*Tree Physiology*, 2026, **46**, tpag004
https://doi.org/10.1093/treephys/tpag004
Advance access publication date 24 January 2026
**Research paper**

# Resin-based defenses in *Pinus edulis* are only reduced after long-term drought

**Drew M.P. Peltier[1,*], Shealyn C. Malone[2], Cameron D. McIntire[3], R. Alex Thompson[4], Sara Pinzon-Navarro[5], Ksenia A. Pereverzeva[6], Andrew D. Richardson[7,8], Nathan G. McDowell[9,10], Henry D. Adams[6], Mariah S. Carbone[7], William T. Pockman[5] and Amy M. Trowbridge[11]**

[1]School of Life Sciences, University of Nevada-Las Vegas, Las Vegas, NV, 4505 S Maryland Pkwy, Las Vegas, NV 89154, USA
[2]Department of Entomology, University of Minnesota, 1980 Folwell Ave, 219 Hodson Hall, Saint Paul, MN 55108, USA
[3]Northeastern Area State, Private, and Tribal Forestry, USDA Forest Service, 271 Mast Road Durham, NH 03824, USA
[4]Life and Environmental Sciences, University of California Merced, 5200 North Lake Rd, Merced CA 95343, USA
[5]Biology Department, University of New Mexico, 219 Yale Blvd NE, Albuquerque, NM 87106, USA
[6]School of the Environment, Washington State University, PO Box 642812, Pullman, WA 99164, USA
[7]Center for Ecosystem Science and Society, Northern Arizona University, PO Box 5620, Flagstaff, AZ 86011, USA
[8]School of Informatics, Computing, and Cyber Systems, Northern Arizona University, 1295 Knoles Dr, Flagstaff, AZ 86011, USA
[9]Atmospheric Sciences and Global Change Division, Pacific Northwest National Lab, PO Box 999, Richland, WA 99352, USA
[10]School of Biological Sciences, Washington State University, PO Box 644236, Pullman, WA 99164-4236, USA
[11]Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, 1630 Linden Dr, Madison, WI 53706, USA

*Corresponding author (drew.peltier@unlv.edu)

Handling Editor: Daniel Epron
Henry Adams and Amy Trowbridge both hold the position of Editor for Tree Physiology and have not peer reviewed or made any editorial decisions for this paper.

Constrained carbon allocation toward secondary metabolites involved in chemical defense is a common explanation for widespread drought-related beetle-kill in conifers—we challenge the generality of this explanation. While monitoring drought stress ($\psi_{pd}$), we tracked both carbon reserves (non-structural carbohydrates) and chemical defenses (terpenes, phenolics, resin flow) in mature *Pinus edulis* Englem. trees experiencing either short-term (3-year) or a 'legacy' long-term (13-year) throughfall exclusion treatments, plus a control. We also quantified the $\Delta^{14}$C-age of resin to measure past allocation to current defense. While 72% of trees in short-term throughfall exclusion plots died (attacked by bark beetles, *Ips confusus* LeConte), mortality patterns were unrelated to throughfall exclusion intensity and all 'legacy' trees survived. We thus assessed trees in four survivorship categories: control, 'legacy', surviving, and dying trees. We found concentrations of certain defense compounds (leaf phenolics, twig monoterpenes) increased with drought stress, particularly in dying trees. In the main stem, dying trees exhibited similar terpene concentrations (94%) and phenolic concentrations (139%) relative to control trees. Compared with control trees, only 'legacy' trees had reduced stem terpenes (−49%, $P < 0.05$) after a decade of drought. $\Delta^{14}$C-age of resin could be up to 10.2 ± 0.5 years old, where the oldest resin was exuded from trees with low sugar concentrations and more negative $\Psi_{pd}$. Our results suggest that drought imposes a weak constraint on carbon allocation to resin-based defense. Instead, we primarily found evidence of increased concentrations of terpene and phenolic compounds under drought, even in dying trees, and only observed reductions in resin-based defenses after 10+ years of drought. $\Delta^{14}$C-ages demonstrate limited resin turnover and/or synthesis of resin from old reserves, suggesting that long-term drought is required to reduce resin-based defenses. Persistent allocation coupled with past investments appears to preserve or enhance concentrations of resin-based defenses even under lethal drought stress in *P. edulis*.

***Keywords:*** carbon allocation, carbon starvation, chemical ecology, mortality, NSC, radiocarbon.

## Introduction

Ongoing and future tree mortality leads to the loss of centuries-old carbon to the atmosphere, presenting challenges to achieving future climate targets and proposed mitigation approaches (Anderegg et al. 2020, Novick et al. 2022). Understanding the mechanisms of tree mortality has thus been a central focus of past research, where evidence has accumulated that the combination of hydraulic damage (Plaut et al. 2012) and carbon starvation (Dickman et al. 2015) contributes to the death spiral in conifers (Allen et al. 2015, Adams et al. 2017, McDowell et al. 2022). In many forests, the interactions of tree physiology with insects like tree-killing bark beetles also play a critical role (Raffa et al. 2008, McDowell et al. 2011, Anderegg et al. 2015, Stephenson et al. 2019). Trees' chemical defenses often influence beetle foraging and reproductive success (Everaerts et al. 1988, Erbilgin et al. 2017*a*, Ott et al. 2021, Korolyova et al. 2022), so an improved understanding of the degree to which drought reduces allocation to defenses, if at all, is essential.

**Received:** July 8, 2025. **Accepted:** January 17, 2026

Trees allocate substantial amounts of carbon to chemical defenses such as carbon-rich terpenes and phenolics that may protect them from beetle attack under drought (Gershenzon 1994, Monson et al. 2022). Conifers produce a wide array of secondary metabolites to entrap, poison, impair, and repel bark beetles and their symbionts, which attack tree stems (Klepzig et al. 1995, Gershenzon and Dudareva 2007). *Pinus* spp. particularly rely on resin for defense, maintaining a complex network of specialized ducts to store and release pressurized resin upon attack (Phillips and Croteau 1999, Luchi et al. 2005). Upon attack, trees can upregulate the production of certain defenses via traumatic resin duct formation (Krokene et al. 2008) and/or terpene synthesis (Lombardero et al. 2000), potentially leveraging large reservoirs of existing resin (Wu and Hu 1997). While adequate resin exudation provides physical defense, certain constituent mono-, sesqui-, and diterpene compounds are toxic or inhibitory to attacking beetles and symbionts (Everaerts et al. 1988, Chiu et al. 2017, Reid et al. 2017). Phenolics are another class of secondary metabolites that defend against some beetle-symbiont complexes, particularly in *Picea* spp. (Sallé et al. 2005, Zhao et al. 2019, Korolyova et al. 2022). However, individual secondary metabolites can also have a broad range of roles beyond physical and chemical defense (Erb and Kliebenstein 2020), where phenolics are important in scavenging reactive oxygen species produced under oxidative stress caused by drought (Karuppanapandian et al. 2011).

Bark beetle outbreaks in conifer forests often co-occur or follow drought events (Raffa et al. 2008), suggesting drought constrains defensive capacity (Lerdau et al. 1994, Gely et al. 2020), but empirical evidence has been mixed. Drought may constrain tree carbon allocation such that sinks like growth and respiration supersede secondary metabolite production (McDowell et al. 2008, 2022, Wotherspoon et al. 2014, Anderegg et al. 2015, Huang et al. 2019). Previous studies have produced mixed findings, suggesting that drought reduces resin production in *Pinus ponderosa* Dougl. ex Laws. (Kolb et al. 2019) but has no impact on resin production in *Pinus edulis* Englem. (Gaylord et al. 2013). In *Pinus sylvestris* L., trees experiencing drier conditions actually allocated more carbon to stem resin and produced greater resin flow than irrigated trees (Rissanen et al. 2021). In leaves and twigs, the allocation of nonstructural carbohydrates toward production of monoterpene chemical defenses has also been shown to increase with drought and heat (Trowbridge et al. 2021). While leaf and twig secondary metabolites may seem incidental to beetle attacks on the main stem, monoterpenes and other low-molecular weight compounds are abundant and readily volatilize (Sharkey and Singsaas 1995, Trowbridge et al. 2019), potentially signaling host quality to searching bark beetles (Huber et al. 2000, Lehmanski et al. 2023). Since the early 2000s, *Ips confusus* LeConte have been observed to attack and reproduce in both the main stem and branches of *P. edulis* (Raffa et al. 2008, Gaylord et al. 2013), suggesting that drought-induced changes to defense compounds throughout the whole tree may be important (Kolb et al. 2006). Shifts in crown secondary metabolites might also be coordinated with resource allocation in the stem, perhaps in anticipation of biotic attack (Mertens et al. 2021). Together, these studies illustrate the capacity for conifers to produce a wide array of chemical defenses across organs and tissues, yet the relative investment in various defense compounds (terpenes and phenolics) and aspects of defense (resin flow) under drought has been variable.

Mixed responses of defense production to drought stress could reflect ecological memory (Ogle et al. 2015), unquantified differences in past allocation to carbon reserves or resin production that may confound experimental attribution. Trees have large supplies of carbon reserves of mixed ages (i.e., non-structural carbohydrates: NSCs; Körner 2003, Hoch et al. 2003, Peltier et al. 2024) that might sustain secondary metabolite production under prolonged stress (Guérard et al. 2007). As such, defense production during a given drought may be decoupled from current carbon status, instead reflecting past carbon investments in reserves. Gaylord et al. (2013) showed that $\delta^{13}$C of resin in drought-stressed pines reflected patterns expected in NSC substrate under reduced stomatal conductance, which could suggest new production of resin during drought stress. However, in *P. edulis*, older carbon reserves (higher $\Delta^{14}$C) were only consumed after many sequential years of drought, suggesting that they are actively preserved or large enough to be resistant to exhaustion (Peltier et al. 2023*b*). Multi-year storage of defensive compounds themselves could also decouple defense production from carbon status during a drought. Resin-based terpenes are suspected to have long residence times when sequestered in specialized ducts spanning the sapwood and inner bark (Krokene et al. 2008, Rissanen et al. 2021). Bark is the first tissue encountered by attacking bark beetles yet is built up by phloem growth over many years—and thus exuded resin may reflect past resource investments. Annually produced xylem resin ducts are interconnected with the phloem (Werker and Fahn 1969), such that beetles may encounter a mix of old resin (Rigling et al. 2003) and newly produced resin (Erbilgin et al. 2021). Bomb-$\Delta^{14}$C measurements of resin could inform our understanding of secondary metabolite turnover and use (i.e., the age of carbon in exuded resin), which has been an obstacle to modeling conifer defense under drought.

