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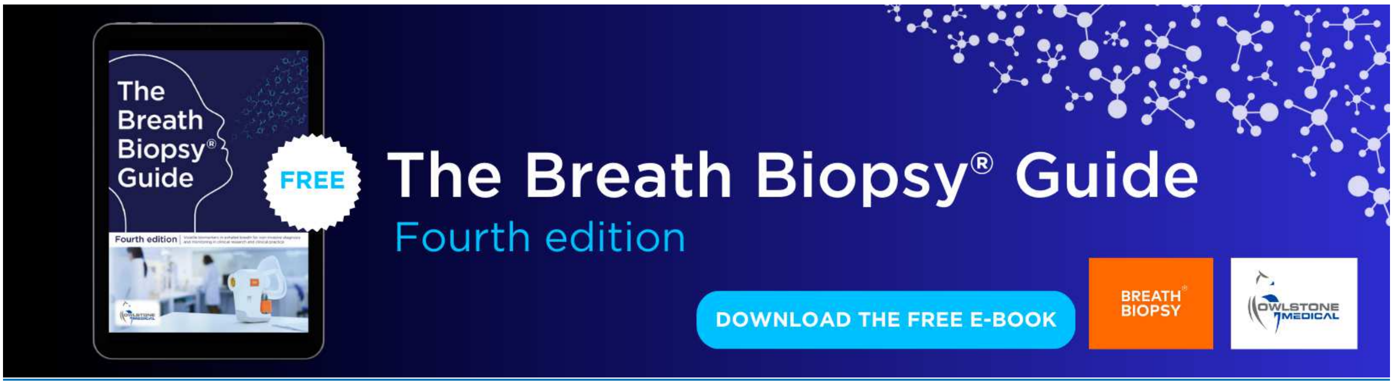

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LETTER

Drought supersedes warming in determining volatile and tissue defenses of piñon pine (*Pinus edulis*)

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Supplementary material for this article is available online

Abstract

Trees are suffering mortality across the globe as a result of drought, warming, and biotic attacks. The combined effects of warming and drought on *in situ* tree chemical defenses against herbivory have not been studied to date. To address this, we transplanted mature piñon pine trees—a well-studied species that has undergone extensive drought and herbivore-related mortality—within their native woodland habitat and also to a hotter-drier habitat and measured monoterpene emissions and concentrations across the growing season. We hypothesized that greater needle temperatures in the hotter-drier site would increase monoterpene emission rates and consequently lower needle monoterpene concentrations, and that this temperature effect would dominate the seasonal pattern of monoterpene concentrations regardless of drought. In support of our hypothesis, needle monoterpene concentrations were lower across all seasons in trees transplanted to the hotter-drier site. Contrary to our hypothesis, basal emission rates (emission rates normalized to 30 °C and a radiative flux of 1000 μmol m^{-2} s^{-1}) did not differ between sites. This is because an increase in emissions at the hotter-drier site from a 1.5 °C average temperature increase was offset by decreased emissions from greater plant water stress. High emission rates were frequently observed during June, which were not related to plant physiological or environmental factors but did not occur below pre-dawn leaf water potentials of −2 MPa, the approximate zero carbon assimilation point in piñon pine. Emission rates were also not under environmental or plant physiological control when pre-dawn leaf water potential was less than −2 MPa. Our results suggest that drought may override the effects of temperature on monoterpene emissions and tissue concentrations, and that the influence of drought may occur through metabolic processes sensitive to the overall needle carbon balance.

Introduction

Trees are suffering mortality related to drought, warming and biotic attacks from pests and pathogens across the globe (Allen *et al* 2010, 2015, Hartmann *et al* 2018). Pines and other tree species use defensive secondary metabolites, such as volatile terpenes, to defend against herbivory and other biotic attacks (Gershenzon and Dudareva 2007, Unsicker *et al* 2009). Monoterpenes ($C_{10}H_{16}$) in particular help plants resist herbivory by acting as oviposition and feeding deterrents (Hummelbrunner and Isman 2001, Abdelgaleil *et al* 2009), negatively affecting larval performance and survival through toxicity (Lerdau *et al* 1994, Thoss and Byers 2006, Ilse and Hellgren 2007), decreasing insect immunocompetency (Trowbridge *et al* 2016), and aiding in parasitoid host location (Thaler *et al* 2002, Kant *et al* 2004, Mithöfer *et al* 2005). Terpenes are multifunctional: on the one hand, high concentrations of particular compounds are toxic to bark beetles and fungal symbionts, but bark beetles can also use volatile monoterpenes to identify preferred host trees and as precursors for aggregate pheromone production (Raffa *et al* 2005, Seybold *et al* 2006). Despite the acknowledged importance of monoterpenes in affecting higher trophic level interactions and contributing to tree resistance, the effect of warming with drought on their synthesis and emissions has not been evaluated despite observed and predicted increases in temperature and drought conditions.

The composition and concentration of monoterpenes in conifer oleoresin is dynamic and can be altered by biotic and abiotic factors, including drought (Zulak and Bohlmann 2010, Keefover-Ring *et al* 2016). The interactive effect of these stressors is expressed through shifts in monoterpene synthesis and emission (or volatilization). Monoterpene emission rate is generally unaffected by mild drought (Staudt *et al* 2002b, Lavoir *et al* 2009, Peñuelas *et al* 2009, Niinemets 2010, Eller *et al* 2016) and decreases only during more severe droughts (Llusià and Peñuelas 1998, Staudt *et al* 2002a, Lavoir *et al* 2009, Trowbridge *et al* 2014). The internal concentration of monoterpenes in the needles—and seasonal variations therein—generally increase under drought stress (Blanch *et al* 2009), but this can vary with drought severity (Niinemets 2015) and may not reflect the composition of released volatiles (Llusià and Peñuelas 1998, Trowbridge *et al* 2014). In other words, drought often reduces monoterpene emission rate without a decrease—or possibly an increase—in the rate of monoterpene synthesis (Lavoir *et al* 2009) and is one of the major uncertainties in models of biogenic volatile organic compound (BVOC) emissions (Seco *et al* 2015). Although drought is expected to reduce plant defensive emissions, the effect of additional warming with drought has not been evaluated. An increase in monoterpene emissions under warming could mitigate drought-related decreases in emissions with implications for plant defense and herbivore success in a changing climate. So, while temperature is commonly thought to be the dominant control over emission rate, which in turn can affect foliar concentrations, plant water status during drought may mask its impact. Unfortunately, we lack a basic understanding of how these two variables interact to affect monoterpene production and emission rate in mature conifers in the field.

