Drought and heat stress interactions modify photorespiration and hydrogen peroxide content in silver fir

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Photorespiration (PR) greatly reduces net carbon assimilation in trees (by c. 25%), but has received recent attention particular for its potential role in stress-signaling through the accumulation of hydrogen peroxide (H_2O_2), a stress signaling agent. Despite an increasing frequency of drought and heat events affecting forests worldwide, little is known about how concurrent abiotic stressors may interact to affect PR and subsequent H_2O_2 accumulation in trees. Here, we sought to identify how drought and a compounded 1-day heat treatment individually and interactively affect PR (determined under variable O_2) in *Abies alba* Mill. seedlings. Additionally, we quantified foliar H_2O_2 accumulation and enzymatic scavenging via peroxidase in relation to PR rates. We found drought stress to slightly increase PR (+5.2%) during mild-drought (12 days, $\Psi_{md} = -0.85$ MPa), but ultimately to decrease PR (−13.6%) during severe-drought (26 days, $\Psi_{md} = -1.70$ MPa) compared with the control, corresponding to increasing non-stomatal limitations of photosynthesis (i.e., decreased electron transport rate). The response of PR to heat stress was dependent on soil water availability as heat stress increased PR in control seedlings (+37.8%), but not in drought-stressed seedlings. Decreased PR during severe-drought corresponded to ∼2× lower foliar H_2O_2 compared with the control. Despite increased PR under heat stress in control seedlings, foliar H_2O_2 decreased to near-zero likely due to enhanced scavenging as observed in ∼2× greater peroxidase activity. Our results demonstrate that carbon loss to PR during drought stress can be highly dynamic, depending on the severity of soil dehydration. Additionally, increased PR under abiotic stress does not necessarily lead to accumulated H_2O_2, as tight regulation by scavenging enzymes instead minimize oxidative stress, reducing stress-signaling potential.

Keywords: *Abies alba*, oxidative scavenging, oxidative stress, photosynthesis, reactive oxygen species (ROS), RuBP oxygenation.

Introduction

The world's forests serve as a global carbon sink, currently accounting for a net removal of -7.6 ± 49 $GtCO_2eyr^{-1}$ from the atmosphere due to photosynthetic carbon assimilation minus respiratory processes (Harris et al. 2021). Photorespiration (PR) has a large impact on the global carbon cycle by reducing carbon assimilation from photosynthesis by c. 25% in nearly all tree species, which predominately utilize C_3 carbon fixation (Sharkey 1988, Raghavendra 2003, Young et al. 2020). Specifically, PR occurs when O_2 rather than CO_2 fills the active site of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), ultimately consuming ATP and NADPH and releasing CO_2 (Bauwe et al. 2010). Abiotic stress events such as drought and heat have long been known to increase rates of PR (Hochberg et al. 2013, Wolf and Paul-Limoges 2023). Despite these associated losses in carbon assimilation, PR has recently received attention primarily for its potential role in stress tolerance affecting the cellular signaling of metabolic-intermediates such as hydrogen peroxide (H_2O_2; (Voss et al. 2013, Sunil et al. 2019, Ünlüsoy et al. 2023). With drought and heat risk expected to increase in forests worldwide, understanding the responses of PR to abiotic stress is important to better model both future forest carbon fluxes and tree stress responses (Allen et al. 2010, Lloyd et al. 2023, Wolf and Paul-Limoges 2023).

Both drought and heat stress can increase PR, as physiological responses to stress in trees modify the interaction of Rubisco and CO_2 (Ku and Edwards 1978). To minimize water loss during periods of drought stress, plants close stomata, increasing stomatal resistance and reducing the amount of intercellular CO_2 available for carboxylation by Rubisco (Ku and Edwards 1978, Martin-StPaul et al. 2017, Petek-Petrik et al. 2023). As Rubisco preferentially draws down CO_2, the resulting higher O_2:CO_2 ratio can increase PR rates during initial drought (Wingler et al. 1999, Fang et al. 2023). As drought progresses, non-stomatal limitations to photosynthesize may increase as processes in the photosynthetic light reactions like electron transport rate (ETR) become limiting, including a reduction in the regeneration of NADPH and ATP, which are essential for the Calvin cycle and overall carbon assimilation (Flexas and Medrano 2002*a*; Lawlor and Tezara 2009). With increased non-stomatal limitations, intracellular CO_2 no longer is the greatest limitation to photosynthesis, which may relieve PR rates. Heat stress can similarly increase PR rates in trees, as the increased solubility of O_2 relative to CO_2 at warmer temperatures leads to higher O_2:CO_2

(Ku and Edwards 1977, Doehlert and Walker 1981). In addition, heat stress can enhance PR by altering Rubisco kinetics, such as by decreasing affinity to CO_2 and/or increasing rates of Rubisco misfire (Jordan and Ogren 1984, Salvucci and Crafts-Brandner 2004, Bracher et al. 2017). However, despite their mechanistic similarities and often simultaneous occurrence, the interacting effects of drought and heat stress on PR in trees remain to be directly experimentally investigated, and may represent a limitation in GPP modeling (Zhou et al. 2013, Dewar et al. 2022).

Photorespiration is suggested to facilitate abiotic stress tolerance through the regulation of excess photochemical products and/or by stress-signaling through the PR intermediate H_2O_2 (Voss et al. 2013, Sunil et al. 2019, Ünlüsoy et al. 2023). H_2O_2 activates transcription factors of stress-response genes (Marinho et al. 2014), and is used as a secondary messenger in plant-hormone pathways, such as abscisic acid-induced stomatal regulation (Bright et al. 2006). During abiotic stress, reduced RuBP-regeneration can lead to an overaccumulation of ATP and NADPH from the light-reactions of photosynthesis, resulting in the inactivation of PSII and increased reactive oxygen species (ROS) production (Kato et al. 2003, Bambach et al. 2020). Photorespiration can help relieve photoinhibition by consuming excess NADPH and ATP to maintain cyclic electron flow (CEF) and dissipate excess energy, which may be particularly important for trees during heat stress (Peñuelas and Llusià 2002, Flexas and Medrano 2002*b*, Takagi et al. 2016). While H_2O_2 can originate from multiple cellular compartments such as chloroplasts during high light, in the mitochondrion during respiration and the metabolism of ROS, 70% is produced via glycolate oxidation in the peroxisome during PR (Noctor et al. 2002, Voss et al. 2013, Strand et al. 2015). H_2O_2 can also upregulate the alternative oxidase pathway, which limits ROS accumulation and maintains redox balance by increasing scavenging enzyme activity (Sunil et al. 2019). As H_2O_2 itself is a ROS that can cause cellular damage, concentrations are continuously regulated by a series of scavenging enzymes like peroxidases and catalases, as well as chemical pathways like the glutathione-ascorbate cycle to minimize oxidative stress. Thus, it remains unclear how potential increases in PR during abiotic stress like drought and heat may affect H_2O_2 accumulation and enzymatic scavenging (Abogadallah 2011).

In this study, we aimed to identify how drought and heat stress individually and interactively affected the contribution of PR to carbon assimilation, as well as to H_2O_2 accumulation and scavenging in silver fir (*Abies alba* Mill.) seedlings. Silver fir is a native conifer widespread throughout Europe. While silver fir is considered as a potential alternative to Norway spruce because it is able to draw water from deeper depths, it may still be particularly sensitive to drought and heat stress (Stangler et al. 2022). To further explore this sensitivity, we exposed silver fir seedlings to a 25-day drought, followed by a 6-h heat wave at 40 °C. Gas exchange and ETRs were determined to estimate PR during mild and severe drought, as well as during a subsequent heat event. Additionally, we analyzed foliar concentrations of H_2O_2 and peroxidase (POD), a major enzymatic H_2O_2 scavenging class. We hypothesize that the relative contribution of PR to net carbon assimilation increases with drought progression, and would be greatest during combined drought and heat stress. Additionally, we hypothesize that foliar H_2O_2 concentration increases with PR during drought and heat stress, outpacing increased POD activity.