In light of inconsistent findings and evidence for long-term storage of carbon reserves and resin, we sought to test the assumption that drought reduces the concentrations of key conifer chemical defenses. Piñon–juniper woodlands are the most widespread old-growth forest type in the USA, in which *P. edulis* experienced widespread drought-induced mortality in the early 2000s with around 25% of populations recently declining (Breshears et al. 2005, Shriver et al. 2022, Barbour and Palmer 2024). Experimental tests in *P. edulis* have suggested that the specialist bark beetle, *I. confusus,* directly kills trees during drought rather than being a secondary driver (Gaylord et al. 2013). *Pinus edulis* may also be resistant to short-term drought treatments (Peltier et al. 2023*b*). We thus exposed mature *P. edulis* trees to both short-term (3 years, 2020–22) and long-term (13 years, 2010–22) experimental throughfall exclusion. The long-term treatment leveraged a pre-existing, intact plot from a previous experiment at the same site (Pangle et al. 2012), which we hereafter refer to as the 'legacy plot'. To quantify defenses, we measured crown (needle and twig) and stem phloem monoterpenes, sesquiterpenes, and phenolics as well as stem resin flow. To characterize tree water and carbon status, we simultaneously monitored pre-dawn water potential ($\Psi_{pd}$), NSC concentrations (all tissues), and needle and twig growth. Finally, to estimate past allocation to defense and/or use of old carbon reserves to produce defenses, we quantified the amount of bomb-$^{14}$C in

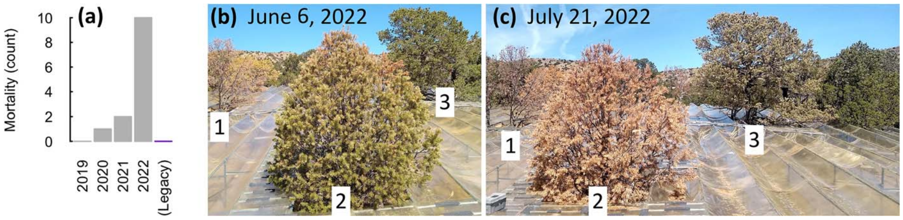

**Figure 1.** (a) Count of dying trees (13 total) across study years; legacy plot trees are broken out separately as 0 died. (b, c) Images of typical progression of browning in trees that died during August 2021 (Tree 1), June 2022 (Tree 2) and late-July 2022 (Tree 3). All dying trees contained bark beetles.

resin exuded in response to fungal inoculation. As described above, trees can upregulate resin production in response to attack, so this presented a way to understand past investments in resin production or the use of old carbon reserves for resin production. Simultaneous measurements of multiple dimensions of defense, growth, NSC, and water status in surviving and dying trees in a natural setting present a unique opportunity to assess the extent to which drought does or does not constrain certain aspects of defense over time and their potential role in resistance against bark beetles.

We hypothesized that (H1) short-term drought (3 years, 2020–22) does not reduce defense concentrations (terpenes, phenolics) or resin flow in surviving or dying trees, as resin pools are built up over many years and persistent production of terpenes and phenolics may be supported by old carbon reserves. Comparison with trees under long-term drought ('legacy' treatment, 2010–22) can help us understand if such resilience is temporary. We also hypothesized that (H2) resin $^{14}$C-ages would be oldest in trees with poor carbon and water status (low NSC, more negative $\Psi_{pd}$), reflecting use of old resin (vs newly produced resin) and/or greater reliance on old NSC to produce new resin under drought. The presence of old resin and/or the use of old NSC to produce resin would both suggest that resin pools are not substantially reduced by short-term drought stress. Preliminary analyses revealed no significant differences in defensive compound concentrations among the newly established throughfall exclusion plots (drought intensities: −45%, −75%, −90%; see Materials and methods) or between these plots and the control, and similar patterns were observed in a prior study (Thompson et al. 2024). To evaluate how chemical defenses vary with drought exposure and tree mortality, we classified trees into four survivorship categories: (i) control (trees under ambient conditions), (ii) survived (trees that survived the 3-year drought treatments), (iii) died (trees that died during the 3-year drought treatments) and (iv) legacy (trees that survived the 13-year drought treatment; Figure 1). This categorization enabled us to assess temporal changes in defense allocation in relation to drought duration and tree mortality, providing insight into the potential mechanisms governing conifer resilience under prolonged drought stress.

## Materials and methods

The study site occurs in low-elevation piñon-juniper woodland within the Sevilleta LTER (New Mexico, USA), collocated with previous drought studies (Plaut et al. 2012), and details of soil, slope, aspect and other plot establishment considerations are well-described therein and in subsequent research (e.g., Gaylord et al. 2013, Dickman et al. 2015, Peltier et al. 2023*a*). Throughfall exclusion is accomplished by rows of large polycarbonate gutters supported by metal posts. Gutters divert rainfall off the plot and cover different proportions of total plot area to produce differing throughfall exclusion intensities. In short, there were five 40 × 40 m plots: control (no gutters), −45% (∼45% of plot area covered by gutters), −75%, −90%, and a long-term −45% each situated on gentle slopes at or near the top of hills. Treatment effectiveness was verified with in situ soil moisture sensors at three different depths (10, 30 and 100 cm) distributed in four pits across each plot. Four of the plots were newly constructed in January 2020 following Pangle et al. (2012) including a control, plus three rainfall exclusion plots (−45%, −75%, −90%). A fifth 40 × 40 m plot (−45%) was previously constructed in 2010 and left largely unmonitored from 2015 to 2019 (the 'legacy' of a previous experiment) and is used here as a long-term drought treatment. While we refer to this as the 'legacy' plot, trees in this plot experienced continuous throughfall exclusion from 2010 to 2022—a chronic, long-term drought treatment. Trees in this legacy plot were infrequently sprayed with a permethrin-based insecticide (environmental half-life ∼30 days; Wang et al. 2012) during the 2010–15 outbreak of *I. confusus*. These insecticides may provide protection for up to 1 year (Fettig et al. 2024), but trees have not been sprayed since 2015, so are unlikely to have retained insecticide-based protection against beetle attack 5–7 years later during our study. Our measurements focused on six target trees per plot ($n$ = 30) at least 5 m from edges. Although only one plot was established per drought level, this design is not considered pseudoreplicated because each plot encompasses many trees that are spatially independent, ensuring substantial within-plot heterogeneity and providing independent sampling units for tree-level responses. The same large-plot throughfall exclusion design has been used and validated in long-term drought experiments over the past 20 years (McDowell et al. 2013, Adams et al. 2015).

Despite variation in throughfall exclusion intensity across the plots, preliminary analysis found no differences in concentrations of defense compounds either among the new throughfall exclusion plots or between the new plots and the control, consistent with previous analyses (Thompson et al. 2024) and ongoing analyses (Malone et al., unpublished data). Instead, we focused on studying whether concentrations of defense compounds and/or resin flow were related to differing mortality outcomes among trees. To address our hypotheses (H1, H2) we compared tree responses to drought among four survivorship categories: control ($n$ = 6, trees that survived ambient conditions, no mortality), survived ($n$ = 5; trees that survived the new treatments), died ($n$ = 13; trees that died in the new treatments), and legacy ($n$ = 6; trees that survived long-term drought, no mortality).

## Physiological measurements

To understand how drought influenced tree physiology, we monitored key metrics of water ($\Psi_{pd}$) and carbon (growth, NSC) status, as well as chemical defense concentrations (specifically terpenes and phenolics). We monitored predawn water potential ($\Psi_{pd}$, Scholander-type chamber, PMS, Albany, OR, USA) and needle and twig growth (following Adams et al. 2015). We monitored at monthly intervals beginning in May (2020; delayed due to the COVID-19 pandemic), February (2021) or March (2022), and bimonthly during winter months. We also collected needle and proximal twig samples for NSC (soluble sugars, starch) and defense concentration measurements (total monoterpenes, sesquiterpenes, and phenolics) three times annually from 2019 to 2022 during peak growing season (May, June, August) on two random sunlit, mid-canopy branches. We avoided sampling near wounds or evidence of previous resin. Main stem phloem (inner bark) and xylem samples for NSC and defense concentration measurements were collected annually in June, using a 10 mm punch and a 5.15 mm increment borer (Haglof, Sweden) to 3 cm, again avoiding wounds. Monthly and bimonthly sampling of $\Psi_{pd}$ and needle/twig growth took place over a single day, while May, June and August measurement campaigns to collect needles, twigs and phloem punches/increment cores for chemical analyses typically lasted 4–5 days (full measurement schedule in Table S1 available as Supplementary Data at *Tree Physiology* Online).

Non-structural carbohydrate samples were microwaved for 180 s within 3–4 h of collection, oven-dried for 72 h at 60 °C and ground to a fine powder using a ball mill (Retsch MM200, Haan, Germany). To analyze NSC concentrations, samples were analyzed following the standard phenol-sulfuric acid method (Landhäusser et al. 2018) via color formation following extraction/digestion and using a spectrophotometer with reference to a standard curve.

Samples for chemical analysis of terpenes and phenolics were immediately flash frozen in liquid nitrogen in the field and stored at −80 °C. Terpene and total phenolic tissue samples were extracted by submerging ground or diced tissue in dimethyl chloride or methanol, respectively, for 24 h before collecting the supernatant for chemical analysis (Cipollini et al. 2011, Trowbridge et al. 2021). Mono- and sesquiterpenes were quantified by gas chromatography–mass spectrometry according to Trowbridge et al. (2021), and total soluble phenolic content was quantified spectrophotometrically using the Folin-Ciocalteu method modified from Cipollini et al. (2011) and Ainsworth and Gillespie (2007). Defense concentrations are reported as mg $g^{-1}$ fresh weight (FW), as there are no statistical differences between dry weight and fresh weight for this species in this region (Trowbridge et al. 2021). Further methodological details are reported in Methods S1 available as Supplementary Data at *Tree Physiology* Online.

## Bark beetle monitoring

We regularly monitored for visual evidence of bark beetle entrance holes and frass. We also monitored populations of flying *I. confusus*, the specialist bark beetle that attacks *P. edulis* trees at this site, using four Lindgren multiple-funnel traps (Lindgren 1983) per plot. Traps were collected monthly in 2019–21 and bimonthly in 2022; samples were sometimes lost to wind damage or contamination with mice. Insects were stored in propylene glycol until identification and quantification could be performed in the lab by a trained insect systematist with expertise in bark beetles (LaBonte and Valley 2019). *Ips confusus* were counted in each trap and summed to produce a study-level metric of flying beetle density. Samples collected August–December 2021 were lost in shipment, and limited resources only allowed analysis of June and August samples in 2022.