While several studies have documented the interaction between drought, increased temperatures, and bark beetles in semi-arid and arid areas (e.g. Negrón and Wilson 2003, Breshears *et al* 2005, Mueller *et al* 2005, Gaylord *et al* 2013), the interactive mechanisms underlying an increased susceptibility to insects and pathogens remain unknown. Retrospective studies show trees that succumbed to drought stress tend to have smaller resin ducts and a lower resin duct area (as a percent of xylem area) relative to trees that lived (Gaylord *et al* 2013, 2015). However, contrary to other conifer systems (e.g. Blanche *et al* 1992, Kane and Kolb 2010), resin flow and resin duct parameters are not correlated in piñon pine (Gaylord *et al* 2013), which complicates our ability to generalize how drought-induced changes in tree defenses influence destructive pests. Furthermore, recent work on lodgepole pine (*P. contorta*) and whitebark pine (*P. albicaulis*) shows that constitutive and induced terpene concentrations are unrelated to resin duct size and abundance (Mason *et al* 2018), suggesting an uncoupling of anatomical and chemical anti-herbivore traits that may also be present in other species of pines in semi-arid areas.

Tree defense against biotic agents affects whether trees die or survive during drought, but defense is rarely studied or integrated into vegetation models (McDowell *et al* 2011, Dietze and Matthes 2014). Furthermore, studies often assume that plants are in a 'steady-state' (Anderegg *et al* 2013), failing to account for seasonal changes in growth or defense despite seasonal changes in their metabolic demand for carbon and subsequent influence against herbivory (Trowbridge *et al* 2014). It is thus critical to account for seasonality and phenology when evaluating how changing environmental conditions interact with plant primary and secondary metabolism to promote susceptibility to biotic agents. Here, we used a field transplant experiment to quantify monoterpene foliar concentrations and emission rates under temperature and moisture conditions that are consistent with global change projections across the vegetative growing season. We focused our study on the piñon pine *Pinus edulis*, a widespread, well-studied species that has undergone extensive drought-herbivore-related mortality (Breshears *et al* 2005, 2018). Trees were transplanted downhill to a hot and dry site outside the species' range (hereafter, 'Hotter-Drier'), and to a site near their origin which served as an ambient temperature control (hereafter, 'Ambient') (Law *et al* 2019). Within each site, piñon pine water status was altered by watering trees and by adding impervious barriers to prevent water from entering the soil as precipitation or leaving through soil evaporation. Foliar monoterpene concentrations and emission rate—in addition to common meteorological drivers and leaf-level physiological variables—were measured throughout the growing season at both sites and across all treatments. We expected that trees transplanted to the Hotter-Drier site would exhibit lower needle monoterpene concentrations due to volatilization and assumed that trees would have limited capacity for investment of C toward secondary metabolism that could offset volatilized losses. We further hypothesized that emission rate will be determined primarily by atmospheric meteorological factors rather than foliar physiological processes as previously determined in Trowbridge *et al* (2014).

Materials and methods

Field study sites

Field campaigns took place monthly during the 2010 May through September growing season at two experimental sites in northern Arizona, USA: A piñon pine-juniper woodland at 35.49 °N 111.85 °W (Ambient), and a hotter-drier site at 35.45 °N 111.50 °W (HFotter-Drier) *ca.* 35 kilometers east of the Ambient site (Law *et al* 2019). Air temperature at the Hotter-Drier site was 1.5 °C warmer on average than the Ambient site when measurements were made. In October 2008, piñon pine trees of similar size and age were randomly selected from the Ambient site and, using a 2.3 m tree spade, were immediately transplanted into open areas of the site as well as at the lower elevation Hotter-Drier site. The transplanted trees were regularly watered until August 2009 when the drought treatments began, resulting in 100% survival following the transplant at both sites (Law *et al* 2019). All selected trees had nearest neighbor canopy-to-canopy distances of at least one meter and were transplanted in a grid of squares with a spacing of at least 10 m. Eighteen trees at each site were randomly assigned to three moisture treatment groups: watered ($n = 3$), ambient ($n = 6$), and barrier ($n = 9$). Ambient trees were left exposed to natural conditions, watered trees received 25 gallons of water one week prior to sampling on a monthly basis, and barrier trees were fitted with a 4.3 m × 4.3 m below-canopy rain-out shelter consisting of a UV resistant tarp elevated just above the soil surface on a PVC frame to prevent direct vertical exchanges of water between the soil and atmosphere of the transplanted trees. These treatments created variability in pre-dawn water potential (Ψ) within each site. Pre-dawn water potential measurements were made within one week of gas exchange and monoterpene flux measurements, which are described below. See appendix A in Law *et al* (2019) for additional details on the field transplant experiment.

Field measurement techniques: gas exchange

Branch gas exchange was measured using a portable photosynthesis system with a transparent conifer chamber (LI-6400, LiCor Inc., Lincoln, NE, USA) to obtain rates of net CO_2 assimilation (A) and stomatal conductance (g_s). During each field campaign, we conducted gas exchange measurements over a four-day period alternating each day between the two sites. The trees we measured throughout the day at each site were randomly selected during each measurement period so as not to introduce diurnal biases in temperature and light over the course of the experiment. All measurements were taken at 400 ppm CO_2 with a flow rate of 500 μmol s^{-1}. Repeated measures were performed on the same trees each month by placing *ca.* 4 cm of the terminal portion of each study branch in the conifer chamber and sealing with a silicone polymer (Silly Putty®, Crayola LLC, Easton PA, USA) to minimize leaks. Because there was no controllable light source in the conifer chamber, measurements were only made once the light, CO_2, and humidity measurements within the chamber were stable on sunny days. Photosynthetic photon flux density (PPFD), leaf temperature (T_L), and air temperature outside of the chamber (T_a) were also recorded. After gas exchange measurements were made and following BVOC sampling, the entire branch was harvested, and the smaller gas exchange portion *versus* larger area used for BVOC measurements were separated to obtain needle dry weight and leaf area using methods for volume displacement according to Chen *et al* (1997) (see also appendix B in Trowbridge *et al* 2014).