Materials and methods

Plant material and growth conditions

We obtained bare-rooted 4-year-old *A. alba* seedlings from a tree nursery in Gunzenhausen, Germany in March 2023. Seedlings had an average aboveground height of 32.29 cm ± 0.78, and an average diameter of 9.53 mm ± 0.30 2 cm above the soil level. Seedlings were planted in 5.7 L pots containing a mixture of peat substrate and perlite (5:1). Osmocote© 5 8–9 month slow-release fertilizer (16–8-12 + 2.2MgO + TE; ICL Specialty Fertilizers) was added to all pots at a rate of 1 g L^{-1}. During the pre-experimental period (March 2023 – August 2023) seedlings were kept outdoors at an experimental greenhouse facility in Garmisch-Partenkirchen, Germany (708 m a.s.l., 47°28′32.9″ N, 11°3′44.2″ E). In August 2023, seedlings were moved inside the greenhouse facility to acclimate to greenhouse conditions for 3 weeks. Seedlings were automatically drip irrigated with 60 mL water twice daily (08:00, 21:00 h; Rain Bird, Azusa, USA), with LED grow lamps maintaining a 15-h photoperiod (LED-KE 400 VSP, DHLicht, Wuelfrath, Germany). Continuously measured photosynthetic active radiation (PAR) reached daytime averages of 550–650 μmol m^{-2}, while temperature and relative humidity were maintained at 23 °C and 55%, respectively (Figure S1 available as Supplementary data at *Tree Physiology* Online, PQS 1, Kipp & Zonen, Delft, The Netherlands).

Experimental conditions

Twenty seedlings were randomly assigned to either a well-watered control ($n = 10$) or drought treatment ($n = 10$). On 26 August 2023, irrigation was withheld completely from the drought treatment while the control treatment continued to receive drip irrigation. Soil volumetric water content (SWC) was measured throughout the experimental period in all pots (10HS, Meter Group, USA), with control seedlings maintaining ∼37% SWC throughout the experimental period. Midday branch water potential (Ψ_{md}) was periodically measured on randomly selected individuals to track drought progression by removing axial branches and immediately measuring with a Scholander-type pressure chamber (Model 600D, PMS Instruments, OR, USA).

Gas exchange and fluorescence measurements were collected on 26 August (experimental start), 6 September (mild-drought) and 20 September 2023 (severe-drought), beginning at midday and lasting c. 3 h. Additionally, leaf temperature (T_{leaf}; PI 450, Optris, Germany), and samples for chemical analysis were collected on all individuals during severe-drought. On 21 September 2023 at 6:00 h, greenhouse daytime air temperature was increased to 40 °C, beyond the thermal optimal in silver fir (Robakowski et al. 2002, Húdoková et al. 2022). Heat stress was maintained for 6 h before repeated collection of gas exchange and fluorescence measurements, leaf temperature and samples for chemical analysis on both control + heat treatment (formerly control) and drought + heat treatment (formerly drought) individuals. All gas exchange and chemical analyses were performed on current-year growth.

Gas exchange and fluorescence measurements

Branches with c. 6 leaves (aligned flat for consistent coverage) were measured for net photosynthetic carbon assimilation rate (A_{net}), stomatal conductance (g_{sw}) and ETR using an LI-6800 portable photosynthesis system equipped with the 6800-01A multiphase flash fluorometer (LI-COR Biosciences, Lincoln, NE, USA). The 2-cm^2 cuvette conditions were set to: 600 μmol m^{-2} s^{-1} photosynthetic photon flux density (estimated saturation point from light-response curves, Figure S2 available as Supplementary data at *Tree Physiology* Online), 400 p.p.m. [CO_2] and a 750 μmol s^{-1} flow rate using ambient humidity levels (~25–55%, Figure S1 available as Supplementary data at *Tree Physiology* Online). Air temperature inside the cuvette was set to 25 °C at measurement timepoints before heat stress, and to 40 °C during heat stress to reflect greenhouse air temperature. Directly following the measurement under ambient air (~21%-O_2), while the cuvette was still attached to the leaves, reduced oxygen air (~2%-O_2) at the same flow rate and same CO_2 concentration was supplied to the cuvette. The measurements were taken after instrumental stability of A_{net} (slope < 0.5 μmol CO_2 m^{-2} s^{-1} min^{-1}) and g_{sw} (slope < 0.01 mol m^{-2} s^{-1} min^{-1}) was reached, with a minimum stabilization time of 1 min. The ~2%-O_2 A_{net} was compared with that measured under ~21%-O_2, with their ratio being an estimation of Rubisco carboxylation efficiency, and their difference being the estimation of PR (Sharkey 1988, Yin et al. 2020). The air source provided to the LI-6800 was modified using a three-way valve connected to an air tank containing 2%-O_2 in N_2, with another valve open to ambient air. All parameters were recalculated using individually measured leaf area within the 2-cm^2 cuvette.

Chemical analysis: hydrogen peroxide and peroxidase activity

Axial branches of c. 10 cm were harvested from all individuals at both severe-drought and during subsequent heat stress, with leaves being removed and immediately flash frozen in liquid nitrogen. Frozen leaf material was ground to a fine powder in liquid nitrogen using mortar and pestle. Hydrogen peroxide (H_2O_2) concentration was quantified using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (ThermoFisher Scientific, Waltham, MA, USA), as described in Chakraborty et al. (2016). In short, the oxidation of Amplex Red reacts in 1:1 stoichiometry with H_2O_2 in the presence of peroxidase resulting in the fluorescent product resorufin, which we measured using a spectrophotometer for absorbance at 560 nm following excitation between 530 and 560 nm (Epoch 2, BioTek, VT, USA). Sample H_2O_2 concentrations were determined by comparing absorbances to a standard curve of known H_2O_2 concentrations, with all samples saturated with horseradish peroxidase. Peroxidase enzymatic activity (POD) was quantified using the same reagents following a similar procedure, where concentrations were determined by comparing absorbances to a POD standard curve, with all samples instead saturated with H_2O_2. POD activity is reported in units (U), defined by the manufacturer as the amount of enzyme that forms 1.0 mg purpurogallin from pyrogallol in 20 s at pH 6.0 and 20 °C. Additional fresh leaf samples were taken at each sampling day to standardize chemical concentrations from fresh material analyses to dry weight (DW) by weighing the fresh and dried (at 60 °C for 48 h) samples.

Statistical analysis

All statistical analyses and data visualizations were conducted in the R statistical programming environment v4.3.2 (R Core Team 2022). The contribution of PR to net carbon assimilation was assessed using linear mixed-effect regression to model the relationship between A_{net} at both 21%-O_2 (ambient) and 2%-O_2 (PR suppressed) using the package *nlme* (Pinheiro et al. 2023). This relationship indicates the relative efficiency of Rubisco carboxylation, where slopes closer to one represent less PR. Differences in slope (interactions) were assessed across levels of drought and heat treatments as main effects, with plant ID as a random effect to account for repeated measures during the heat treatment. Similar mixed effect models were used to evaluate the additive and interactive effects of drought and heat treatment on T_{leaf}, H_2O_2 and POD. Family-wise comparisons across treatment combinations were performed using Tukey's Honest Significant Difference (*emmeans* package; Lenth et al. 2024) and informed compact letter displays in figures with a 0.05 significance level. Parametric modeling assumptions of normality, equal variance and influential points (Cook's distance > 0.5) were verified using diagnostic plots. Due to heteroscedasticity when modeling H_2O_2, weights were assigned to each observation based on variance of the residuals. All parameter means are reported with standard error.