## Mortality monitoring

Coincident with monthly or bi-monthly monitoring, we estimated the percentage of canopy that was brown (5% increments) and investigated trees for signs of bark beetle attack. We only estimated brownness of remaining needles (ignoring dropped needles). No trees in our study experienced 35% brownness and survived, so we selected 35% brown as a threshold for imminent mortality for subsequent analyses (e.g., Tree 2 in Figure 1). All dying trees were attacked by beetles (see Results), but trees took an additional ~30–60 days to exceed 95% brown, so we consider 35% a lethal beetle attack threshold or biotic 'point of no return' (*sensu* Hammond et al. 2019), rather than a mortality threshold.

To understand whether water, carbon and defensive status differed in dying trees, we assembled 'terminal' physiology for dying trees. Thus, for each dying tree, we assembled physiological data (e.g., $\Psi_{pd}$, leaf and twig NSC, phloem terpenes and phenolics) from the last timepoint prior to crossing the 35% brownness threshold. We consider these physiological measurements to approximate the conditions foraging beetles encountered when selecting trees. We recalculated this terminal physiological data for each tree as a percentage of average cotemporaneous values for all six control trees.

## Resin flow measurements

We quantified bulk resin flow (g $h^{-1}$; Lorio 1993, Gaylord et al. 2013) annually in June from 2019 to 2022 and in July and August in 2021 and 2022, respectively. A 3D-printed collector with a 15 mL falcon tube (Sigma) was affixed directly over a 13 mm hole in the phloem. Resin was collected for 48 h.

In June and July 2022, resin flow collections were coincident with fungal inoculations using a newly described species of blue-stain fungus (*Ophiostoma* spp.) consistently associated (~97%) with *I. confusus* at this site (Thompson et al. 2024). Inoculations induce a defense response (e.g., Stewart et al. 2020). *Ophiostoma* fungus was grown on 2% malt agar using single spore isolates from *I. confusus* beetles collected at the study site in 2021. To inoculate, outer bark in a randomly selected stem aspect (N, S, E, W) was removed with a chisel, and a 5 mm punch of inner bark (hereafter 'phloem') was replaced with a 5 mm fungal plug contacting the sapwood. Phloem was replaced on top of the fungus to reduce desiccation. A larger phloem punch (13 mm) was removed directly below this inoculated tissue, where resin flow was collected (design depicted in Figure S1 available as Supplementary Data at *Tree Physiology* Online). Phloem NSC collections were performed immediately before inoculation to assess how trees allocated NSC toward resin-based defenses in response to an attack. We emphasize that only two inoculations were applied per tree (one in June 2022, a second in July 2022). Other samples taken to quantify phloem and xylem chemistry (e.g., NSC and terpenes) described above were collected from different parts of the stem. Some trees had already died prior to the inoculation work, so sample sizes for this set of measurements were reduced relative to previous campaigns ($n$ = 27 in June 2022; $n$ = 21 in July 2022).

## Accelerator mass spectrometry and $^{14}$C-dating

Following July 2022 inoculations, we subsampled collected resin exuded from target trees that produced resin ($n$ = 20) to assess the $^{14}$C-age of carbon in resin. This included six trees in the control, six dying, five surviving, and three legacy. We note the punches for resin flow removed phloem only and did not puncture the sapwood. Collected resin was stored at −80 °C. For $^{14}$C measurement, we quickly removed samples from a freezer and smeared a small drop of resin (target 4–12 mg) on the inside of a 6 mm quartz tube with a metal spatula (ethanol-washed and dried), confirming proper sample mass on a balance. Cupric oxide was added to catalyze the conversion of resin carbon to $CO_2$ (Boutton et al. 1983). Tubes were then rapidly sealed (<20 min after smearing) on a vacuum purification line while tube bottoms were simultaneously immersed in liquid nitrogen to prevent resin volatilization. Sealed tubes were subsequently baked at 450 °C, converting all carbon to $CO_2$ (Boutton et al. 1983). Accelerator mass spectrometry details (standard procedures) are provided in Methods S2 available as Supplementary Data at *Tree Physiology* Online. Measurements were performed at the Arizona Climate and Ecosystems (ACEs) isotope lab at Northern Arizona University on a Mini Carbon Dating System (Synal et al. 2007) where $\Delta^{14}$C measurement, correction, and reporting were performed by ACE following standard methods (Trumbore et al. 2016). $^{14}$C-ages are estimated by matching $\Delta^{14}$C to the atmospheric bomb-spike (Hua et al. 2022) as in Peltier et al. (2023*b*). Note that $\Delta^{14}$C cannot distinguish old resin from new resin built with old NSC.

## Statistical analyses

As previously described, we aggregated trees into four categories: control ($n$ = 6), survived (survived new drought treatments, $n$ = 5), died (died in new drought treatments, $n$ = 13), and legacy (legacy treatment, all survived, $n$ = 6). To quantify the effects of drought on concentrations of key chemical defenses in various tissues (H1), we compared total monoterpenes, total sesquiterpenes, total phenolics, and resin flow among the four survivorship categories using a hierarchical-Bayesian Analysis of variance type model (HB-ANOVA). We chose this approach due to the complex structure of the data (missing data, limited time points for some response variables, not all trees sampled at all time points if they died, heteroscedasticity, etc.). We similarly assessed differences in physiological covariates ($\Psi_{\text{pd}}$, NSC, leaf/twig growth). Because leaf and twig growth accumulate throughout a growing season, we conducted statistical tests using the end-of-season growth (maximum measured leaf or twig lengths).

In the HB-ANOVA, to account for the repeated measures of individual trees across time, individual observations (e.g., one observation of total phloem monoterpenes in one tree) are normally distributed, and modeled with tree-level means, $\alpha_{\text{tree}}$. These means, $\alpha_{\text{tree}}$, are themselves normally distributed around survivorship-level means, $\mu_s$, where s = 1 for control, s = 2 for survived trees, s = 3 for dying trees, and s = 4 for legacy trees. We compared posterior distributions among these survivorship-level means with contrasts (differences, e.g., $\mu_4 - \mu_1$) and report associated Bayesian $P$-values. We describe differences as significant when the Bayesian $P$-value is <0.05. Model code is provided in Methods S3 available as Supplementary Data at *Tree Physiology* Online.

Chemical defense concentrations in canopy tissues (leaves and twigs) were highly temporally variable (see random effects model described in Methods S4 available as Supplementary Data at *Tree Physiology* Online), so we also modeled concentrations (leaf and twig monoterpenes, leaf and twig phenolics) on contemporaneous $\Psi_{\text{pd}}$ for trees in each survivorship category (across time points and trees) with linear mixed effects models (random slopes and effects for each tree).

To further understand if dying trees differed, while accounting for temporal variability, we also relativized defenses in dying trees by contemporaneous values in control trees. That is, for each dying tree, *tree*, we scaled terpene and phenolic concentrations, $X$, by the same concentration in control trees (on average) at the same time, $t$, to produce $X^*$, such that

$$X^*_{tree,t} = \frac{100 * X_{tree,t}}{\overline{X_{control,t}}} \tag{1}$$

where $X$ is the leaf/twig monoterpenes and leaf/twig phenolics, and $\overline{X_{control,t}}$ indicates the mean control tree value for compound $X$ during time period $t$. We also regressed these normalized means $X^*$ at each time period, $t$, against the contemporaneous $\Psi_{\text{pd}}$.

To determine if variation across target trees in the $^{14}$C-age of resin was related to carbon and water status (H2), after preliminary analysis, we regressed resin $^{14}$C-age against contemporaneous phloem NSC concentrations (sugar and starch) and $\Psi_{\text{pd}}$. We report results from a multiple regression of $^{14}$C-age on contemporaneous phloem sugars, $\Psi_{\text{pd}}$ and their interaction, such that

$$\begin{aligned} {}^{14C}Age_{tree} \sim \alpha_1 + \alpha_2 sugar_{tree} + \alpha_3 \Psi_{pd_{tree}} \\ + \alpha_4 sugar_{tree} \times \Psi_{pd_{tree}} \end{aligned} \tag{2}$$

where ‘sugar’ indicates phloem sugar concentrations measured in July 2022 (immediately prior to resin sampling) and $\alpha_{1\text{–}4}$ indicate the regression effects. Because some phloem sugar and water potential measurements were missing, we also fit this model by filling in missing values with prior month values for a given tree and report results from both approaches. All statistical analyses were performed in R (R Core Team 2025). Linear mixed effects models were conducted and interpreted with the lme4, MuMIn and lmerTest packages.

# Results

Thirteen of 18 study trees in new, short-term drought plots died (72%, 2020–22), all 13 of which were attacked by *I. confusus* beetles evidenced by numerous entrance holes and abundant frass. All trees in the ‘legacy’ long-term drought plot survived (six trees, 2010–22). On all 13 dying trees, abundant entrance holes and frass on main stems and branches were detected (Figure S2 available as Supplementary Data at *Tree Physiology* Online) during or immediately following 35% brown, when beetles were also found in associated traps. Mortality was greatest in 2022, when the largest amounts of beetles were captured (hundreds of beetles in single traps, Figure S3 available as Supplementary Data at *Tree Physiology* Online). Zero trees died in the legacy plot (Figure 1a), where associated traps never detected a single *I. confusus* beetle

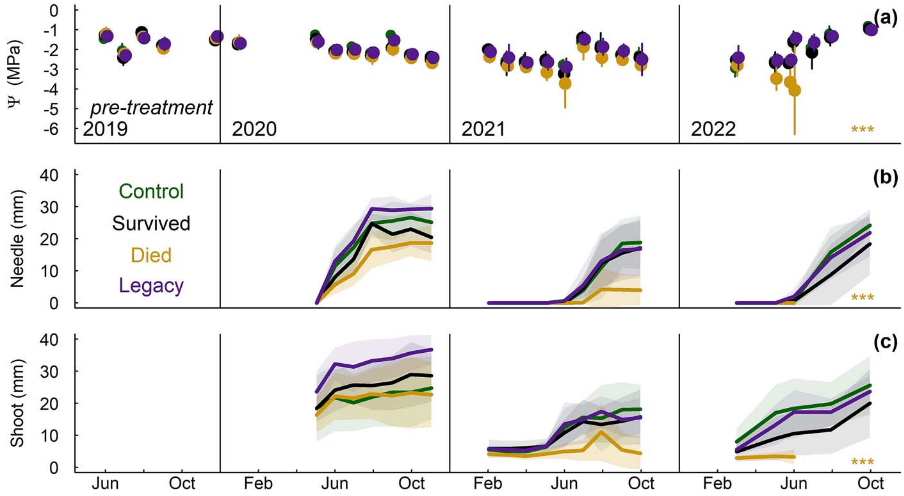

**Figure 2.** Pre-dawn water potential and growth in needles and twigs were reduced in trees that died; needle/twig NSC and needles/twig defenses did not differ among groups. Lines and shading denote mean ± standard deviation for control trees (green), legacy 45% trees (purple), droughted trees that survived (black), or droughted trees that died (yellow). (a) Pre-dawn water potential, (b) needle length and (c) shoot length. Data were collected monthly excepting a gap in 2020. *** indicates significant annual differences between dying trees and the control at $P < 0.001$ (HB-ANOVA).