Monoterpene volatile emissions sampling

A dynamic headspace branch-level enclosure was used to measure the flux of monoterpenes emitted from piñon pine branches (see supplemental materials is available online at stacks.iop.org/ERL/14/065006/mmedia and Trowbridge *et al* 2014). All monoterpene flux measurements were conducted over a four-day period during each field campaign in the same manner as the gas exchange measurements described above. Monoterpenes were sampled onto custom-made glass adsorbent cartridges (7.6 cm in length, 0.635 cm OD, Allen Scientific Inc., Boulder, CO, USA) packed with 25 mg Tenax® GR adsorbent (20/35 mesh, Alltech Associates Inc., Deerfield, IL, USA) between plugs of quartz wool at a flow rate of 150 ml min^{-1} for 10 min. Inlet and sample flow rates were set and controlled using mass flow controllers and a four-channel power supply readout box (MKS Instruments Inc., Methuen, MA, USA) to enable two branches to be measured simultaneously. Three branches were measured per tree plus one control (an empty chamber). Immediately after sampling, the glass tube was disconnected from the outlet and both ends were capped with Swagelok fittings, cooled to 0 °C, transported back to the lab, and stored in a −20 °C freezer for chemical analysis. Sample branches were then immediately cut and dry weight was measured after drying the needles at 60 °C for 48 h.

Volatile chemical analysis: thermal desorption

Sample tubes were analyzed for identification of emissions and emission rate determination within 7–21 d using thermal desorption (Perkin-Elmer ATD400) GC-FID/MS (Hewlet-Packard 5890/5970, Wilmington, DE, USA). Instrumental and analytical details are provided in the Supplemental Materials and in Helmig *et al* (2004), Ortega *et al* (2008), and Baghi *et al* (2012).

Foliar samples and monoterpene chemical analysis

At the time of collection, one-year old needles were flash frozen and stored in liquid nitrogen, transported to the laboratory, and placed in a −80 °C freezer. Details for monoterpene extraction are provided in the Supplemental Material.

Statistical analyses

We fit an exponential model between Ψ and: (1) net assimilation rates (A), and (2) stomatal conductance rates (g_s), across all treatments and sites using a nonlinear least square curve fitting procedure. Total and individual monoterpene tissue concentrations and emission rate observations were log transformed to meet assumptions of normality. To determine the effect of site on emission rate and foliar monoterpene concentrations over time, total and individual emission rate and monoterpene concentration data from the two sites were analyzed using a repeated measured ANOVA (SAS v 9.3; PROC MIXED statement) with tree listed as a random effect nested within each water treatment and applying a Bonferroni correction. We used Welch's two-sample t-test to detect any differences in average environmental and physiological variables between the two sites.

To quantify the variables responsible for the seasonal variability of emission rate and monoterpene concentrations in piñon pine needles over the growing season, we constructed linear models of meteorological variables (temperature, vapor pressure deficit [D], PPFD), plant physiological variables (namely A, g_s, Ψ, and leaf internal CO_2 concentration, C_i, as a surrogate for photosynthesis that excludes g_s), and site-level information (tree, drought treatment, and site). Models were fit for each month using all available observations, and for the entire growing season using month as an explanatory variable. Concentration was included as an input for the emission rate models, and emission rates were included as an input for concentration models. The parsimonious model with the minimum value of Akaike's Information Criterion corrected for finite sample sizes (AICc) (Akaike 1974, Burnham and Anderson 2002) was selected using the *dredge* command in the 'MuMln' package (Bartoń 2018) that follows the model selection routines of Burnham and Anderson (2002) using R (R Core Team 2017). In brief, *dredge* explores all combinations of linear models and selects the one with the lowest AICc to penalize models with additional parameters (k) and favor models with high values of the likelihood function L while accounting for sample size n. We also relaxed the assumption of linearity between driver and response by creating linear models for the natural logarithm of emission rate and monoterpene concentrations. Observations from May lacked leaf-level ecophysiological observations and were excluded from the modeling analysis.

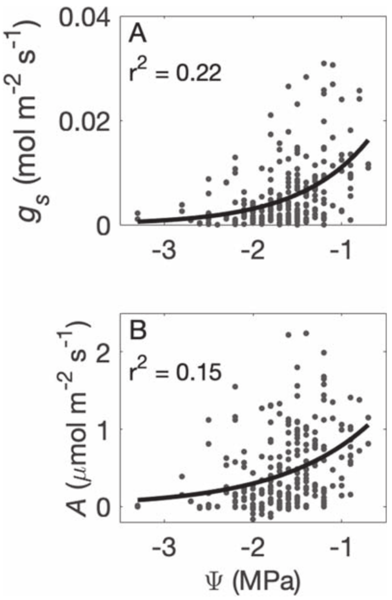

Figure 1. The exponential relationship between pre-dawn water potential (Ψ), stomatal conductance (g_s, subplot A), and photosynthesis (A, subplot B). The r^2 values refer to Pearson's correlation coefficients.

Results

Environmental and plant physiological differences between sites

During the sampling periods, the Hotter-Drier site was, on average, 1.5 °C warmer than the Ambient site (28.7 °C versus 27.2 °C; $P < 0.03$) as noted and sampling chamber measurements were 1.7 °C warmer (29.4 °C *versus* 27.7 °C; $P < 0.003$). Ψ was significantly lower at the Hotter-Drier site (−1.80 MPa *versus* −1.54 MPa; $P < 0.0001$). A and g_s exhibited exponential relationships to Ψ (figure 1) and as a consequence were ~2.5-fold greater, on average, at the Ambient site *versus* the Hotter-Drier site ($P < 0.0001$). Values for A and g_s averaged 0.26 μmol m^{-2} s^{-1} and 0.0031 mol m^{-2} s^{-1} at the Hotter-Drier site, and 0.64 μmol m^{-2} s^{-1} and 0.0078 mol m^{-2} s^{-1} at the Ambient site, respectively.