Results

Treatment progression

During the experiment drought, SWC, midday plant water potential (Ψ_{md}) and stem radial diameter progressively declined (Figure 1), and were significantly different from control trees during both mild- and severe-drought (both $P < 0.001$). By severe-drought, volumetric SWC had declined to 2.28% ± 3.26 compared with 37.01% ± 2.46 in the control, while Ψ_{md} had declined to −1.70 MPa ± 0.08 compared with −0.75 MPa ± 0.05 in the control. Stem radial diameter in the drought treatment decreased by 0.61% ± 1.10 during the experimental period, while control tree diameter increased by 6.50% ± 1.20 over the same period.

During the 1-day heat event, midday T_{air} increased by 16 °C to 39 °C, while RH decreased from 58 to 31.5% (Figure S1 available as Supplementary data at *Tree Physiology* Online), corresponding to an increase of ambient vapor pressure deficit from 1.18 to 4.75 kPa. Both drought and heat stress individually increased T_{leaf} (both $P < 0.001$): in the drought + heat treatment it was highest with 42.4 °C ± 0.36, followed by the control + heat treatment with 40.5 °C ± 0.41, the drought treatment with 28.5 °C ± 0.11, and the control with 25.4 °C ± 0.27 (Figure S3 available as Supplementary data at *Tree Physiology* Online).

Photorespiration and chlorophyll fluorescence

During mild-drought we found relative PR to increase ($P < 0.001$, Figure 2a, Table 1) as Rubisco carboxylation efficiency in the drought treatment slightly decreased to 76.3% ± 1.50 compared with 81.46% ± 1.74 in the control. Contrastingly, by severe-drought, we found relative PR to decrease to near zero ($P < 0.001$, Figure 2b, Table 1), as Rubisco carboxylation efficiency increased to 99.02% ± 7.23 compared with 85.43% ± 2.25 in the control. Overall, Rubisco carboxylation efficiency decreased slightly during the

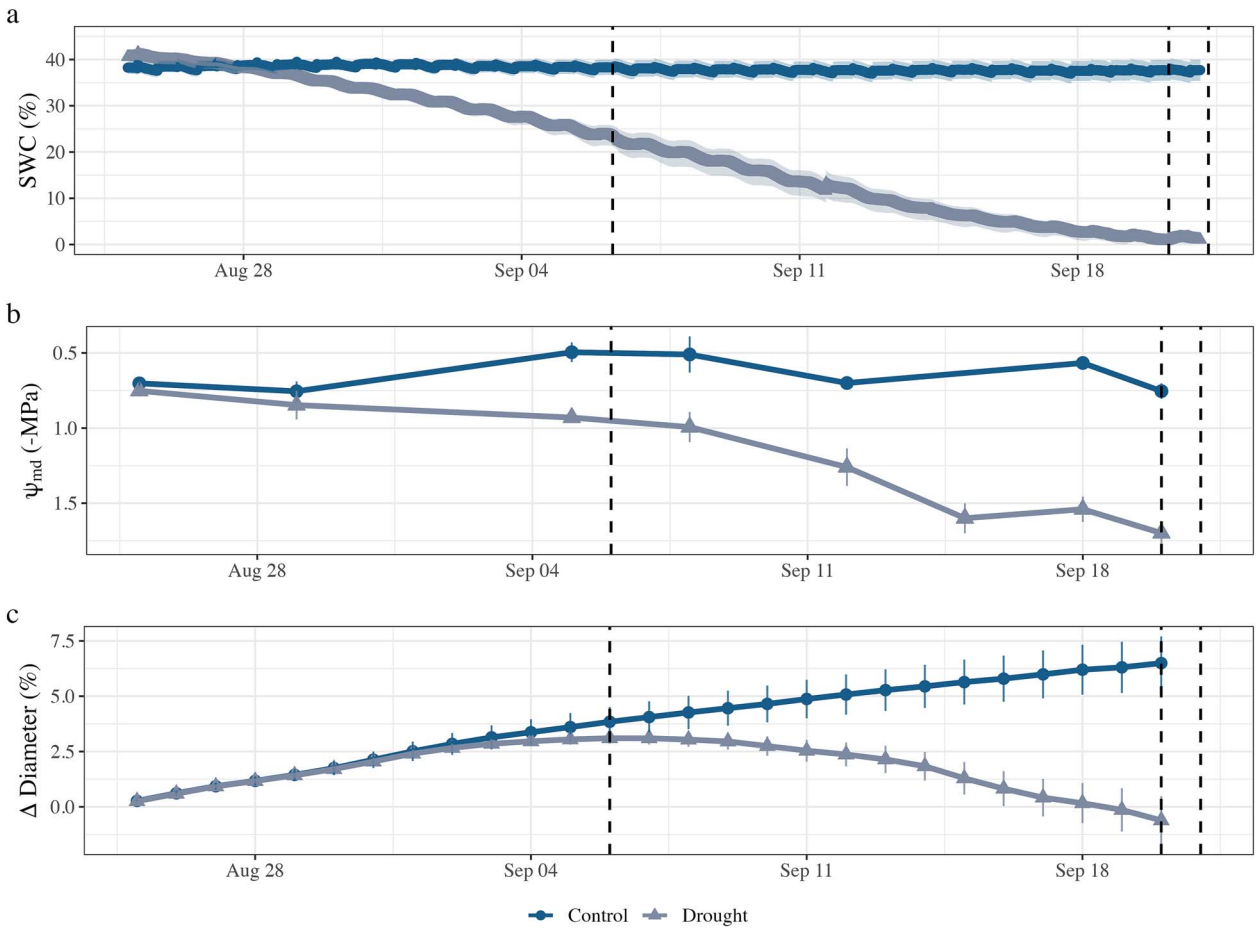

Figure 1. Time series visualization of volumetric SWC (a), midday branch water potential (Ψ_{md}, b) and percent change in stem diameter (c) during the experimental period. Data points are displayed with standard error, while vertical black lines represent mild-drought, severe-drought and heat treatment timepoints, respectively.

initial drought stress, but carboxylation ultimately increased to nearly 100% as SWC declined to 0% (Figure 3).

During the 1-day heat event, relative PR in the control + heat treatment increased greatly ($P < 0.001$, Figure 2b, Table 1), as Rubisco carboxylation efficiency decreased to 47.62% ± 8.66. However, under combined drought and heat stress, A_{net} was near or below zero and relative PR no longer increased ($P = 0.689$, Figure 2b, Table 1), leaving Rubisco carboxylation similar to the control.

Similarly, while ETR was unaffected during mild-drought, it largely declined during severe-drought ($P = 0.008$, Figure 4b, Table 1). Additionally, we found ETR to increasingly explain the variation in A_{net} with drought progression (estimated using sums of squares ratio), suggesting an increase in photochemical limitations with increasing drought. Heat stress further decreased ETR ($P < 0.001$, Table 1), with the greatest decrease in ETR occurring under drought + heat stress (Figure 4b), at which point A_{net} was near or below zero. Additional gas exchange data including stomatal conductance and transpiration are available in Figure S4 (available as Supplementary data at *Tree Physiology* Online).