(Figure S2 available as Supplementary Data at *Tree Physiology* Online) and where we did not observe any entrance holes or frass.

As already stated, defense concentrations were unrelated to throughfall exclusion intensity (e.g., phloem terpenes did not differ across new throughfall exclusion plots, Figure S4 available as Supplementary Data at *Tree Physiology* Online). Instead, we investigated whether dying trees ($n = 13$) substantially differed from control trees ($n = 6$), trees which survived new drought treatments ($n = 5$), or trees in the 'legacy' long-term drought treatment ($n = 6$). We found trees that died exhibited lower $\Psi_{PD}$ than control trees across all 3 years ($P < 0.001$, Figure 2a). On average, $\Psi_{pd}$ in trees that died was more negative than in control trees by 0.7 MPa (August 2020), 1.0 MPa (June 2021), and 2.4 MPa (June 2022). $\Psi_{pd}$ in control, survived, and legacy trees also appeared to recover to less negative values than trees that died during the summer monsoon periods in 2021 and 2022. In 2020, trees that survived new throughfall exclusion plots experienced similarly low $\Psi_{pd}$ to trees that died, but recovered to similar values as control trees in 2021, except at peak water stress in June (Figure 2a). Seasonal growth was significantly reduced in dying trees, where some trees in 2021 and particularly 2022 failed to grow needles at all ($P < 0.001$, Figure 2b; $P < 0.001$, Figure 2c). Non-structural carbohydrate concentrations did not differ among throughfall exclusion plots or survivorship categories in leaves, twigs, or phloem ($P > 0.05$, and see Peltier et al. 2023*b*, Thompson et al. 2024), and were more strongly driven by temporal variation across all trees (Figure S5 available as Supplementary Data at *Tree Physiology* Online). Secondary metabolite concentrations were highly variable across time, but we detected no differences that did not pre-date treatment (Figures S5–S7 available as Supplementary Data at *Tree Physiology* Online), and variability in defense concentrations was not explained by NSC concentrations (Figure S8 available as Supplementary Data at *Tree Physiology* Online).

## H1: Crown defenses increase with drought stress

Concentrations of certain classes of secondary metabolites increased with drought stress (more negative $\Psi_{pd}$, Figure 3). Twig monoterpene concentrations in dying trees increased as $\Psi_{pd}$ declined, when relativized as a percentage of values in control trees (Eq. (1), $P < 0.01$ from linear mixed-effects, conditional $R^2 = 0.62$, marginal $R^2 = 0.20$, Figure 3a). Across all trees, leaf phenolic concentrations also increased with more negative $\Psi_{pd}$ in control ($P < 0.001$, conditional $R^2 = 0.31$, marginal $R^2 = 0.26$, Figure 3b). According to the average sensitivity of leaf phenolic concentrations to $\Psi_{pd}$, trees exhibiting $\Psi_{pd} = -4$ MPa had 62% more leaf phenolics than trees with $\Psi_{pd} = -1$ MPa. According to the average sensitivity of relativized dying tree twig monoterpenes to $\Psi_{pd}$, dying trees at $\Psi_{pd} = -1$ MPa had around 24% less twig monoterpenes than control trees, but 26% more twig monoterpenes than control trees at −4 MPa.

## H1: Phloem terpenes only reduced after 10+ years of drought

Concentrations of total phloem monoterpenes and sesquiterpenes did not differ between surviving and dying trees nor were they significantly different from control trees (Figure 4a–d). For monoterpenes, control trees contained $2.5 \pm 1.7$ mg g$^{-1}$ FW compared with $1.8 \pm 1.2$ (surviving) or $1.8 \pm 1.4$ (dying) mg g$^{-1}$ FW (mean ± sd; Figure 4a). Time-series demonstrate substantial variability within treatments (Figure 4b). Trends for sesquiterpenes among different survivorship categories were similar to those in monoterpenes (Figure 4c and d). Phloem phenolic concentrations were significantly higher in surviving trees than in control trees ($P < 0.05$) but did not differ from dying trees or legacy trees (Figure 4e). Terminal phloem concentrations (that is, just prior to mortality) showed no drought-associated reductions compared with control trees: dying trees contained 94 ± 65% (monoterpenes), 94 ± 42% (sesquiterpenes), and 139 ± 53% (phenolics) of contemporaneous control concentrations (mean ± sd). These terminal concentrations are an estimate of conditions encountered by attacking beetles, measured around 1 month prior to crossing the 35% browning threshold ('point of no return'). We note that there were no significant differences among phloem secondary metabolite concentrations prior to our 2020–22 drought

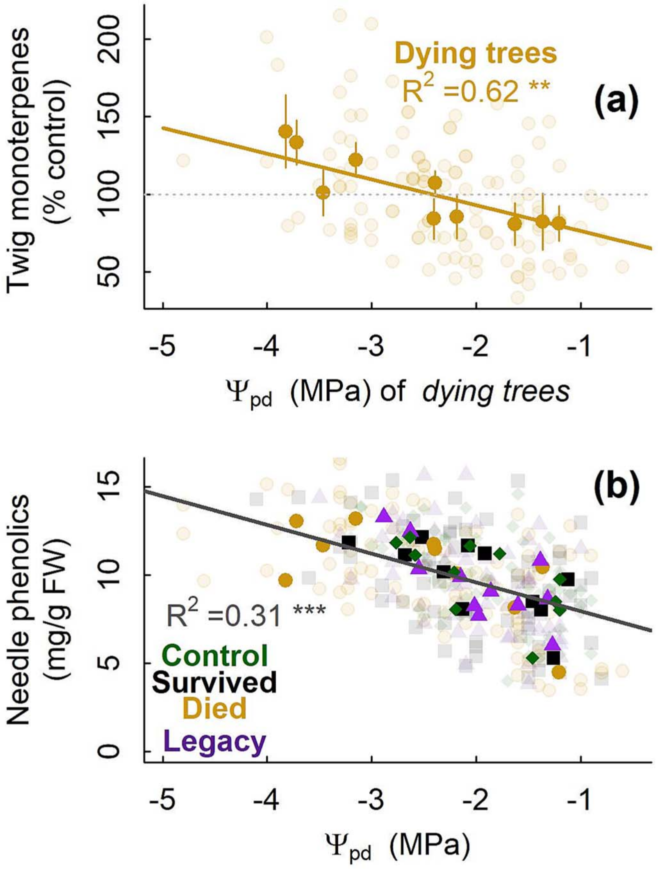

**Figure 3.** Relationships of twig total monoterpenes and needle phenolics with water potential ($\Psi_{pd}$). (a) Dying tree twig monoterpenes as a percentage of control tree twig monoterpenes on the same sampling date (see Eq. (1)). Opaque symbols denote unique category-date mean ± sd (vertical lines). Line denotes fit of a linear mixed effects model to all observations in dying trees. (b) Needle phenolics, where opaque symbols denote unique category-date means. ** and *** indicate significance levels of $P < 0.01$ or $P < 0.001$ (linear mixed effects model), respectively. Conditional $R^2$ values are displayed on each panel, and marginal $R^2$ values are reported in Results.

treatments (Figure S9 available as Supplementary Data at *Tree Physiology* Online). We also found no differences among survivorship categories in induced defense responses (e.g., total terpene concentration) in the phloem in 2022 following induction with *Ophiostoma* fungus (Figure S10 available as Supplementary Data at *Tree Physiology* Online).

However, extended experimental drought roughly halved the concentrations of phloem terpenes as compared with the control. Legacy trees contained 49% lower phloem monoterpene concentrations (1.27 ± 0.98 mg $g^{-1}$FW, $P < 0.05$, Figure 4a) and 45% lower phloem sesquiterpene concentrations (1.0 ± 0.67 mg $g^{-1}$FW, $P < 0.05$, Figure 4c) than control trees. There were no significant differences in phloem phenolic concentrations between legacy and control trees (average across trees = 6.6 ± 2.4 mg $g^{-1}$FW, Figure 4e and f).

## H1: Induced resin flow was greater in surviving than dying trees

We found no significant differences in resin flow among survivorship categories during 2020–21, nor in resin flow after induction in 2022 (2022 results in Figure 5a, 2019–21 in Figure S11 available as Supplementary Data at *Tree Physiology* Online) likely due to high variability. While we report quantitative comparisons among treatments, none of the reported differences is statistically significant ($P > 0.05$, HB-ANOVA). During 2022, after 3 years of treatment and following inoculation with *Ophiostoma* fungus to simulate beetle attack, trees that survived exuded 154% more resin than trees that died (Figure 5a). Across all sampling dates, the number of cases of zero resin flow during a single sampling period was 4 (control), 2 (survive), 16 (dying), and 9 (legacy). At the same time, despite dying trees exhibiting the most zero-flow events, the highest observed resin flow was also observed in a dying tree (15.4 mg in 48 h, July 2021). Legacy trees in 2022 exuded the least amount of resin at 0.85 ± 0.9 mg per 48 h, less than surviving trees (−68%) and control trees (−49%, Figure 5a).