Composition of foliar monoterpene emissions and concentrations

The compound α-pinene averaged 30% of the total monoterpene emission rate across all months, sites, and water treatments. β-myrcene, β-phellandrene, limonene and δ-carene each averaged 13%–14% of the total monoterpene emission rate, and β-pinene and camphene each averaged 7%–8% (figures 2(A) and (B)). α-pinene comprised 48% of total needle tissue monoterpene concentration on average (figures 2(C) and (D)), and up to *ca.* 66% on a monthly basis. β-pinene averaged 19% of the total monoterpene foliar concentration, β-myrcene and limonene each averaged 13%, β-phellandrene and camphene each averaged 2%–4%, and δ-carene fell below the

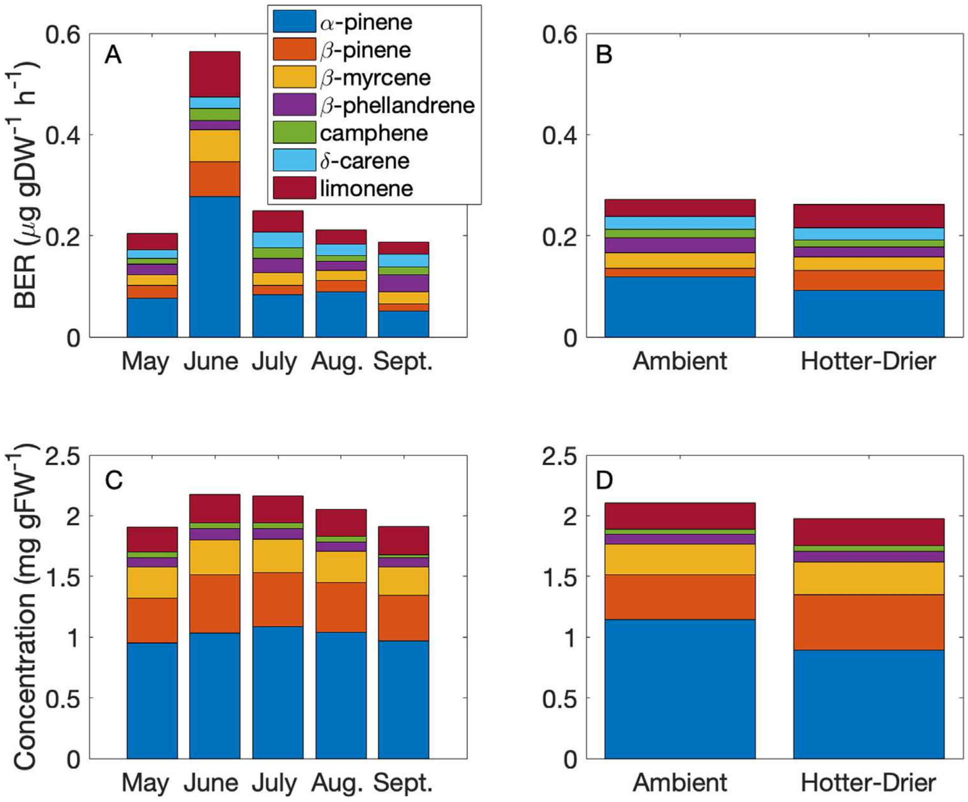

Figure 2. Average monoterpene basal emission rates (BER, μg C gDW^{-1} h^{-1}) by month (A) and by site (B) and needle monoterpene concentrations (mg gFW^{-1}) by month (C) and site (D) from piñon pine transplanted near the original piñon-juniper woodland ('Ambient'), and transplanted to a hotter and drier site ('Hotter-Drier', H-D) in northern Arizona, USA.