Foliar hydrogen peroxide and peroxidase

Foliar H_2O_2 concentration was sensitive to water-limitation as well as heat stress. We found drought to decrease foliar H_2O_2 concentration by ~50% (0.68 mg gDW^{-1} ± 0.12) compared with the control (1.29 mg gDW^{-1} ± 0.18; $P < 0.001$, Figure 5a, Table 1). Surprisingly, the 1-day heatwave largely reduced foliar H_2O_2 to near zero in both control + heat as well as drought + heat ($P < 0.001$, Figure 5a, Table 1), indicating that H_2O_2 was quickly scavenged. While drought stress alone did not affect POD activity ($P = 0.419$, Figure 4b, Table 1), heat stress approximately doubled POD activity in both control + heat and drought + heat treatments (635.33 U gDW^{-1} ± 57.59) compared with the control (320.33 U gDW^{-1} ± 40.17; $P < 0.001$, Figure 4b, Table 1).

Discussion

We found that mild drought stress can increase PR when stomatal conductance primarily limits photosynthesis, but that severe drought stress may lead to decreases in PR as non-stomatal limitations of photosynthesis intensify, as observed with decreased ETR, ultimately decreasing photosynthetic CO_2 demand. We also found that heat stress can greatly increase PR, but in combination with drought this increase was no longer observed. Surprisingly, we found that foliar H_2O_2 concentrations were near zero under both heat and drought + heat treatments despite an increase in PR under heat. Analysis of POD activity suggests that heat stress led to rapid H_2O_2 scavenging, demonstrating a tight oxidative regulation independent of photosynthesis and thus also

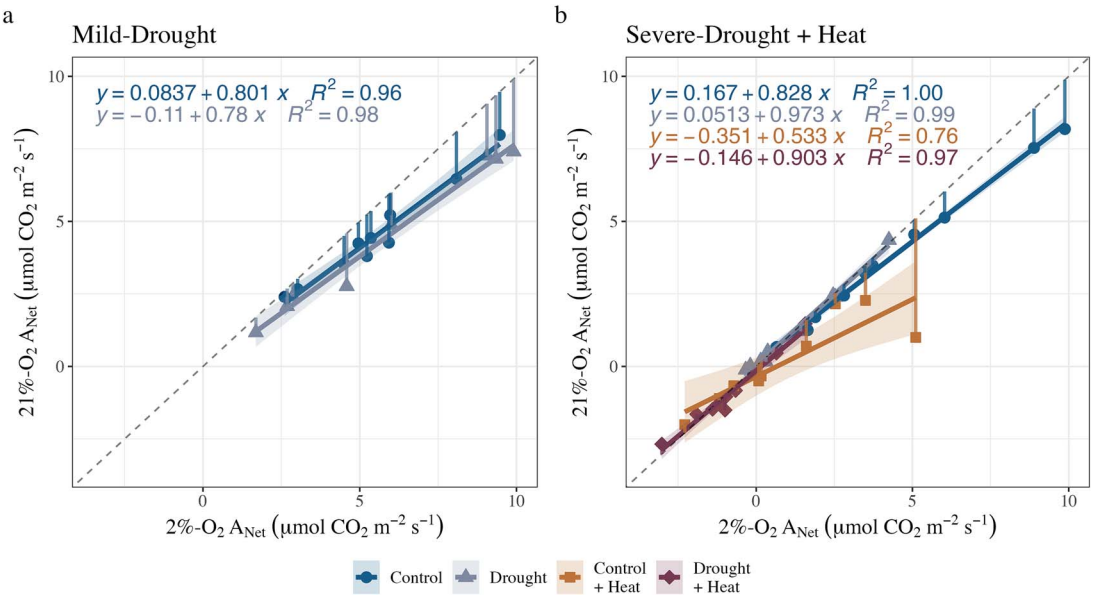

Figure 2. Photorespiration and Rubisco carboxylation efficiency derived from measurements of photosynthetic carbon assimilation (A_{net}) under 21% and 2%-O_2 in silver fir. Shown are the data points from the measurements of all individuals ($n = 8$ per treatment) during both mild-drought (a), as well as during severe-drought and a 1-day heat event (b). The ratio (slope) of these measures estimates Rubisco carboxylation efficiency. The 1:1 dotted line represents 100% carboxylation efficiency, while, the regression line represents within treatment variation in photosynthetic assimilation. The length of the vertical line above each point estimates net PR flux for each individual. Shaded area around regression lines represents 95% confidence intervals.

Table 1. Summary of mixed-effect models comparing the individual and interactive effects of drought (mild-drought, severe-drought) and heat treatment on leaf temperature (T_{leaf}), PR, ETR, hydrogen peroxide (H_2O_2) and peroxidase enzyme activity (POD). Reported values include estimated effect, t-value with degrees of freedom (not-applicable for H_2O_2 due to weighted analysis) and the respective *P*-value.

Mild-drought	**Drought**								
Response	**Estimate**	***t*-value**	***P*-value**						
PR	0.052	$50.761_{(17)}$	<.001						
ETR	−3.212	$-0.489_{(18)}$	0.631						
Severe-drought + heat	**Drought**			**Heat**			**Drought∗heat**		
Response	Estimate	*t*-value	*P*-value	Estimate	*t*-value	*P*-value	Estimate	*t*-value	*P*-value
T_{Leaf}	2.404	$3.735_{(18)}$	0.002	15.056	$39.500_{(48)}$	<0.001	−0.507	$-0.801_{(48)}$	0.427
PR	−.159	$13.421_{(10)}$	<0.001	0.378	$-6.219_{(10)}$	<0.001	−0.258	$1.810_{(10)}$	0.100
ETR	−15.563	$-2.906_{(23)}$	0.008	−46.486	$-10.206_{(12)}$	<0.001	6.087	$0.912_{(12)}$	0.377
H_2O_2	−.416	−7.759	<0.001	−1.032	−19.262	<0.001	.415	7.700	<0.001
POD	5.818	$0.066_{(17)}$	0.948	324.661	$8.657_{(12)}$	<0.001	95.327	$1.536_{(12)}$	0.150

independent of water availability. Together, these results provide key insights into the response of the PR pathway and its stress-signaling potential to combined drought and heat stress. In the following we will further elaborate on the finding that the magnitude and direction of PR changes during drought and/or heat stress depend on the timing and intensity of stress events, and that increased PR does not necessarily lead to accumulated H_2O_2 due to enzymatic scavenging.

Photorespiration depends on timing and interactions between drought and heat

We observed that PR slightly increased at mild drought, with declines in SWC from ~40% down to 25%, but only small changes in Ψ_{md} and radial growth indicating mild drought stress (Figure 1). Photorespiration is commonly assumed to increase during mild to moderate drought stress, as reduced CO_2 uptake from higher stomatal resistance increases the rate of Rubisco oxygenation relative to carboxylation (Bai et al. 2008, Voss et al. 2013, Tsonev et al. 2014). In contrast, more severe drought stress decreased relative PR, suggesting that reduced CO_2 uptake was no longer the main limiting factor of photosynthesis. As drought stress progresses, other factors such as reduced photochemical efficiency and/or limited regeneration of RuBP can additionally limit photosynthesis (Dias and Brüggemann 2010), demonstrated in our results by reduced ETR at severe-drought. Under severe drought stress, stomatal closure may not necessarily lead to decreased intercellular CO_2 as non-stomatal limitations may instead limit the ability of the Calvin–Benson cycle to utilize existing, resulting in intercellular CO_2 accumulating due to cellular respiration and cuticular permeability (Cornic and Briantais 1991, Flexas and Medrano 2002*a*, Pirasteh-Anosheh et al. 2016). As such, while Rubisco efficiency may approach 100%, reduced net gas exchange limits the benefit of increased carboxylation efficiency.