## H2: Drought stressed trees exude higher $^{14}$C-mean age resin

The $\Delta^{14}$C of resin was between 0.2 ± 1.4‰ and 30.5 ± 1.5‰, reflecting $^{14}$C-mean ages of 3–10 years at this site (average 5.2 ± 1.8 years, Figure 5b). Average resin $^{14}$C-mean age in the control plot was 5.6 ± 2.1 years, 4.8 ± 1.8 years in surviving trees, 6.0 ± 1.8 years in dying trees, and 4.3 ± 0.9 years in legacy trees. There was no evidence resin age significantly differed among survivorship categories, and resin age was uncorrelated with resin flow mass (data not shown). Consistent with (H2), resin $^{14}$C-mean age was highest in trees with low phloem sugar concentrations (Figure 5c, marginal $P < 0.05$) and more negative water potentials (Figure 5d, marginal $P < 0.01$; $R^2 = 0.58$). This multiple regression

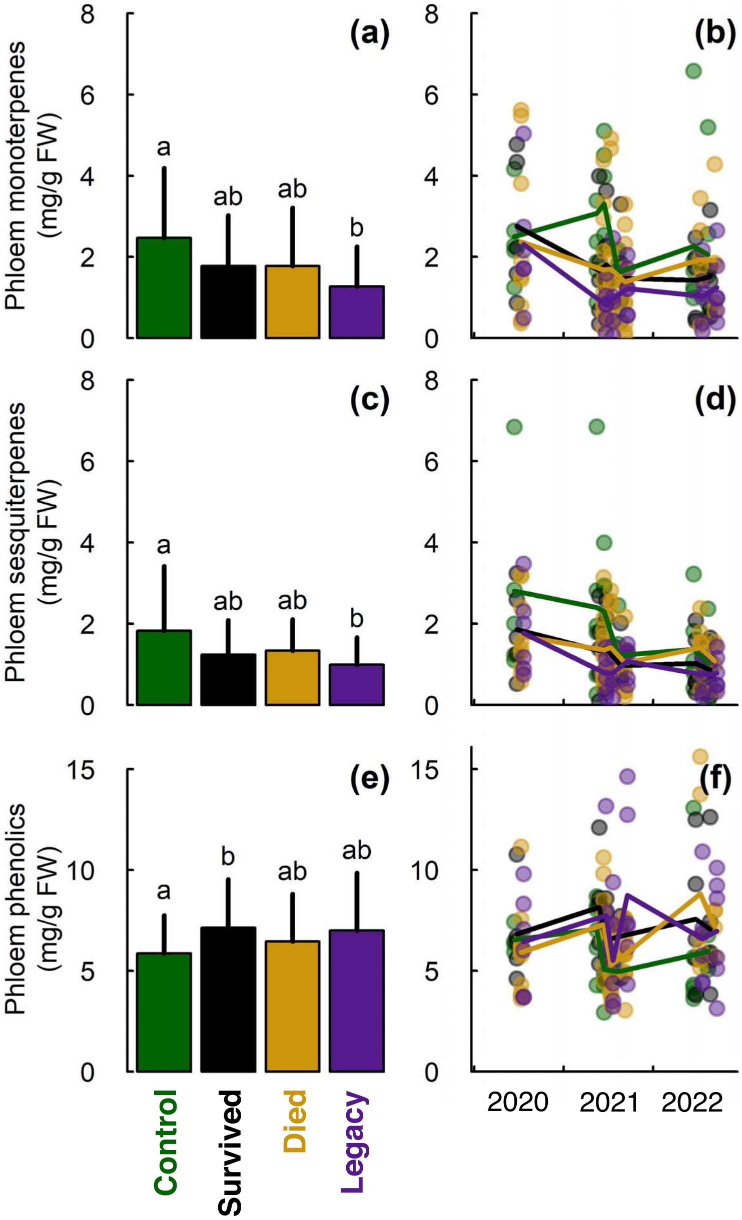

**Figure 4.** Phloem secondary metabolites during the treatment period. (a, c, e) Mean ± sd and (b, d, f) time-series of phloem secondary for control (green), survived (black), died (yellow), and legacy (purple) trees. Lowercase letters denote significant differences among survivorship categories ($p < 0.05$, HB-ANOVA).

relationship was not robust to the replacement of missing observations with 1-month lagged values; however, a univariate regression on $\Psi_{pd}$ with replacement of observations was significant ($P < 0.05$, $R^2 = 0.21$) and similarly described a negative effect of $\Psi_{pd}$ on resin age.

## Discussion

We found no evidence that carbon allocation to chemical defenses was limited by short-term drought, even in dying trees. Instead, similar to previous findings of carbon starvation after long-term drought, we found that reduction of resin-based defenses only occurs after a decade of water limitation (Peltier et al. 2023*b*). Drought-induced mortality of *P. edulis* in the absence of *I. confusus* attack appears rare in our study site, even under extremely prolonged drought. All dying trees were attacked by bark beetles, with main stems covered with entrance holes (Figure 1, Figure S2 available as Supplementary Data at *Tree Physiology* Online). Because bark beetles impose intense and recurrent herbivore pressure, investment in defense against such specialized and often lethal herbivores in this system may be paramount for tree survival (Gaylord et al. 2013). Our measurements across organs and classes of secondary metabolites show that *P. edulis* trees maintain concentrations of defensive compounds during severe and ultimately lethal drought, suggesting active prioritization of chemical defense under drought stress. Relative to controls, even dying trees exhibited similar or increased concentrations of monoterpenes and phenolics in leaves and twigs as water potential declined (Figure 3). In the main stem, trees subjected to drought for 2–3 years had similar or enhanced secondary metabolite concentrations when compared with

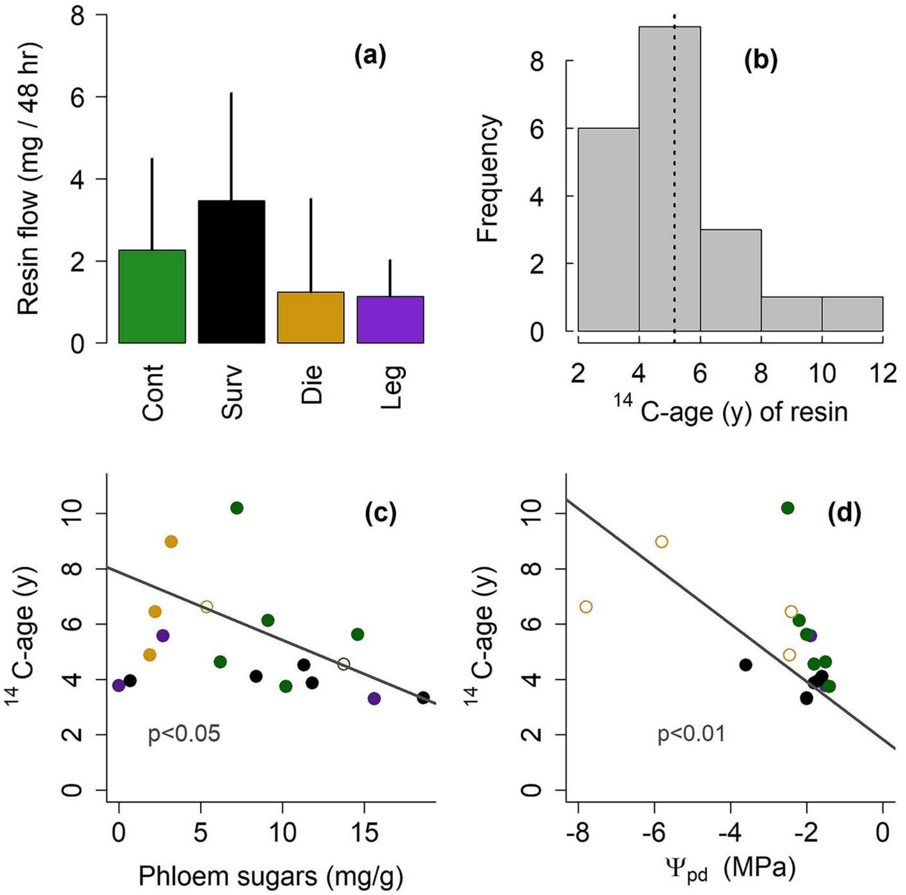

**Figure 5.** Post-inoculation resin flow rate and $^{14}$C-mean age, and response of $^{14}$C-mean age to both sugars and $\Psi_{pd}$ from Eq. (2). All data are from the inoculation experiment in 2022; resin flow did not differ among categories during the pre-treatment period, or in 2020 or 2021 (Figure S11 available as Supplementary Data at *Tree Physiology* Online). (a) Mean ± sd of 2022 resin flow across control (green), surviving (black), dying (yellow), and legacy 45% trees (purple). (b) Average $^{14}$C-mean age of oleoresin is 5.2 ± 1.8 years (dotted vertical line); $^{14}$C-mean age did not differ among categories. In (c, d), filled circles are contemporaneous (c) phloem sugar concentrations or (d) $\Psi_{pd}$, where 1-month prior values are also shown as unfilled circles for trees where contemporaneous values were missing (see Materials and methods). Oleoresin is older in trees with (c) lower contemporaneous phloem sugar concentrations ($\alpha_2$, effect $P < 0.05$) and (d) more negative contemporaneous $\Psi_{pd}$ ($\alpha_3$, effect $P < 0.01$). In (c,d), results are from one multiple regression where total $R^2 = 0.58$ (see Eq. (2)); lines denote marginal slopes.

control trees (Figure 4), and some of the highest concentrations of phenolics were observed in dying trees late in the experiment (Figure 4b and f). The only evidence of reduced concentrations of defense compounds occurred after more than a decade of drought in the legacy plot—a chronic, long-term drought plot (Figure 4). Like tree carbon reserves, pools of resin-based defenses in mature trees are large, built up over many years and have long residence times (Figures 4 and 5). Reduction of these pools under transient drought conditions may then be unlikely, and challenges the idea that trees are attacked by beetles during drought because the amounts or concentrations of defenses are constrained by carbohydrate allocation.