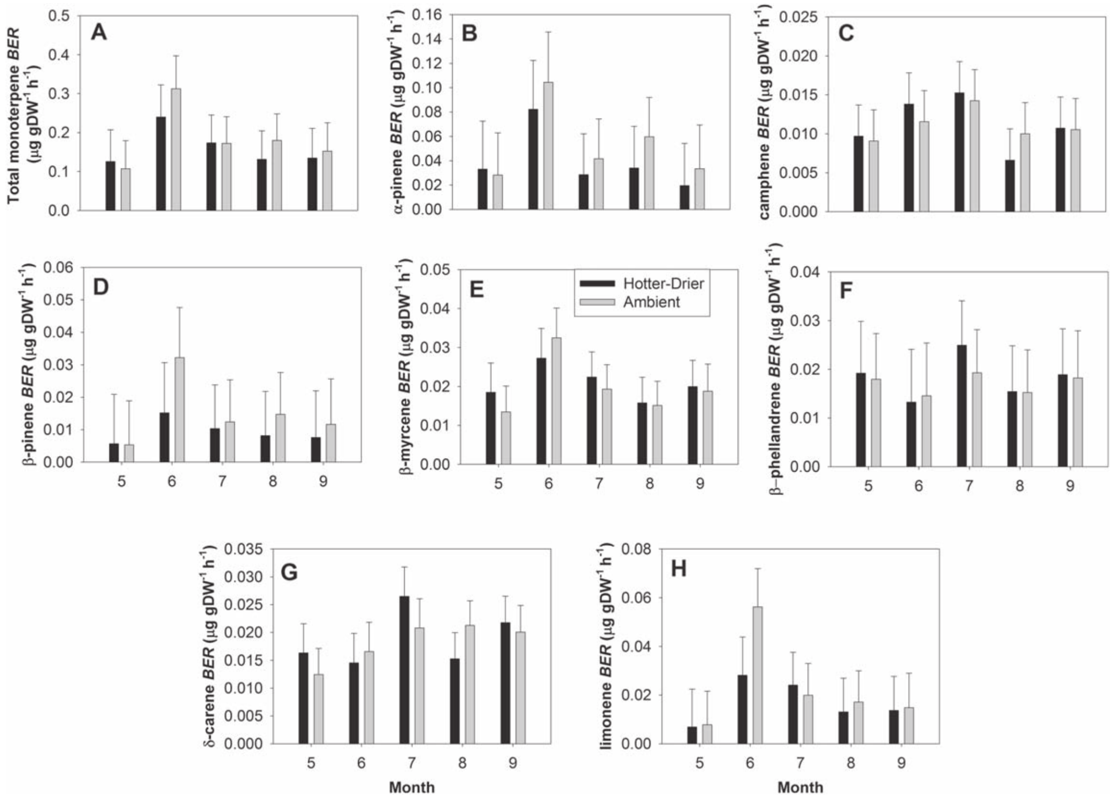

Figure 3. The back-transformed average and standard error of the mean (SEM) of monthly monoterpene basal emission rates (BER, μg C gDW^{-1} h^{-1}) from piñon pine branches transplanted near the original site in a piñon pine juniper woodland (gray bars; Ambient) and a hotter-drier site (white bars; Hotter-Drier) located in northern Arizona, USA.

Table 1. Linear models of monoterpene basal emission rates (μg C gDW^{-1} h^{-1}) selected using the minimum value of the adjusted Akaike's information criterion (AICc) found using the *dredge* function in R. *F*: *F*-statistic, df: degrees of freedom, Adj. r^2: adjusted coefficient of variation, *P*: *P*-value, *T*: air temperature within the sampling chamber (°C), *D*: vapor pressure deficit (kPa), g_s: stomatal conductance (mol m^{-2} s^{-1}), *C*: total leaf monoterpene concentration (mg gFW^{-1}), *M*: month, *E*: experimental treatment (1: Dry, 2: Ambient, 3: Wet).

Time period	Model	*F*	df	Adj. r^2	*P*
Growing season	$1.4-0.011\,T + 0.0005\,C_i + 0.10\,\mathrm{A}-0.11\,M-0.061\,E$	5.65	5, 194	0.10	<0.0001
Growing season excluding outliers	$0.74-0.0088\,T + 3.63\,g_s-0.033\,M-0.030\,E$	6.79	3, 181	0.11	<0.0001
June	$0.048-0.0024\,C_i + 99\,g_s + 24\,C-0.27\,D$	5.74	4, 35	0.33	0.001
June excluding outliers	$0.72-8.1\mathrm{e}\text{-}4\,C_i-0.20\,D$	6.07	2, 27	0.26	0.007
July	$0.58-0.017\,T + 0.044\,D$	4.62	2, 53	0.12	0.01
August	$0.51-0.008\,T + 0.087\,A + 6.4\,C-0.062\,E$	6.03	4, 46	0.33	0.0001
September	$0.58-0.015\,T$	9.59	1, 47	0.15	0.003

Table 2. Linear models of the logarithm of monoterpene basal emission rates (μg C gDW^{-1} h^{-1}) selected using the minimum value of the adjusted Akaike's information criterion found using the *dredge* function in R. *F*: *F*-statistic, df: degrees of freedom, Adj. r^2: adjusted coefficient of variation, *P*: *P*-value, *T*: air temperature within the sampling chamber (°C), *D*: vapor pressure deficit (kPa), g_s: stomatal conductance (mol m^{-2} s^{-1}), *C*: total leaf monoterpene concentration (mg gFW^{-1}), *M*: month, *E*: experimental treatment (1: Dry, 2: Ambient, 3: Wet), $S =$ site (1: Hotter-drier, 2: Ambient).

Time period	Model	*F*	df	Adj. r^2	*P*
Growing season	$2.0-0.08\,T + 0.0013\,C_i-0.26\,M + 0.32\,A-0.20\,E + 0.14\,D$	8.63	6, 193	0.19	<1e-7
Growing season excluding outliers	$1.5-0.057\,T + 0.0011\,C_i-0.21\,M-0.22\,E$	10.9	4, 193	0.17	<1e-7
June	$0.69 + 0.0040\,C_i-0.52\,E-0.68\,D$	7.63	3, 36	0.34	0.0005
June excluding outliers	$0.44 + 0.0038\,C_i-0.51\,E-0.61\,D$	6.55	3, 35	0.30	0.001
July	$0.40-0.10\,T + 0.30\,D$	9.75	2, 54	0.24	0.0002
August	$3.2-0.16\,T-0.005\,C_i + 44\,g_s-0.05\,S + 40\,C-0.27\,T_r + 0.32\,D$	7.91	7, 44	0.49	<1e-5
September	$0.96-0.12\,T + 0.0011\,C_i$	11.1	2, 47	0.29	0.0001

measurement detection limit in all instances (figures 2(C) and (D)).

Seasonal patterns of monoterpene emissions

The total monoterpene emission rate did not differ between the Ambient and Hotter-Drier sites but did differ as a function of time ($P < 0.0001$). Total emission rate was significantly greater in June at both sites relative to other months (figure 2) due to increases in emissions of α-pinene, β-pinene, β-myrcene, and limonene (figure 3). Month was also a significant main effect ($P < 0.0001$) for the emission rates of all individual compounds, and the relative contribution of different monoterpene compounds to total emissions changed throughout the growing season because the emission rate of each compound followed different seasonal patterns (figure 3).

Models of the seasonal variability of monoterpene emission rates

The linear model with the lowest AICc value explained only ~10% of the variance across all emission rate observations regardless of the exclusion of the statistical outliers, considered here to be >1 μg C gDW^{-1} h^{-1} (i.e. five standard deviations greater than the mean emission rate), that were frequently observed in June. This model included temperature, month, treatment (dry, ambient, or watered), and intercellular CO_2 concentration (C_i) as a surrogate for leaf physiology (table 1). The linear model for the logarithm of emission rate for the entire season explained nearly 20% of the variance of observations and included similar variables (table 2).