Thus, the presented results demonstrate that PR may increase or decrease during drought depending on the influence of non-stomatal limitations such as the slowdown of

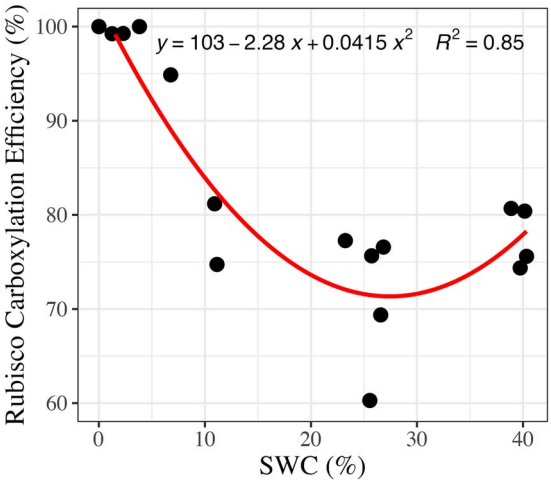

Figure 3. Rubisco carboxylation efficiency in response to SWC in silver fir. Data points present measured values collected on individual drought treatment seedlings ($n = 4$–6 per measurement campaign) at the experimental start, mid-drought and end-drought. The provided equation represents a second-degree polynomial regression model, where Rubisco carboxylation efficiency is modeled as a function of SWC.

photosynthetic light reactions, and indicate that any ability of PR to mitigate stress may decrease with drought progression (Noctor et al. 2002, Lawlor and Tezara 2009). Additionally, this likely represents an inaccuracy in models of GPP which do not consider non-stomatal limitations of photosynthesis (Rödig et al. 2017, Yin and Struik 2017, Nadal-Sala et al. 2021, Asargew et al. 2024), despite being coordinated with stomatal control to maximize photosynthesis during drought (Salmon et al. 2020). Such models of GPP therefore do not account for potential fluctuations of PR's contribution to net carbon assimilation with soil dehydration, and may therefore be less accurate predicting GPP during drought events (Zhou et al. 2013). As stomatal and non-stomatal coordination during drought varies across forest species (Dewar et al. 2022), a better species-level understanding of such coordination is needed to improve models of GPP.

We observed the largest increase in PR relative to A_{net} following 6 h of 40 °C T_{air}, decreasing average Rubisco carboxylation efficiency by nearly half. Individual PR responses to heat stress had increased variance than under other treatments, particularly with higher A_{net} values. Heat stress often increases PR despite constant atmospheric CO_2 due to an increased relative solubility of O_2 at higher temperatures, by decreasing Rubisco CO_2 affinity, and/or by increasing Rubisco misfire (Zhang and Sharkey 2009, Bracher et al. 2017). This increase in PR during heat stress could potentially be amplified by mild drought, when the ratio of O_2:CO_2 can be further increased by limited CO_2 uptake. However, we did not observe an amplification of PR under combined severe drought and heat stress when most plants had stomata closed and A_{net} was close to zero (Figure S4 available as Supplementary data at *Tree Physiology* Online), instead finding that severe drought stress eliminated the heat induced increase in PR. This suggests that despite changes in O_2:CO_2 solubility or Rubisco kinetics during heat that would otherwise favor PR, intercellular CO_2 may no longer limit photosynthesis during severe drought when non-stomatal limitations are increased, such as reduced ETR and greater NPQ (Flexas and Medrano 2002*a*, Zhou et al. 2013). Studies combining drought and heat stress also suggest a greater role of biochemical limitations rather than stomatal limitations on photosynthesis, supporting our observations (Flexas and Medrano 2002*a*, Ruehr et al. 2016, Perdomo et al. 2017). Additionally, drought stress can lower the optimal temperature of photosynthesis, meaning heat stress combined with drought is more likely to reach temperatures beyond this optimal photosynthesis threshold than just heat alone, increasing biochemical limitations (Fang et al. 2023). Taken together, our results demonstrate that while heat alone can increase PR, compounding non-stomatal limitations in the light-dependent reactions from additional drought stress may eliminate this increase.

H_2O_2 accumulation is tightly regulated despite variable PR during stress

Drought stress tended to reduce foliar H_2O_2 by c. 50% on average, suggesting either lower production and/or an increase in enzymatic scavenging from stress. Drought stress often leads to an accumulation in H_2O_2 due to increased PR which can outpace upregulated scavenging enzyme activity (Luna et al. 2005, Luyssaert et al.al. 2008, Pan et al. 2017). Other studies demonstrate that drought stress instead reduces scavenging enzyme activity, resulting in a greater accumulation of H_2O_2 from PR (Silva et al. 2012). As we observed no significant changes in POD activity of silver fir leaves in the drought treatment, we attribute lower foliar H_2O_2 concentration either to nearly-eliminated PR at severe-drought, and/or to increased activity of other scavengers like peroxisomal catalases. The remaining H_2O_2 concentration in the drought treatment may originate in cellular compartments not associated with PR, including in the thylakoid membrane during the light-dependent reactions of photosynthesis (Khorobrykh et al. 2015). With ETR reduced during drought stress, electrons may increasingly transfer to O_2 forming superoxide radicals, which are then quickly converted to H_2O_2. However, increased Non-Photochemical Quenching (NPQ) can mitigate this by dissipating excess excitation energy as heat, reducing H_2O_2 accumulation in the chloroplast. Our observation of small concentrations of H_2O_2 and increased leaf temperatures (Figure S3 available as Supplementary data at *Tree Physiology* Online) under stress conditions additionally suggest increased NPQ under our severe stress conditions. While the expectation that the proportion of total H_2O_2 originating from PR increases during drought (Noctor et al. 2002, Silva et al. 2012), this likely is only true under mild-to moderate-drought stress when stomatal closure primarily limits photosynthesis.

Heat stress reduced H_2O_2 to near-zero concentration while greatly increasing POD activity, suggesting an efficient scavenging of H_2O_2 despite likely higher production from increased PR. The potential of H_2O_2 from PR as a stress-signaling molecule is dependent on the balance between accumulation and scavenging (Petrov and Van Breusegem 2012). H_2O_2 accumulated in the peroxisome during PR can diffuse into the cytosol, where it can be converted to hydroxyl radicals and cause cellular membrane damage, DNA mutations and cell death (Richards et al. 2015, Carvalho and Silveira 2020). In addition to the measured POD enzymatic increase, increased H_2O_2 scavenging by catalases in the peroxisome, or enhanced regulation by the glutathione-ascorbate cycle in various cellular compartments such as chloroplasts, mitochondria, peroxisomes and the