Similar to Trowbridge et al. (2021), we found no evidence for drought constraints on defense concentrations in leaves or twigs, despite this study coinciding with the most severe meteorological drought in a millennium (Williams et al. 2022). Consistent with the major role of hydraulic failure in drought mortality (Adams et al. 2017, McDowell et al. 2022), $\Psi_{pd}$ in dying trees declined to values below critical thresholds ($p_{50} = -4.4$ MPa, Hudson et al. 2018), and we observed $\Psi_{pd}$ below −6 MPa in some dying trees. Allocation to secondary metabolites may be prioritized under stress, as suggested for Norway spruce that maintained secondary metabolite concentrations even under reduced $CO_2$ (Huang et al. 2019). Here, some droughted trees failed to grow new leaves in 2021 and 2022 (Figure 2), where only minimal allocation may be required to maintain concentrations of defenses in existing leaves. Observations of increased canopy twig monoterpene concentrations (Figure 3a) could be particularly adaptive as *I. confusus* attacked branches here and in previous studies (Gaylord et al. 2013). Plants may anticipate certain biotic stress through perception of (and correlation with) abiotic conditions and/or phenology (Mertens et al. 2021). However, maintenance or passive increases of defense concentrations under drought could also result from reduced volatile emissions at more negative $\Psi_{pd}$ (Trowbridge et al. 2019) and the long residence times of many defense compounds (discussed later). The induction of new defense production is also an important response to attack. But while resin flow in dying trees was low, resin flow in droughted trees did not differ significantly from the control, providing no clear evidence for a drought effect on physical resin exudation (Figure 5a). We also found no difference in induced terpene concentrations following inoculations in the phloem (i.e., inner bark) among survivorship categories (Figure S10 available as Supplementary Data at *Tree Physiology* Online). Regardless of mechanism, maintenance or enhanced production of chemical defenses like terpenes during drought would be advantageous, as the probability of bark beetle attack becomes more likely. At the same time, not all secondary metabolites are produced strictly for defense and may not serve identical roles across different beetle–host systems. Phenolics are important for scavenging of reactive oxygen species (Karuppanapandian et al. 2011). And while phenolics inhibit beetle-vectored fungal symbionts in the European beetle *Ips typographus* (Sallé et al. 2005, Zhao et al. 2019, Korolyova et al. 2022) the role of phenolics in defense against other beetles such as *Dendroctonus ponderosae* is less clear (Erbilgin et al. 2017*b*) and is completely unknown for *I. confusus*.

One explanation for how trees are overwhelmed by beetles during drought is that drought stress reduces the production of defensive compounds, but while we found nearly 50% reductions in mono- and sesquiterpene concentrations in the main stem, this required more than a decade of experimental drought (Figure 4). Short-term drought appeared to have weak impacts on resin-based defense concentrations. If old carbon reserves support the production of chemical defenses, or defense compounds have long residence times (as suggested by $\Delta^{14}$C measurements of resin, discussed below), defense concentrations may be buffered against short-term drought. Stem carbon reserve pools are large (Hoch et al. 2003) and in *P. edulis* may require a decade of drought to show substantial reductions (Peltier et al. 2023*b*). Meta-analysis also suggests that long drought durations are required to reduce NSC concentrations (He et al. 2020). Consistent with this interpretation, total monoterpene concentrations were reduced by around 49% after 10 + years of drought in the legacy plot relative to control trees (Figure 4a and b), but there were no significant differences among control trees and trees under short-term drought ('surviving' or 'dying' trees). If these results are general, widespread observations of bark beetle-associated mortality may partly be an outcome of drier average conditions and shorter inter-drought return times, rather than single extremely dry years (Peltier et al. 2022, Williams et al. 2022).

Consistent with slow reductions of defense concentrations in tree stems, resin-based terpene pools were up to a decade old, suggesting that reductions in resin amounts may only occur after persistent, sustained drought stress (Figure 5; Kolb et al. 2019). Differentiating existing, constitutive resin stores from newly produced resin is challenging if only measuring resin flow, which is often extremely variable across trees and time points (Gaylord et al. 2013, Hood and Sala 2015). Short-term drought did not reduce stem resin exudation in surviving or dying trees compared with control trees (Figure 5a). While dying trees often produced no resin, many such 'zeroes' reflect the last measurement of dying individuals, when resin networks were potentially already disrupted by attacking beetles. The single largest resin flow measurement we observed also occurred in a dying tree. Old $^{14}$C-mean ages of resin suggest either that exuded resin is largely constitutive at 3–10 years old, derived from xylem and phloem duct networks (Krokene and Nagy 2012), or that 3- to 10-year-old reserves were used to synthesize new resin via an induced defense response (Thompson et al. 2024). Trees can remobilize very old carbon reserves in response to disturbance, previously observed toward new tissue growth (Vargas et al. 2009, Carbone et al. 2013, Muhr et al. 2016, D'Andrea et al. 2019, Peltier et al. 2023*a*) or respiration (Muhr et al. 2018). Regardless of whether $^{14}$C-mean ages reflect old resin storage, old carbon reserves or some combination thereof, $^{14}$C-mean ages suggest that reduction of resin amounts is unlikely under short-term drought. Consistent with this interpretation, individual trees with lower sugars and more negative $\Psi_{\mathrm{pd}}$ exuded resin with older carbon (Figure 5c and d), again implying either old resin or new resin produced from old reserves was mobilized in drought stressed trees.

Given both no differences in terpene or phenolic concentrations between trees that died from control trees and no mortality in legacy plot trees despite reduced stem terpene concentrations, other dimensions of host suitability should be considered. While beetles may simply associate with trees that are already dying, the lack of mortality or beetles in the legacy plot, despite reduced $\Psi_{\mathrm{pd}}$ (Figure 2a), reduced growth (Figure 2b and c), and reduced sapwood NSC and radial growth (Peltier et al. 2023), suggests that beetle attack is required to kill trees at this site. Focus on specific compounds with known roles in bark beetle ecology, such as $\alpha$-pinene, limonene, myrcene, etc., could be more informative for understanding tree defense against bark beetles (Erbilgin et al. 2017*b*, Trowbridge et al. 2021). However, in extensive preliminary analyses, we found no significant differences among throughfall exclusion plots in concentrations of specific compounds measured here. Perhaps very small differences may be important, but functional information on specific metabolites is lacking for *I. confusus*, among other species that are becoming emerging threats under novel conditions (e.g., *Phloeosinus* spp.: Stephenson et al. 2018, Verrier 2022, Wuenschel et al. 2023). Quantification of dose-dependent toxicity of specific secondary metabolites to *I. confusus* beetles is essential, as the modes of action and lethal concentrations of specific metabolites are almost certainly species-specific or perhaps even population-specific (Chiu et al. 2017). Beetle–conifer host systems represent the outcome of long co-evolutionary processes such that other aspects of beetle biology and behavior may also be relevant (Krokene and Nagy 2012). Beetle foraging and detection of suitable host trees via volatile emissions of terpenes from the crown could have thus played a role in which trees were ultimately selected and killed by bark beetles (Trowbridge et al. 2019). It is also possible that trees in the legacy plot may no longer be nutritionally valuable to beetles, and so are not attacked.

Thus, there are many potential reasons beetles attack certain trees (e.g., nutritional quality Basile et al. 2024), but our results demonstrate that a reduction in the total concentrations of terpene and phenolic defenses or even specific metabolites is not a major determinant of bark beetle-induced mortality in *P. edulis*. One hypothesis that could be investigated in the future is that differences in the mobility of resin-based defenses may impact survival, as suggested by a greenhouse study in *P. edulis* (Malone et al. 2025). There was a tendency for dying trees to be more likely to have no resin flow during a sampling period, suggesting that resin flow may distinguish survivors during dry conditions. Across all sampling dates, the number of cases of zero resin flow during a single sampling period was 4 (control), 2 (survive), 16 (dying), and 9 (legacy). There is older evidence that *I. confusus* and closely related species like *Ips paraconfusus* cannot successfully colonize trees with appreciable resin flow (Wood 1962). Entrance holes we observed in dying *P. edulis* trees only contained small amounts of visible resin (Figure S2 available as Supplementary Data at *Tree Physiology* Online). Low water potentials (Figure 2a) and limited sapwood NSC (see Peltier et al. 2023*b*) could limit turgor in the epithelial cells lining resin ducts: these cells are partially responsible for generating the positive pressure required to exude resin (Vité 1961, Cabrita 2018). Then, we hypothesize that dying trees could have contained adequate amounts of resin, but insufficient turgor pressure to exude it (Malone et al. 2025). Future work would be required to assess this hypothesis.

*Pinus edulis* is a model species for tree mortality research due to its small stature and occurrence in dry forests, yet study of the piñon–*I. confusus* relationship is relatively new, as these beetles were not widely observed to be lethal prior to the early 2000s in the Southwest (Raffa et al. 2008). Widespread beetle-associated mortality in numerous pine species under drought has often been presumed to reflect limited production of defense compounds under moisture stress (e.g., Anderegg et al. 2015). While we find evidence for drought-induced reductions in stem terpene concentrations, these are only evident after more than a decade of drought—and did not lead to tree death from bark beetle attack (Figure 1a). Instead, even trees that ultimately died had enhanced production of terpenes and phenolics in leaves and twigs as drought stress progressed (Figure 3). Under short drought duration, stem terpene concentrations did not differ from the control, suggesting that secondary metabolite production for defense is only weakly constrained by drought, consistent with trees maintaining large pools of old resin and/or building new resin using old carbon reserves (Figure 5). Our results suggest that, like carbon reserves, tree defenses are resilient to drought stress, maintained at high levels right up to the point of mortality.

## Acknowledgments

We thank the Sevilleta LTER for supporting the study. We thank Diana Six for isolating the *Ophiostoma* fungus used in inoculations. Henry Adams and Amy Trowbridge both hold the position of Editor for *Tree Physiology* and have not peer reviewed or made any editorial decisions for this paper.

## Supplementary Data

Supplementary data for this article are available at *Tree Physiology* Online.

## Author contributions

S.C.M. quantified secondary metabolites. R.A.T. quantified nonstructural carbohydrates. D.M.P.P. performed radiocarbon preparation steps. D.M.P.P., C.D.M., S.C.M., R.A.T., K.A.P., H.D.A. and A.M.T. collected field data. D.M.P.P. wrote the first draft of the manuscript and conducted the analysis with input from N.G.M., H.D.A. and A.M.T. All authors contributed to revisions.

## Conflict of interest

None declared.

## Funding

We acknowledge awards from NSF-IOS #1936205, 1755345 and 1755346, and NSF GRFP 1-842493. H.D.A. was also supported by the US Department of Agriculture, National Institute of Food and Agriculture, McIntire Stennis project 1019284.

## Data availability

All data will be made available in a Zenodo repository upon acceptance for publication.

## References

Adams HD, Collins AD, Briggs SP, Vennetier M, Dickman LT, Sevanto SA, Garcia-Forner N, Powers HH, McDowell NG (2015) Experimental drought and heat can delay phenological development and reduce foliar and shoot growth in semiarid trees. Glob Change Biol 21:4210–4220.

Adams HD, Zeppel MJ, Anderegg WR et al. (2017) A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. Nat Ecol Evol 1:1285–1291. https://doi.org/10.1038/s41559-017-0248-x.

Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc 2:875–877.