Linear models with the lowest AICc values explained ~30% of the variance of emission rate during June and August, but only 12%–15% during July and September (table 1). Models for July and September included only *T* (and in the case of July also *D*), but not leaf-level physiological variables or experimental treatments (table 1). Models for June and August suggested that leaf-level variables C_i and g_s, as well as total leaf monoterpene concentrations, and *D* (for the case of June) and experimental treatment (for the case of August), should not be excluded from an emission rate model. Models for the logarithm of monoterpene emission rate included similar variables but tended to explain a higher amount of the observed variance, notably in August when a model that included temperature, *D*, C_i, g_s, site, treatment, and needle monoterpene concentration explained nearly 50% of the observed variance in emission rate (table 2). Regardless of month, emission rate was not related to stomatal conductance, photosynthesis or any observed micrometeorological variable when Ψ was below -2 MPa. The model with the lowest AICc under these conditions only contained an intercept value of 0.19 mg C g DW^{-1} h^{-1}.

Ψ, an integrated measure of plant water status, was not included in the selected models for emission rate during any time period despite its significant exponential relationship to both g_s and *A* (figure 1). Emission rates in excess of 1 μg C gDW^{-1} h^{-1}, however, were

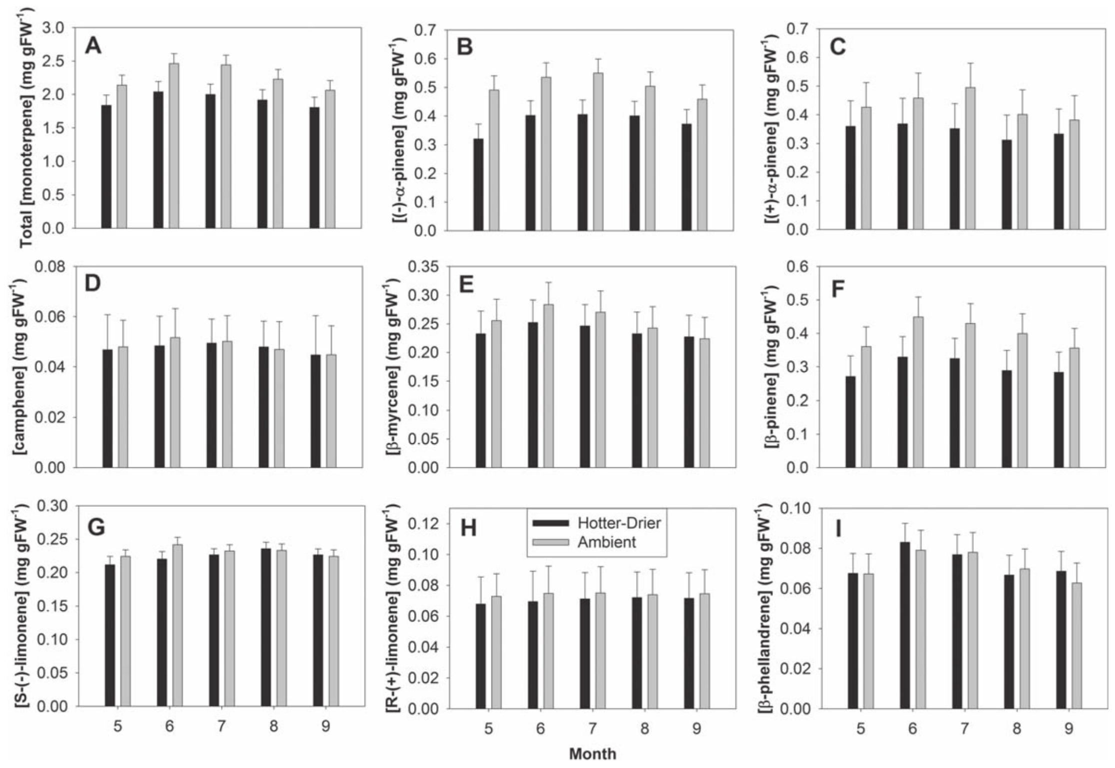

Figure 4. The back-transformed average and standard error of the mean (SEM) of monthly total and individual monoterpene foliar concentrations (mg gFW^{-1}) from one-year old piñon pine needles from trees transplanted near the original site in a piñon pine juniper woodland (gray bars; Ambient) and a hotter-drier site (white bars; Hotter-Drier) located in northern Arizona, USA.

Table 3. Linear models of monoterpene concentrations (mg gDW^{-1}) selected using the minimum value of the adjusted Akaike's information criterion (AICc) found using the *dredge* function in R. *F*: *F*-statistic, df: degrees of freedom, Adj. r^2: adjusted coefficient of variation, *P*: *P*-value, *D*: vapor pressure deficit, g_s: stomatal conductance (mol m^{-2} s^{-1}), *C*: total leaf monoterpene concentration (mg gFW^{-1}), *D* vapor pressure deficit (kPa), *E*: experimental treatment (1: Dry, 2: Ambient, 3: Wet), S = site (1: Hotter-drier, 2: Ambient), emission rate: total monoterpene emissions rate (μg C gDW^{-1} h^{-1}), *M*: month, Ψ: pre-dawn water potential (MPa).

Time period	Model	*F*	df	Adj. r^2	p
Growing season	$0.0085–0.0033\,\Psi + 0.0047\,S$	14.23	2 (197)	0.12	<1e-5
Growing season excluding outliers	$0.0092–0.0034\,\Psi + 0.0042\,S$	11.04	2 (183)	0.09	<1e-4
June	$0.017–1.4\text{e-}5\,C_i - 1.2\,g_s + 6.1\text{e-}3\,S + 3.5\text{e-}3$ emission rate	4.89	4 (35)	0.29	0.003
June excluding emission rate outliers	$0.017–0.9\,g_s + 0.0044\,S$	2.88	2 (27)	0.11	**0.07**
July	$0.014 + 0.0046\,S$	6.55	1 (56)	0.09	0.01
August	$0.014 + 0.004\,S + 0.0084$ emission rate	3.94	2 (49)	0.10	0.03
September	$0.0048–0.0050\,\Psi - 0.0028\,E + 0.0020\,D$	2.94	4 (45)	0.14	0.03

not observed when Ψ was below *ca.* −2 MPa. There were no other significant relationships between these June emission rate outliers and measured environmental and plant physiological variables.