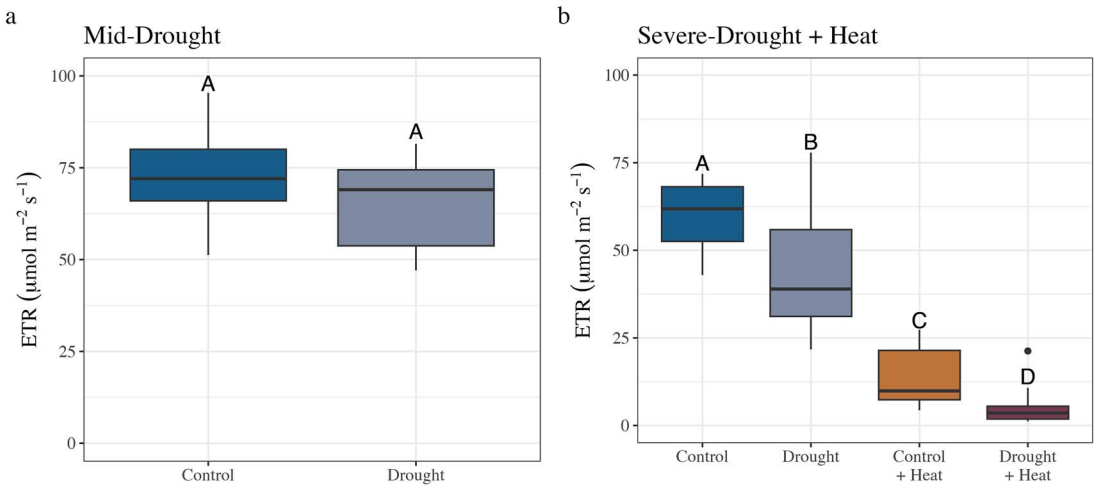

Figure 4. Treatment effects on ETR in silver fir. Shown are boxplots of ETR during mild-drought (a) and severe-drought plus a 1-day heat event (b, $n = 8$). Uppercase letters indicate significant pairwise differences determined post hoc using Tukey's Honest Significant Difference.

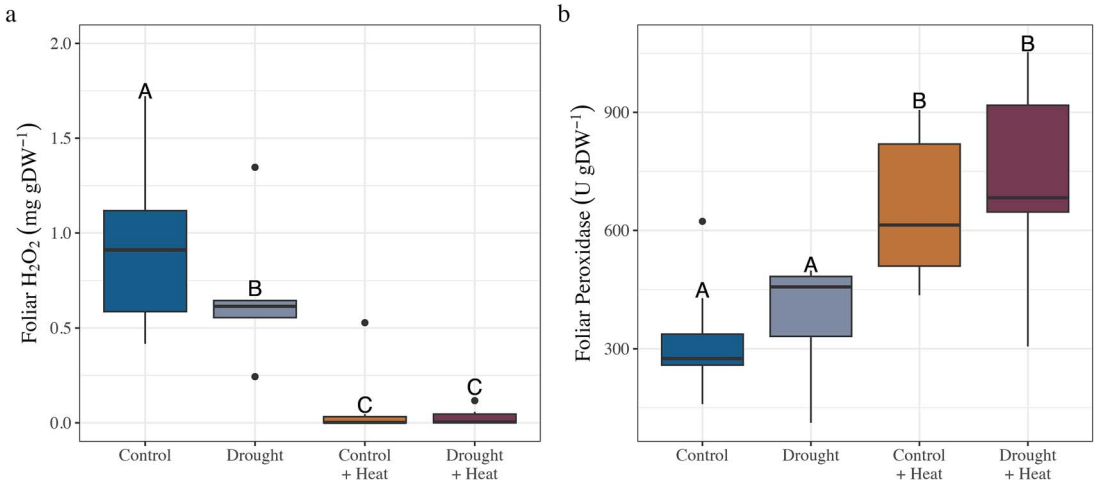

Figure 5. Treatment effects on foliar hydrogen peroxide (H_2O_2) and peroxidase levels in silver fir. Shown are boxplots of H_2O_2 concentration (a) and peroxidase (b) during severe-drought and a 1-day heat stress in silver fir ($n = 8$). Uppercase letters indicate significant pairwise differences determined post hoc using Tukey's Honest Significant Difference.

cytosol, could have also contributed to the decreased H_2O_2 we observed. Quick scavenging of ROS like H_2O_2 during abiotic stress mitigates oxidative stress and maintains cell function, but may reduce the potential of ROS stress-signaling. Our results show that H_2O_2 can be effectively scavenged despite increases in PR during short-term heat stress, likely attributable to the high temperature stability of POD enzymes (Plieth and Vollbehr 2012). Furthermore, POD increases under combined drought and heat stress demonstrate tight regulation of H_2O_2 despite compounding stress, reducing H_2O_2 stress-signaling potential during abiotic stress. This regulation may limit the induction of stress responsive genes by H_2O_2 as well as limit the effectiveness of H_2O_2-mediated hormonal pathways. As prolonged heat stress may ultimately reduce the production of ROS enzymatic scavengers (Foster et al. 2015), stress-signaling via H_2O_2-accumulation may have an increased role during longer-term heat events.

Conclusion

This study demonstrates that the effects of drought and heat stress on PR as well as foliar H_2O_2 in silver fir strongly depend on the timing and intensity of the stress event. We found that PR increased during mild drought stress but ultimately decreased during severe drought stress, likely due to intensifying non-stomatal limitations of photosynthesis such as reduced ETR. Additionally, while heat stress alone increased PR, greater non-stomatal limitations during severe drought corresponded to lower PR under combined stress. H_2O_2 accumulation was limited by scavenging enzyme activity during heat stress despite increases in PR. This demonstrates a tight regulation of ROS production during abiotic stress while limiting the potential for cellular stress-signaling. To understand tree-level stress signaling, it is necessary to consider PR and the underlying physiological processes which determine its dynamic nature.

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Authors' contributions

F.A., N.K.R. and P.P. conceived and designed the experiment. F.A. analyzed the data and drafted the initial manuscript, with input from N.K.R. and P.P. All authors have read and approved the final manuscript.

Conflict of interest

None declared.

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Data availability

All experimental data are available upon request to the corresponding author.

References

Abogadallah GM (2011) Differential regulation of photorespiratory gene expression by moderate and severe salt and drought stress in relation to oxidative stress. Plant Sci 180:540–547. https://doi.org/10.1016/j.plantsci.2010.12.004.

Allen CD, Macalady AK, Chenchouni H et al. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage 259:660–684. https://doi.org/10.1016/j.foreco.2009.09.001.

Asargew MF, Masutomi Y, Kobayashi K, Aono M (2024) Water stress changes the relationship between photosynthesis and stomatal conductance. Sci Total Environ 907:167886. https://doi.org/10.1016/j.scitotenv.2023.167886.

Bai J, Xu DH, Kang HM, Chen K, Wang G (2008) Photoprotective function of photorespiration in *Reaumuria soongorica* during different levels of drought stress in natural high irradiance. Photosynthetica 46:232–237. https://doi.org/10.1007/s11099-008-0037-5.

Bambach N, Kyaw Tha Paw U, Gilbert ME (2020) A dynamic model of RuBP-regeneration limited photosynthesis accounting for photoinhibition, heat and water stress. Agric For Meteorol 285-286:107911. https://doi.org/10.1016/j.agrformet.2020.107911.

Bauwe H, Hagemann M, Fernie AR (2010) Photorespiration: players, partners and origin. Trends Plant Sci 15:330–336. https://doi.org/10.1016/j.tplants.2010.03.006.

Bracher A, Whitney SM, Ulrich Hartl F, Hayer-Hartl M (2017) Biogenesis and metabolic maintenance of Rubisco. Annu Rev Plant Biol 68:29–60. https://doi.org/10.1146/annurev-arplant-043015-111633.

Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ (2006) ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H2O2 synthesis. Plant J 45:113–122. https://doi.org/10.1111/j.1365-313X.2005.02615.x.

Carvalho FEL, Silveira JAG (2020) Chapter 4 - H2O2-retrograde signaling as a pivotal mechanism to understand priming and cross stress tolerance in plants. In: Hossain MA, Liu F, Burritt DJ, Fujita M, Huang B (eds) Priming-mediated stress and cross-stress tolerance in crop plants Academic Press, Elsevier, pp 57–78. https://doi.org/10.1016/B978-0-12-817892-8.00004-0.

Chakraborty S, Hill AL, Shirsekar G, Afzal AJ, Wang G-L, Mackey D, Bonello P (2016) Quantification of hydrogen peroxide in plant tissues using Amplex Red. Methods 109:105–113. https://doi.org/10.1016/j.ymeth.2016.07.016.

Cornic G, Briantais J-M (1991) Partitioning of photosynthetic electron flow between CO2 and O2 reduction in a C3 leaf (*Phaseolus Vulgaris* L.) at different CO2 concentrations and during drought stress. Planta 183:178–184. https://doi.org/10.1007/BF00197786.

Dewar R, Hölttä T, Salmon Y (2022) Exploring optimal stomatal control under alternative hypotheses for the regulation of plant sources and sinks. New Phytol 233:639–654. https://doi.org/10.1111/nph.17795.

Dias MC, Brüggemann W (2010) Limitations of photosynthesis in *Phaseolus Vulgaris* under drought stress: gas exchange, chlorophyll fluorescence and Calvin cycle enzymes. Photosynthetica 48:96–102. https://doi.org/10.1007/s11099-010-0013-8.

Doehlert DC, Walker RB (1981) Photosynthesis and photorespiration in Douglas-fir as influenced by irradiance, CO2 concentration, and temperature. For Sci 27:641–650. https://doi.org/10.1093/forestscience/27.4.641.

Fang L, Martre P, Jin K, Xinmiao D, van der Putten PEL, Yin X, Struik PC (2023) Neglecting acclimation of photosynthesis under drought can cause significant errors in predicting leaf photosynthesis in wheat. Glob Chang Biol 29:505–521. https://doi.org/10.1111/gcb.16488.

Flexas J, Medrano H (2002*a*). Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. Ann Bot 89:183–189. https://doi.org/10.1093/aob/mcf027.

Flexas J, Medrano H (2002*b*). Energy dissipation in C3 plants under drought. Funct Plant Biol 29:1209–1215. https://doi.org/10.1071/fp02015.

Foster NL, Lukowiak K, Henry TB (2015) Time-related expression profiles for heat shock protein gene transcripts (HSP40, HSP70) in the central nervous system of *Lymnaea stagnalis* exposed to thermal stress. Commun Integr Biol 8:3. https://doi.org/10.1080/19420889.2015.1040954.

Harris NL, Gibbs DA, Baccini A et al. (2021) Global maps of twenty-first century forest carbon fluxes. Nat Clim Change 11:234–240. https://doi.org/10.1038/s41558-020-00976-6.

Hochberg U, Degu A, Fait A, Rachmilevitch S (2013) Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. Physiol Plant 147:443–452. https://doi.org/10.1111/j.1399-3054.2012.01671.x.

Húdoková H, Petrik P, Petek-Petrik A, Konôpková A, Leštianska A, Střelcová K, Kmeť J, Kurjak D (2022) Heat-stress response of photosystem II in five ecologically important tree species of European temperate forests. Biologia 77:671–680. https://doi.org/10.1007/s11756-021-00958-9.

Jordan DB, Ogren WL (1984) The CO2/O2 specificity of ribulose 1,5-bisphosphate carboxylase/oxygenase. Planta 161:308–313. https://doi.org/10.1007/BF00398720.

Kato MC, Hikosaka K, Hirotsu N, Makino A, Hirose T (2003) The excess light energy that is neither utilized in photosynthesis nor dissipated by photoprotective mechanisms determines the rate of photoinactivation in photosystem II. Plant Cell Physiol 44:318–325. https://doi.org/10.1093/pcp/pcg045.

Khorobrykh SA, Karonen M, Tyystjärvi E (2015) Experimental evidence suggesting that H2O2 is produced within the thylakoid membrane in a reaction between plastoquinol and singlet oxygen. FEBS Lett 589:779–786. https://doi.org/10.1016/j.febslet.2015.02.011.

Ku S-B, Edwards GE (1977) Oxygen inhibition of photosynthesis: I. Temperature dependence and relation to O2/CO2 solubility ratio 1. Plant Physiol 59:986–990. https://doi.org/10.1104/pp.59.5.986.

Ku S-B, Edwards GE (1978) Oxygen inhibition of photosynthesis. Planta 140:1–6. https://doi.org/10.1007/BF00389372.

Lawlor DW, Tezara W (2009) Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Ann Bot 103:561–579. https://doi.org/10.1093/aob/mcn244.

Lenth RV, Bolker B, Buerkner P et al. (2024) Emmeans: estimated marginal means, aka least-squares means. https://cran.r-project.org/web/packages/emmeans/index.html.

Lloyd MK, Stein RA, Ibarra DE, Barclay RS, Wing SL, Stahle DW, Dawson TE, Stolper DA (2023) Isotopic clumping in wood as a proxy for photorespiration in trees. Proc Natl Acad Sci USA 120:e2306736120. https://doi.org/10.1073/pnas.2306736120.

Luna CM, Pastori GM, Driscoll S, Groten K, Bernard S, Foyer CH (2005) Drought controls on H2O2 accumulation, catalase (CAT) activity and CAT gene expression in wheat. J Exp Bot 56:417–423. https://doi.org/10.1093/jxb/eri039.

Luyssaert S, Schulze ED, Börner A, Knohl A, Hessenmöller D, Law BE, Ciais P, Grace J (2008) Old-growth forests as global carbon sinks. Nature 455:213–215. https://doi.org/10.1038/nature07276.

Marinho HS, Real C, Cyrne L, Soares H, Antunes F (2014) Hydrogen peroxide sensing, signaling and regulation of transcription factors. Redox Biol 2:535–562. https://doi.org/10.1016/j.redox.2014.02.006.

Martin-StPaul N, Delzon S, Cochard H (2017) Plant resistance to drought depends on timely stomatal closure. Ecol Lett 20: 1437–1447. https://doi.org/10.1111/ele.12851.

Nadal-Sala D, Grote R, Birami B, Knüver T, Rehschuh R, Schwarz S, Ruehr NK (2021) Leaf shedding and non-stomatal limitations of photosynthesis mitigate hydraulic conductance losses in scots pine saplings during severe drought stress. Front Plant Sci 12:715127. https://doi.org/https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2021.715127.

Noctor G, Veljovic-Johanovic S, Driscoll S, Novitskaya L, Foyer CH (2002) Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? Ann Bot 89:841–850. https://doi.org/10.1093/aob/mcf096.

Pan D, Li QX, Lin Z, Chen Z, Tang W, Pan C, Tan H, Zeng D (2017) Interactions between salicylic acid and antioxidant enzymes tilting the balance of H2O2 from photorespiration in non-target crops under halosulfuron-methyl stress. Pestic Biochem Physiol 143: 214–223. https://doi.org/10.1016/j.pestbp.2017.09.007.

Peñuelas J, Llusià J (2002) Linking photorespiration, monoterpenes and thermotolerance in *Quercus*. New Phytol 155:227–237. https://doi.org/10.1046/j.1469-8137.2002.00457.x.