Allen CD, Breshears DD, McDowell NG (2015) On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere 6:art129.

Anderegg WR, Hicke JA, Fisher RA et al. (2015) Tree mortality from drought, insects, and their interactions in a changing climate. New Phytol 208:674–683. https://doi.org/10.1111/nph.13477.

Anderegg WRL, Trugman AT, Badgley G et al. (2020) Climate-driven risks to the climate mitigation potential of forests. Science 368:eaaz7005.

Barbour J, Palmer M (2024) Mature and old-growth forests: definition, identification, and initial inventory on lands managed by the Forest Service and Bureau of Land Management. US Department of Agriculture, Forest Service.

Basile S, Stříbrská B, Kalyniukova A, Hradecký J, Synek J, Gershenzon J, Jirošová A (2024) Physiological and biochemical changes of *Picea abies* (L.) during acute drought stress and their correlation with susceptibility to *Ips typographus* (L.) and *I. duplicatus* (Sahlberg). Front For Glob Change 7:1436110.

Boutton TW, Wong WW, Hachey DL, Lee LS, Cabrera MP, Klein PD (1983) Comparison of quartz and pyrex tubes for combustion of organic samples for stable carbon isotope analysis. Anal Chem 55:1832–1833.

Breshears DD, Cobb NS, Rich PM et al. (2005) Regional vegetation die-off in response to global-change-type drought. Proc Natl Acad Sci USA 102:15144–15148.

Cabrita P (2018) Resin flow in conifers. J Theor Biol 453:48–57. https://doi.org/10.1016/j.jtbi.2018.05.020.

Carbone MS, Czimczik CI, Keenan TF, Murakami PF, Pederson N, Schaberg PG, Xu X, Richardson AD (2013) Age, allocation and availability of nonstructural carbon in mature red maple trees. New Phytol 200:1145–1155. https://doi.org/10.1111/nph.12448.

Chiu CC, Keeling CI, Bohlmann J (2017) Toxicity of pine monoterpenes to mountain pine beetle. Sci Rep 7:8858.

Cipollini D, Wang Q, Whitehill JG, Powell JR, Bonello P, Herms DA (2011) Distinguishing defensive characteristics in the phloem of ash species resistant and susceptible to emerald ash borer. J Chem Ecol 37:450–459. https://doi.org/10.1007/s10886-011-9954-z.

D'Andrea E, Rezaie N, Battistelli A et al. (2019) Winter's bite: beech trees survive complete defoliation due to spring late-frost damage by mobilizing old C reserves. New Phytol 224:625–631. https://doi.org/10.1111/nph.16047.

Dickman LT, McDowell NG, Sevanto S, Pangle RE, Pockman WT (2015) Carbohydrate dynamics and mortality in a piñon-juniper woodland under three future precipitation scenarios. Plant Cell Environ 38:729–739. https://doi.org/10.1111/pce.12441.

Erb M, Kliebenstein DJ (2020) Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. Plant Physiol 184:39–52. https://doi.org/10.1104/pp.20.00433.

Erbilgin N, Cale JA, Hussain A, Ishangulyyeva G, Klutsch JG, Najar A, Zhao S (2017*a*) Weathering the storm: how lodgepole pine trees survive mountain pine beetle outbreaks. Oecologia 184:469–478. https://doi.org/10.1007/s00442-017-3865-9.

Erbilgin N, Cale JA, Lusebrink I, Najar A, Klutsch JG, Sherwood P, Bonello P, Evenden ML (2017*b*) Water-deficit and fungal infection can differentially affect the production of different classes of defense compounds in two host pines of mountain pine beetle. Tree Physiol 37:338–350. https://doi.org/10.1093/treephys/tpw105.

Erbilgin N, Zanganeh L, Klutsch JG et al. (2021) Combined drought and bark beetle attacks deplete non-structural carbohydrates and promote death of mature pine trees. Plant Cell Environ 44: 3866–3881.

Everaerts C, Grégoire J-C, Merlin J (1988) The toxicity of Norway spruce monoterpenes to two bark beetle species and their associates. In: Mattson WJ, Levieux J, Bernard-Dagan C (eds) Mechanisms of woody plant defenses against insects. Springer New York, New York, NY, pp 335–344.

Fettig CJ, Grosman DM, Munson AS, Moan JE (2024) Protecting conifers from bark beetles (Coleoptera: Curculionidae, Scolytinae) with insecticides in the western United States. J Entomol Sci 60:157–185.

Gaylord ML, Kolb TE, Pockman WT, Plaut JA, Yepez EA, Macalady AK, Pangle RE, McDowell NG (2013) Drought predisposes piñon–juniper woodlands to insect attacks and mortality. New Phytol 198: 567–578. https://doi.org/10.1111/nph.12174.

Gely C, Laurance SGW, Stork NE (2020) How do herbivorous insects respond to drought stress in trees? Biol Rev 95:434–448. https://doi.org/10.1111/brv.12571.

Gershenzon J (1994) Metabolic costs of terpenoid accumulation in higher plants. J Chem Ecol 20:1281–1328. https://doi.org/10.1007/BF02059810.

Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3:408–414.

Guérard N, Maillard P, Bréchet C, Lieutier F, Dreyer E (2007) Do trees use reserve or newly assimilated carbon for their defense reactions? A 13C labeling approach with young Scots pines inoculated with a bark-beetle-associated fungus (*Ophiostoma brunneo ciliatum*). Ann For Sci 64:601–608.

Hammond WM, Yu K, Wilson LA, Will RE, Anderegg WR, Adams HD (2019) Dead or dying? Quantifying the point of no return from hydraulic failure in drought-induced tree mortality. New Phytol 223: 1834–1843. https://doi.org/10.1111/nph.15922.

He W, Liu H, Qi Y, Liu F, Zhu X (2020) Patterns in nonstructural carbohydrate contents at the tree organ level in response to drought duration. Glob Change Biol 26:3627–3638.

Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. Plant Cell Environ 26:1067–1081.

Hood S, Sala A (2015) Ponderosa pine resin defenses and growth: metrics matter. Tree Physiol 35:1223–1235. https://doi.org/10.1093/treephys/tpv098.

Hua Q, Turnbull JC, Santos GM et al. (2022) Atmospheric radiocarbon for the period 1950–2019. Radiocarbon 64:723–745.

Huang J, Hammerbacher A, Weinhold A et al. (2019) Eyes on the future–evidence for trade-offs between growth, storage and defense in Norway spruce. New Phytol 222:144–158. https://doi.org/10.1111/nph.15522.

Huber DP, Gries R, Borden JH, Pierce HD Jr (2000) A survey of antennal responses by five species of coniferophagous bark beetles (Coleoptera: Scolytidae) to bark volatiles of six species of angiosperm trees. Chemoecology 10:103–113.

Hudson PJ, Limousin JM, Krofcheck DJ, Boutz AL, Pangle RE, Gehres N, McDowell NG, Pockman WT (2018) Impacts of long-term precipitation manipulation on hydraulic architecture and xylem anatomy of piñon and juniper in Southwest USA. Plant Cell Environ 41:421–435. https://doi.org/10.1111/pce.13109.

Karuppanapandian T, Moon J-C, Kim C, Manoharan K, Kim W (2011) Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Aust J Crop Sci 5:709–725.

Klepzig KD, Kruger EL, Smalley EB, Raffa KF (1995) Effects of biotic and abiotic stress on induced accumulation of terpenes and phenolics in red pines inoculated with bark beetle-vectored fungus. J Chem Ecol 21:601–626. https://doi.org/10.1007/BF02033704.

Kolb T, Keefover-Ring K, Burr SJ, Hofstetter R, Gaylord M, Raffa KF (2019) Drought-mediated changes in tree physiological processes weaken tree defenses to bark beetle attack. J Chem Ecol 45:888–900. https://doi.org/10.1007/s10886-019-01105-0.

Kolb TE, Guerard N, Hofstetter RW, Wagner MR (2006) Attack preference of *Ips pini* on *Pinus ponderosa* in northern Arizona: tree size and bole position. Agric For Entomol 8:295–303.

Körner C (2003) Carbon limitation in trees. J Ecol 91:4–17.

Korolyova N, Buechling A, Lieutier F, Yart A, Cudlín P, Turčáni M, Jakuš R (2022) Primary and secondary host selection by *Ips typographus* depends on Norway spruce crown characteristics and phenolic-based defenses. Plant Sci 321:111319.

Krokene P, Nagy NE (2012) Anatomical aspects of resin-based defences in pine. Pine Resin Biol Chem Appl 661:67–86.

Krokene P, Nagy NE, Krekling T (2008) Traumatic resin ducts and polyphenolic parenchyma cells in conifers. In: Induced plant resistance to herbivory. Dordrecht: Springer Netherlands, pp 147–169.

LaBonte JR, Valley SA (2019) Illustrated key to the species of *Ips*, *Orthotomicus*, and *Pseudips* of North America (or spines, spines, and more spines). Salem, OR: Oregon Department Agriculture.

Landhäusser SM, Chow PS, Dickman LT et al. (2018) Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. Tree Physiol 38:1764–1778. https://doi.org/10.1093/treephys/tpy118.

Lehmanski LMA, Kandasamy D, Andersson MN, Netherer S, Alves EG, Huang J, Hartmann H (2023) Addressing a century-old hypothesis – do pioneer beetles of *Ips typographus* use volatile cues to find suitable host trees? New Phytol 238:1762–1770. https://doi.org/10.1111/nph.18865.

Lerdau M, Litvak M, Monson R (1994) Plant chemical defense: monoterpenes and the growth-differentiation balance hypothesis. Trends Ecol Evol 9:58–61. https://doi.org/10.1016/0169-5347(94)90269-0.

Lindgren BS (1983) A multiple funnel trap for scolytid beetles (Coleoptera). Can Entomol 115:299–302.

Lombardero MJ, Ayres MP, Lorio PL Jr, Ruel JJ (2000) Environmental effects on constitutive and inducible resin defences of *Pinus taeda*. Ecol Lett 3:329–339.

Lorio PL Jr. 1993. Environmental stress and whole-tree physiology. In: Beetle-pathogen interactions in conifer forests. Academic Press; p 81–101.

Luchi N, Ma R, Capretti P, Bonello P (2005) Systemic induction of traumatic resin ducts and resin flow in Austrian pine by wounding and inoculation with *Sphaeropsis sapinea* and *Diplodia scrobiculata*. Planta 221:75–84.