Seasonal patterns of foliar monoterpene concentrations

The total monoterpene concentration was greater in needles at the Ambient site than at the Hotter-Drier site ($P < 0.05$) due to higher concentrations of α-pinene and β-pinene (figures 4(B), (C), and (F)). Total monoterpene concentration and all individual compounds, except *R*-(+)-limonene, differed significantly as a function of time. Unlike total emission rate, total monoterpene concentrations did not show a pronounced change during June (figures 2 and 4).

Models of the seasonal variability of monoterpene concentrations

Selected models for monoterpene concentration for the entire growing season and for all months (table 3) always included site (Ambient or Hotter-Drier) as a variable but explained only 9%–14% of the observed variance with the exception of the model for June, which explained 29% of the observed variance and also included C_i, g_s, and emission rate. Models of the logarithm of monoterpene concentrations (not shown) did not increase the percent of variance explained. Notably, Ψ was included in models for monoterpene concentrations the entire growing season regardless of the treatment of emission rate outliers, as well as the model for September. Monoterpene concentrations in plants with $\Psi > -2$ MPa were significantly greater than those with $\Psi < -2$ MPa ($P < 0.05$).

Discussion

Experimental hypotheses

As predicted, we observed lower needle monoterpene concentrations from trees transplanted to the Hotter-Drier site relative to Ambient site (figure 4). While we hypothesized that this decrease in concentration would result from a loss via volatilization at higher temperatures, emission rates did not differ between sites consistent with an overriding effect of increased water limitation on emissions, especially as Ψ decreased below a limiting value determined to be −2 MPa. The environmental and phenological factors that determined monoterpene concentrations and emission rate were dynamic throughout the study period (tables 1–3) as discussed in greater detail in the following.

Seasonal patterns of monoterpene emissions

Few studies have explored the consequences of interactions between altered water availability and temperature on emission rate and monoterpene concentrations *in situ* across the vegetative growing season (Staudt *et al* 2002a, Geron *et al* 2016). A striking feature of our observations is the significant increase of emission rate during June, when nearly 25% of the emission rate measurements exceeded the growing season mean plus four times the variance of emission rate observations (figures 2a and 3). When considering emission rates from trees across the water stress gradient in June (−1 to −2.5 MPa), none of the high emission rate measurements occurred when Ψ was below *ca.* −2 MPa, the approximate zero-assimilation point of piñon pine (Lajtha and Barnes 1991).

Although we did not measure the timing of leaf and shoot growth in our study, others have observed that needle emergence (bud break) in piñon occurs in June (Grossiord *et al* 2017), which could have driven the high emission rates we measured in this month. Multiple lines of evidence suggest that periods of leaf differentiation and growth are of particular importance to BVOC flux. For example, monoterpene emissions were greatest in the early growing season for both *P. rigida* and *P. koraiensis* (Son *et al* 2015). Furthermore, peaks in atmospheric particle formation rates during the early growing season in boreal ecosystems have been associated with springtime increases in BVOC fluxes from evergreen conifers (Dal Maso *et al* 2009). Models based on summertime observations underestimated BVOC emissions during earlier times of year (Holzinger *et al* 2006), suggesting that unique springtime processes or relationships between BVOC emissions and environmental drivers are important (Bäck *et al* 2005). Monoterpene emission bursts have been demonstrated in *Pinus sylvestris* due to new foliage growth (Aalto *et al* 2014), the recovery of photosynthesis (Aalto *et al* 2015), and possibly the refilling of embolized tracheid elements (Vanhatalo *et al* 2015). These observations are also consistent with recent findings that the presence of exposed resin in the axils of needles—especially when sap pressure is relatively high—is associated with high BVOC emission rates (Eller *et al* 2013).

We cannot exclude resin exposure as a potential contributor to the transient increases in emission rate observed in the present study, which can occur during shoot extension and needle emergence. Resin exposure is related to positive xylem pressure potentials that cause exudation (Eller *et al* 2013), and emission rate spikes were not observed when Ψ was below −2 MPa as noted. Our results demonstrate the need to further study different states of plant water relations and physiological function—including those that increase the likelihood of resin exposure—on BVOC emissions from piñon pine shoots and needles.

Models for monoterpene emission rates are determined by water thresholds

Monoterpene basal emission rate was not related to stomatal conductance, photosynthesis, or any measured micrometeorological variable when Ψ was below −2 MPa. A and g_s are strongly limited in piñon pine when Ψ is lower than −2 MPa (figure 1, Lajtha and Barnes 1991), and the best model of total monoterpene basal emission rate under these conditions is a constant 0.19 mg C g $\mathrm{DW}^{-1}\,\mathrm{h}^{-1}$. Above the −2 MPa threshold, a model with T, C_i, monoterpene concentration, and water treatment had the lowest AICc, but explained only 14% of the observed variance in emission rate. The model for the logarithm of emission rate with the lowest AICc included similar variables and explained 5% more of the observed variance. Combined, these results suggest that emission rate is controlled by measured physiological and environmental factors above the −2 MPa Ψ threshold, but not below. These results contrast the findings of Eller *et al* (2016), who found that BVOC emissions are correlated with leaf physiology below a plant physiological threshold, namely a net CO_2 assimilation rate $A < ca.\ 2\ \mu\mathrm{mol\ m^{-2}\,s^{-1}}$ and stomatal conductance $g_s < 0.02\ \mathrm{mol\ m^{-2}\,s^{-1}}$ in the needles of mature *P. ponderosa* trees. These results point to the importance of studying physiological controls over BVOC efflux across different *Pinus* species to understand why differences emerge.