Perdomo JA, Capó-Bauçà S, Carmo-Silva E, Galmés J (2017) Rubisco and Rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. Front Plant Sci 8:490. https://doi.org/https://www.frontiersin.org/articles/10.3389/fpls.2017.00490.

Petek-Petrik A, Petrík P, Lamarque LJ, Cochard H, Burlett R, Delzon S (2023) Drought survival in conifer species is related to the time required to cross the stomatal safety margin. J Exp Bot 74: 6847–6859. https://doi.org/10.1093/jxb/erad352.

Petrov VD, Van Breusegem F (2012) Hydrogen peroxide—a central hub for information flow in plant cells. AoB Plants 2012:pls014. https://doi.org/10.1093/aobpla/pls014.

Pinheiro J, Bates D, R Core Team (2023) Nlme: linear and nonlinear mixed effects models https://cran.r-project.org/web/packages/nlme/index.html.

Pirasteh-Anosheh H, Saed-Moucheshi A, Pakniyat H, Pessarakli M (2016) Stomatal responses to drought stress. In: Ahmad P (ed), Water stress and crop plants: A Sustainable Approach. John Wiley & Sons, Ltd, pp 24–40. https://doi.org/10.1002/9781119054450.ch3.

Plieth C, Vollbehr S (2012) Calcium promotes activity and confers heat stability on plant peroxidases. Plant Signal Behav 7:650–660. https://doi.org/10.4161/psb.20065.

R Core Team (2022) R: a language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Raghavendra AS (2003) Photosynthesis and partitioning | C3 plants. In: Thomas B (ed), Encyclopedia of applied plant sciences Elsevier, Oxford, pp 673–680. https://doi.org/10.1016/B0-12-227050-9/00094-6.

Richards SL, Wilkins KA, Swarbreck SM, Anderson AA, Habib N, Smith AG, McAinsh M, Davies JM (2015) The hydroxyl radical in plants: from seed to seed. J Exp Bot 66:37–46. https://doi.org/10.1093/jxb/eru398.

Robakowski P, Montpied P, Dreyer E (2002) Temperature response of photosynthesis of silver fir (*Abies alba* Mill.) seedlings. Ann For Sci 59:163–170. https://doi.org/10.1051/forest:2002003.

Rödig E, Huth A, Bohn F, Rebmann C, Cuntz M (2017) Estimating the carbon fluxes of forests with an individual-based forest model. For Ecosyst 4:4. https://doi.org/10.1186/s40663-017-0091-1.

Ruehr NK, Gast A, Weber C, Daub B, Arneth A (2016) Water availability as dominant control of heat stress responses in two contrasting tree species. Tree Physiol 36:164–178. https://doi.org/10.1093/treephys/tpv102.

Salmon Y, Lintunen A, Dayet A, Chan T, Dewar R, Vesala T, Hölttä T (2020) Leaf carbon and water status control stomatal and nonstomatal limitations of photosynthesis in trees. New Phytol 226:690–703. https://doi.org/10.1111/nph.16436.

Salvucci ME, Crafts-Brandner SJ (2004) Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiol Plant 120:179–186. https://doi.org/10.1111/j.0031-9317.2004.0173.x.

Sharkey TD (1988) Estimating the rate of photorespiration in leaves. Physiol Plant 73:147–152. https://doi.org/10.1111/j.1399-3054.1988.tb09205.x.

Silva EN, Ribeiro RV, Ferreira-Silva SL, Vieira SA, Ponte LFA, Silveira JAG (2012) Coordinate changes in photosynthesis, sugar accumulation and antioxidative enzymes improve the performance of *Jatropha curcas* plants under drought stress. Biomass Bioenergy 45:270–279. https://doi.org/10.1016/j.biombioe.2012.06.009.

Stangler DF, Miller TW, Honer H, Larysch E, Puhlmann H, Seifert T, Kahle H-P (2022) Multivariate drought stress response of Norway spruce, silver fir and Douglas fir along elevational gradients in Southwestern Germany. Front Ecol Evol 10:907492. https://doi.org/https://www.frontiersin.org/articles/10.3389/fevo.2022.907492.

Strand DD, Livingston AK, Satoh-Cruz M, Froehlich JE, Maurino VG, Kramer DM (2015) Activation of cyclic electron flow by hydrogen peroxide in vivo. Proc Natl Acad Sci USA 112:5539–5544. https://doi.org/10.1073/pnas.1418223112.

Sunil B, Saini D, Bapatla RB, Aswani V, Raghavendra AS (2019) Photorespiration is complemented by cyclic electron flow and the alternative oxidase pathway to optimize photosynthesis and protect against abiotic stress. Photosynth Res 139:67–79. https://doi.org/10.1007/s11120-018-0577-x.

Takagi D, Hashiguchi M, Sejima T, Makino A, Miyake C (2016) Photorespiration provides the chance of cyclic electron flow to operate for the redox-regulation of P700 in photosynthetic electron transport system of sunflower leaves. Photosynth Res 129:279–290. https://doi.org/10.1007/s11120-016-0267-5.

Tsonev T, Wahbi S, Sun P, Sorrentino G, Centritto M (2014) Gas exchange, water relations and their relationships with photochemical reflectance index in *Quercus ilex* plants during water stress and recovery. Int J Agric Biol 16:335–341.

Ünlüsoy AG, Yolcu S, Bor M, Özdemir F, Türkan İ (2023) Activation of photorespiration facilitates drought stress tolerance in *Lotus corniculatus*. J Plant Growth Regul 42:2088–2101. https://doi.org/10.1007/s00344-022-10683-5.

Voss I, Sunil B, Scheibe R, Raghavendra AS (2013) Emerging concept for the role of photorespiration as an important part of abiotic stress response. Plant Biol 15:713–722. https://doi.org/10.1111/j.1438-8677.2012.00710.x.

Wingler A, Quick WP, Bungard RA, Bailey KJ, Lea PJ, Leegood RC (1999) The role of photorespiration during

drought stress: an analysis utilizing barley mutants with reduced activities of photorespiratory enzymes. Plant Cell Environ 22:361–373. https://doi.org/10.1046/j.1365-3040.1999.00410.x.

Wolf S, Paul-Limoges E (2023) Drought and heat reduce forest carbon uptake. Nat Commun 14:6217. https://doi.org/10.1038/s41467-023-41854-x.

Yin X, Struik PC (2017) Can increased leaf photosynthesis be converted into higher crop mass production? A simulation study for rice using the crop model GECROS. J Exp Bot 68:2345–2360. https://doi.org/10.1093/jxb/erx085.

Yin X, van der Putten PEL, Belay D, Struik PC (2020) Using photorespiratory oxygen response to analyse leaf mesophyll resistance. Photosynth Res 144:85–99. https://doi.org/10.1007/s11120-020-00716-z.

Young SNR, Sack L, Sporck-Koehler MJ, Lundgren MR (2020) Why is C4 photosynthesis so rare in trees? J Exp Bot 71:4629–4638. https://doi.org/10.1093/jxb/eraa234.

Zhang R, Sharkey TD (2009) Photosynthetic electron transport and proton flux under moderate heat stress. Photosynth Res 100:29–43. https://doi.org/10.1007/s11120-009-9420-8.

Zhou S, Duursma RA, Medlyn BE, Kelly JWG, Colin Prentice I (2013) How should we model plant responses to drought? An analysis of stomatal and non-stomatal responses to water stress. Agric For Meteorol 182-183:204–214. https://doi.org/10.1016/j.agrformet.2013.05.009.