Malone SC, Thompson RA, Chow PS, De Oliveira CR, Landhäusser SM, Six DL, McCulloh KA, Adams HD, Trowbridge AM (2025) Water, not carbon, drives drought-constraints on stem terpene defense against simulated bark beetle attack in *Pinus edulis*. New Phytol 245:318–331. https://doi.org/10.1111/nph.20218.

McDowell N, Pockman WT, Allen CD et al. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178: 719–739. https://doi.org/10.1111/j.1469-8137.2008.02436.x.

McDowell NG, Beerling DJ, Breshears DD, Fisher RA, Raffa KF, Stitt M (2011) The interdependence of mechanisms underlying climate-driven vegetation mortality. Trends Ecol Evol 26:523–532. https://doi.org/10.1016/j.tree.2011.06.003.

McDowell NG, Fisher RA, Xu C et al. (2013) Evaluating theories of drought-induced vegetation mortality using a multimodel–experiment framework. New Phytol 200:304–321. https://doi.org/10.1111/nph.12465.

McDowell NG, Sapes G, Pivovaroff A et al. (2022) Mechanisms of woody plant mortality under rising drought, CO2, and vapor pressure deficit. Nat Rev Earth Environ 3:294–308.

Mertens D, Boege K, Kessler A, Koricheva J, Thaler JS, Whiteman NK, Poelman EH (2021) Predictability of biotic stress structures plant defence evolution. Trends Ecol Evol 36:444–456. https://doi.org/10.1016/j.tree.2020.12.009.

Monson RK, Trowbridge AM, Lindroth RL, Lerdau MT (2022) Coordinated resource allocation to plant growth–defense tradeoffs. New Phytol 233:1051–1066. https://doi.org/10.1111/nph.17773.

Muhr J, Messier C, Delagrange S, Trumbore S, Xu X, Hartmann H (2016) How fresh is maple syrup? Sugar maple trees mobilize carbon stored several years previously during early springtime sap-ascent. New Phytol 209:1410–1416. https://doi.org/10.1111/nph.13782.

Muhr J, Trumbore S, Higuchi N, Kunert N (2018) Living on borrowed time–Amazonian trees use decade-old storage carbon to survive for months after complete stem girdling. New Phytol 220:111–120. https://doi.org/10.1111/nph.15302.

Novick KA, Metzger S, Anderegg WRL et al. (2022) Informing nature-based climate solutions for the United States with the best-available science. Glob Change Biol 28:3778–3794.

Ogle K, Barber JJ, Barron-Gafford GA, Bentley LP, Cable JM, Huxman TE, Loik ME, Tissue DT (2015) Quantifying ecological memory in plant and ecosystem processes. Ecol Lett 18:221–235. https://doi.org/10.1111/ele.12399.

Ott DS, Davis TS, Mercado JE (2021) Interspecific variation in spruce constitutive and induced defenses in response to a bark beetle–fungal symbiont provides insight into traits associated with resistance. Tree Physiol 41:1109–1121. https://doi.org/10.1093/treephys/tpaa170.

Pangle RE, Hill JP, Plaut JA, Yepez EA, Elliot JR, Gehres N, McDowell NG, Pockman WT (2012) Methodology and performance of a rainfall manipulation experiment in a piñon–juniper woodland. Ecosphere 3:1–20.

Peltier DMP, Anderegg WRL, Guo JS, Ogle K (2022) Contemporary tree growth shows altered climate memory. Ecol Lett 25:2663–2674. https://doi.org/10.1111/ele.14130.

Peltier DMP, Carbone MS, Enright M, Marshall MC, Trowbridge AM, LeMoine JM, Koch GW, Richardson AD (2023*a*) Old reserves and ancient buds fuel regrowth of coast redwood after catastrophic fire. Nat Plants 9:1978–1985. https://doi.org/10.1038/s41477-023-01581-z.

Peltier DMP, Carbone MS, McIntire CD et al. (2023*b*) Carbon starvation following a decade of experimental drought consumes old reserves in *Pinus edulis*. New Phytol 240:92–104. https://doi.org/10.1111/nph.19119.

Peltier DMP, Carbone MS, Ogle K, Koch GW, Richardson AD (2024) Decades-old carbon reserves are widespread among tree species, constrained only by sapwood longevity. New Phytol 245:1468–1480. https://doi.org/10.1111/nph.20310.

Phillips MA, Croteau RB (1999) Resin-based defenses in conifers. Trends Plant Sci 4:184–190.

Plaut JA, Yepez EA, Hill J, Pangle R, Sperry JS, Pockman WT, Mcdowell NG (2012) Hydraulic limits preceding mortality in a piñon–juniper woodland under experimental drought. Plant Cell Environ 35:1601–1617. https://doi.org/10.1111/j.1365-3040.2012.02512.x.

R Core Team (2025) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raffa KF, Aukema BH, Bentz BJ, Carroll AL, Hicke JA, Turner MG, Romme WH (2008) Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. Bioscience 58:501–517.

Reid ML, Sekhon JK, LaFramboise LM (2017) Toxicity of monoterpene structure, diversity and concentration to mountain pine beetles, *Dendroctonus ponderosae*: beetle traits matter more. J Chem Ecol 43:351–361. https://doi.org/10.1007/s10886-017-0824-1.

Rigling A, Brühlhart H, Bräker OU, Forster T, Schweingruber FH (2003) Effects of irrigation on diameter growth and vertical resin duct production in *Pinus sylvestris* L. on dry sites in the central Alps, Switzerland. For Ecol Manage 175:285–296.

Rissanen K, Hölttä T, Bäck J, Rigling A, Wermelinger B, Gessler A (2021) Drought effects on carbon allocation to resin defences and on resin dynamics in old-grown scots pine. Environ Exp Bot 185:104410.

Sallé A, Monclus R, Yart A, Lieutier F (2005) Effect of phenolic compounds on the in vitro growth of two fungi associated with *Ips typographus*. For Pathol 35:298–304.

Sharkey TD, Singsaas EL (1995) Why plants emit isoprene. Nature 374:769.

Shriver RK, Yackulic CB, Bell DM, Bradford JB (2022) Dry forest decline is driven by both declining recruitment and increasing mortality in response to warm, dry conditions. Glob Ecol Biogeogr 31:2259–2269.

Stephenson NL, Das AJ, Ampersee NJ, Cahill KG, Caprio AC, Sanders JE, Williams AP (2018) Patterns and correlates of giant sequoia foliage dieback during California's 2012–2016 hotter drought. For Ecol Manage 419–420:268–278.

Stephenson NL, Das AJ, Ampersee NJ, Bulaon BM, Yee JL (2019) Which trees die during drought? The key role of insect host-tree selection Edwards D (ed). J Ecol 107:2383–2401.

Stewart JE, Harris FL, Otto K, Davis TS (2020) Responses of Engelmann spruce to inoculation with *Leptographium abietinum*, a symbiotic fungus of the North American spruce beetle. Can J For Res 50:465–472.

Synal H-A, Stocker M, Suter M (2007) MICADAS: a new compact radiocarbon AMS system. Nucl Instrum Methods Phys Res B 259:7–13.

Thompson RA, Malone SC, Peltier D et al. (2024) Local carbon reserves are insufficient for phloem terpene induction during drought in *Pinus edulis* in response to bark beetle-associated fungi. New Phytol 244:654–669. https://doi.org/10.1111/nph.20051.

Trowbridge AM, Adams HD, Collins A et al. (2021) Hotter droughts alter resource allocation to chemical defenses in piñon pine. Oecologia 197:921–938. https://doi.org/10.1007/s00442-021-05058-8.

Trowbridge AM, Stoy PC, Adams HD, Law DJ, Breshears DD, Helmig D, Monson RK (2019) Drought supersedes warming in determining volatile and tissue defenses of piñon pine (*Pinus edulis*). Environ Res Lett 14:065006.

Trumbore SE, Sierra CA, Hicks Pries CE (2016) Radiocarbon nomenclature, theory, models, and interpretation: measuring age, determining cycling rates, and tracing source pools. In: Schuur EAG, Druffel ERM, Trumbore SE, (eds.) Radiocarbon and climate change. Cham, Switzerland: Springer International Publishing, 45–82.

Vargas R, Trumbore SE, Allen MF (2009) Evidence of old carbon used to grow new fine roots in a tropical forest. New Phytol 182:710–718. https://doi.org/10.1111/j.1469-8137.2009.02789.x.

Verrier JT (2022) Size matters: mortality and decline in a regionally uncommon coniferous tree (*Hesperocyparis arizonica*) population, southeastern Arizona. Desert Plants 37:5–17.

Vité JP (1961) The influence of water supply on oleoresin exudation pressure and resistance to bark beetle attack in *Pinus ponderosa*. Inst Plant Res 21:37–66.

Wang D, Naito H, Nakajima T (2012) The toxicity of fenitrothion and permethrin. Insecticides—Pest Engineering. 85–98.

Werker E, Fahn A (1969) Resin ducts of *Pinus halepensis* Mill.–their structure, development and pattern of arrangement. Bot J Linn Soc 62:379–411.

Williams AP, Cook BI, Smerdon JE (2022) Rapid intensification of the emerging southwestern North American megadrought in 2020–2021. Nat Clim Change 12:232–234.

Wood DL (1962) Experiments on the interrelationship between oleoresin exudation pressure in *Pinus ponderosa* and attack by *Ips confusus* (Lec.) (Coleoptera: Scolytidae). Can Entomol 94:473–477.

Wotherspoon K, Wardlaw T, Bashford R, Lawson S (2014) Relationships between annual rainfall, damage symptoms and insect borer populations in mid-rotation *Eucalyptus nitens* and *Eucalyptus globulus* plantations in Tasmania: can static traps be used as an early warning device? Aust For 77:15–24. https://doi.org/10.1080/00049158.2013.871090.

Wu H, Hu Z (1997) Comparative anatomy of resin ducts of the Pinaceae. Trees 11:135.

Wuenschel A, Bartel JA, Bernal A (2023) Forty years of change in Piute cypress (*Hesperocyparis nevadensis*), a rare California tree, after frequent fire and drought. Aliso 41:52–66.

Zhao T, Kandasamy D, Krokene P, Chen J, Gershenzon J, Hammerbacher A (2019) Fungal associates of the tree-killing bark beetle, *Ips typographus*, vary in virulence, ability to degrade conifer phenolics and influence bark beetle tunneling behavior. Fungal Ecol 38: 71–79.

*Tree Physiology*, 2026, 46, tpag004
https://doi.org/10.1093/treephys/tpag004
Research paper