lower carbon reserves and/or nutrient availability (Sampedro *et al*., 2011; Trowbridge *et al*., 2021), potentially limiting their induced defensive capacity, as well as resilience to other stressors and disturbance (Vargas *et al*., 2009; Villari *et al*., 2014; D'Andrea *et al*., 2019; Reed & Hood, 2023). Thus, trees that maximize constitutive resin flow may be better at fending off attacking beetles simply because the minimum concentration of induced phloem terpenes required for survival is lower (Vázquez-González *et al*., 2020; Fig. 3). Identifying tradeoffs in conifer constitutive and induced defense in the context of carbon reserves should be explored in future studies. Other constitutive defenses such as heartwood formation, bark thickness, oxaloacetate crystals, or thorns may represent additional areas where species-level tradeoffs with carbon reserve dynamics and growth may occur (Shigo & Hillis, 1973; Hudgins *et al*., 2003; Hanley *et al*., 2007). Quantifying the carbon cost of these tradeoffs may reveal key traits associated with vulnerability to herbivory under progressively severe drought conditions.

While our results demonstrate that local carbon reserves are insufficient to support the induced response of conifers during drought, there are several key limitations that should be discussed. First, our model only classifies trees as vulnerable or not vulnerable while predicting mortality itself requires integration of multiple exogenous factors, such as beetle behavior and population dynamics. A tree's carbon balance is only one part of the herbivory dynamic since trees must be discovered and attacked at sufficient levels for mortality to occur (Raffa & Berryman, 1983; Boone *et al*., 2011.). Phytochemical diversity, in addition to total terpenes, has been linked to tree survival since different combinations of chemicals can enhance (or reduce) conifer resin toxicity (Trowbridge *et al*., 2016). While the direct incorporation of chemical diversity or effective defenses is not addressed here, it is important for future studies to consider. Finally, we also assumed zero photosynthesis during this time, given our measurements of zero assimilation in these trees, but it is possible we missed brief and small amounts of early morning carbon uptake that may contribute to defense production exceeding measured carbon reserves. However, we argue this is unlikely to explain the large mismatch between local carbon reserves and defense production, given translocation rates were probably also limited (Sevanto, 2014) during this extremely dry part of the season which was amplified by throughfall removal treatments ($\psi_{min} = -3.2$ MPa).

Conclusion

Our model-data fusion provides rare insight into the energetic costs of induced conifer terpene synthesis in response to simulated bark beetle attack. Our results suggest that a tree's local carbon reserves are insufficient to meet the defensive needs of conifers during attack by bark beetles (Fig. 3). The current range of *P. edulis* is experiencing a megadrought, afflicting the western US for the past two decades (Williams *et al*., 2020, 2022) which has already predisposed this species to widespread bark beetle-induced tree mortality (Breshears *et al*., 2005). While drought-induced vulnerability to bark beetles is well documented for many conifer species, the mechanisms by which this vulnerability occurs (and the drivers of its temporal variation) remains poorly understood (McDowell *et al*., 2022). Our model and data provide strong evidence that drought-exposed trees have a reduced ability to synthesize new terpenes in their phloem and are thus more vulnerable to bark beetles (Boone *et al*., 2011; McDowell *et al*., 2019). Yet, the finding that carbon reserves played only a minor role in the observed induced phloem terpene concentrations indicates that mortality is not just a function of a tree's carbon balance at the time of attack. Instead, our results highlight the role of a tree's predisposition toward investment in constitutive defense (either genetic or legacy effects; Clark *et al*., 2012; Blumstein & Hopkins, 2021; Six *et al*., 2021) coupled with its ability to synthesize new phloem terpenes are interacting elements that together determine whether a tree can defend itself from attacking bark beetles (Bryant *et al*., 1983; Tuomi *et al*., 1988). Improved empirical evidence of these theoretical tradeoffs across taxa and ecosystems will improve our ability to predict and potentially manage against future outbreaks of destructive herbivores.

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Competing interests

None declared.

Author contributions

SM conducted the secondary metabolite analysis and fungal inoculations. RAT conducted NSC analysis, collected resin flow samples, devised the model and wrote the paper. SM and CO calculated the carbon cost of defenses. DS isolated the fungus. RAT, SM, DMP, NR, CM, NGM, AT and HD conducted fieldwork. All authors helped to edit and revise the manuscript.

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Data availability

All data are available in Dataset S1.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Terpene, carbon reserves, and resin flow data used to parameterize this model.

Fig. S1 Effect of mechanical damage on the induced response of *Pinus edulis.*

Fig. S2 Graphical representation of the energetic accounting procedure used to calculate the carbon cost of monoterpenes made via the MEP pathway.

Fig. S3 Sensitivity analysis of random walk chain showing invariance of the results as chain length of number of chains increases.

Fig. S4 Relationship between alpha and cusp point.

Fig. S5 Response of phloem terpene concentrations, and whole-tree carbon reserves to fungal inoculation.

Fig. S6 Even if trees could leverage whole-tree NSC concentrations to support the *de novo* synthesis of phloem terpenes, it would still not be enough.

Fig. S7 Altering the time to maximum resin flow for trees that produced no resin during our 2-wk measurement period does not change our model's correct classification rate.

Notes S1 Energetic accounting of the MVA and MEP pathways.

Notes S2 Linear stability analysis of Eqns 1 and 2.