The role of temperature and leaf level monoterpene emissions

Monoterpene emission rate tends to increase 2–3 fold for every 10 °C increase in temperature (Lerdau *et al* 1994, Lerdau *et al* 1997), and this relationship is used to construct global atmospheric models of forest monoterpene emissions (Guenther *et al* 1991, 1993, 2006, Keenan *et al* 2009). Thus, we hypothesized that trees in the hotter-drier site would exhibit higher emission rate due to increased volatility. However, we did not observe a significant difference in emission rate between sites despite a 1.5 °C average air temperature increase (and 1.7 °C average chamber temperature increase) at the Hotter-Drier site, where Ψ was on average 0.3 MPa lower. This lack of relationship also held when emission rate was not normalized by temperature (i.e. when raw emission rate data rather than basal emission rate data were analyzed), emphasizing that temperature increases did not result in emission rate increases. Temperature was included as an explanatory variable in most models of emission rate, but the relationship, when present, was negative (tables 1 and 2). Previous studies have demosnstrated that BVOC efflux from piñon needles responds to temperature during certain parts of the growing season, but also found that water stress was a more important control over emissions in this semi-arid system (Trowbridge *et al* 2014).

Past studies, in addition to citing temperature as a primary driver of emission rate from conifers, have also shown monoterpene emission rate to be a function of foliar monoterpene concentrations through a Henry's Law relationship (Lerdau *et al* 1994, Lerdau *et al* 1997). Other studies have found no relationship between monoterpene concentrations and emission rate (Constable *et al* 1999, Trowbridge *et al* 2014, Eller *et al* 2016). In addition to our inability to find the expected positive temperature-emissions relationship in this study, we also failed to verify a relationship between monoterpene concentrations and emission rate across most time periods studied except for June (table 1). Observations point to a dynamic system where monoterpene concentrations are coupled to emission rate during periods of more positive plant water status during the growing season but are otherwise unrelated as leaf monoterpene concentrations do not factor into the most parsimonious model for emission rate across the rest of the study period.

Combined, our observations are in contrast to other studies and atmospheric models that have derived algorithms based solely on the effects of foliar monoterpene concentrations and temperature for estimating monoterpene emission rate. Our observations are consistent with empirical and modeling studies demonstrating that monoterpene emissions are not under stomatal control when averaged over longer periods of time (Harley 2013, Grote *et al* 2013, Eller *et al* 2016); g_s rarely entered models of emission rate (tables 1 and 2). As noted, when Ψ was above −2 MPa, a model with T_a, C_i (a surrogate for A apart from g_s), needle monoterpene concentration, and experimental water treatment were necessary to explain observed emission rate variability. These observations suggest that plant water status via Ψ acts as a switch between monoterpene emission rates that are not under environmental or biotic control (below −2 MPa) and those that are, although we cannot exclude unmeasured physiological factors when interpreting these results.

The impact of drought and heat on individual compounds

The compound α-pinene was the primary constituent of total monoterpene concentrations and emission rate (figure 2, table 1), in agreement with past studies on piñon pine (e.g. Smith 2000, Trowbridge *et al* 2016), and drove many of the patterns that we observed for both total emissions and concentrations. Although many individual compounds exhibit similar concentration and/or emission patterns to α-pinene (e.g. β-pinene and β-myrcene, figures 3 and 4), the magnitude and percent change over time, between sites, and in response to water status, were often different among compounds, likely due to variation in physiochemical properties and controls over their synthesis (Niinemets *et al* 2004). Furthermore, some compounds show little or no change in either concentration or emissions across treatments or across time (e.g. β-phellandrene, figures 3 and 4), perhaps due to lower relative volatility and/or deterministic gene expression patterns that are little affected by environmental variability.

Piñon pine mortality and herbivory

Piñon pine populations have declined in mass mortality events across the southwestern US, and herbivore damage to compromised trees is often attributed as the cause of tree death (Cobb *et al* 1997, Gaylord *et al* 2013). Synergistic effects among monoterpenes and the ratio of compounds to one another can influence herbivores directly by affecting growth and immunocompetence (Dyer *et al* 2003, Trowbridge *et al* 2016), but also indirectly by attracting parasitoids (e.g. Havill and Raffa 2000). However, over time and under more severe and consistent drought stress, we may expect to see a shift in monoterpene concentrations as piñon pines become more carbon limited, which may increase tree susceptibility to insect pests. The dynamics of monoterpene emission rate and concentrations under prolonged drought is uncertain and must be studied further to understand the interplay between drought, defense, and herbivory.

Conclusions

Our study represents an initial step to add tree defensive chemistry to a comprehensive understanding of piñon pine forest response to drought and temperature stress. We find that the Ψ threshold associated with zero net photosynthetic carbon assimilation in piñon pine trees, −2 MPa, also acts as a threshold below which monoterpene emission rate is not under apparent control by measured plant physiological or micrometeorological variables. Plant water status thus plays an important role in emission rate and should be incorporated into regional and global models of monoterpene efflux. We failed to observe increased emission rate with increased temperature, likely due to the overriding impact of limited water availability, indicating that such relationships need to be factored into global models. Research should focus on the underlying phenological mechanisms responsible for the frequent spikes in emission rate during conditions in which water was not yet limiting (e.g. budburst, resin exposure, or other factors) to improve our inventories of emissions under predicted global change. Future studies should also explore the dynamics of plant secondary compounds as carbon limitation and water stress reach values sufficient to cause tree death to understand how plants allocate resources toward maintenance and defense during periods of acute and/or prolonged stress, and how these changes alter herbivore dynamics. In summary, our results suggest that drought may override the effects of temperature on monoterpene emissions and tissue concentrations, and that the influence of drought may occur through processes sensitive to overall needle carbon balance. Consequently, added warming does not worsen drought-induced suppression of defensive pine emissions—findings that need to be incorporated into global biogeochemical and biogeographic models.

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Author contributions

AMT, DDB, HDA, DJL, and RKM conceived and designed the experiment. AMT, HDA, and DJL performed the experiment with assistance from DH. AMT and PCS analyzed the data. AMT, PCS and RKM wrote the manuscript with contributions from all other authors.

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