Tansley review

High-temperature acclimation of photosystem II in land plants

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Summary

The effect of high temperature on plant performance and survival is a topic of great interest given the ongoing rise in global heatwave frequency, duration, and intensity. The temperature at which photosystem II (PSII) is disrupted is often used as a proxy for photosynthetic heat tolerance. Our current understanding of PSII heat tolerance is predominantly shaped by 'snapshot' measurements that capture heat tolerance at a single point in time. However,

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growing evidence of dynamic thermal acclimation of PSII raises questions about the accuracy of current estimates of photosynthetic heat tolerance based on snapshot measurements. We believe that failing to account for acclimation may result in the underestimation of PSII heat tolerance and that the extent of acclimation can be predicted from leaf economic traits, leaf habit, plant water use strategies, photosynthetic pathway, and habitat. We also explore efforts to use spectroscopy techniques to predict acclimation, and the biotic and abiotic factors that may influence these predictions. Finally, we provide recommendations for the future incorporation of PSII heat tolerance and acclimation into models of the thermal limits of plant performance.

I. Introduction

Projected increases in subcontinental-scale mean annual, seasonal, and monthly temperatures due to climate change are well-established, with relatively strong agreement among general circulation models (Dufresne *et al.*, 2013; Zhao *et al.*, 2021). Consequently, global warming is predicted to produce more frequent and intense heatwaves around the world (Robinson *et al.*, 2021). In recent decades, the number of record-breaking monthly temperature extremes across the globe is five times larger than during the late-19th century through the mid-20th century (Coumou *et al.*, 2013). More recently, July 2024 was the hottest month ever recorded by the National Oceanic and Atmospheric Administration, with global mean temperature 1.2°C warmer than average across the 20th century (NOAA, 2024). As a result, between July 2023 and July 2024, maximum air temperatures reached or exceeded 50°C in at least 10 countries, including the United States, China, Mexico, India, and Iran (Kacprzyk *et al.*, 2025). The vulnerability of plant communities and ecosystems to extreme high temperatures may depend on their capacity to acclimate to high-temperature exposure, particularly at the leaf level (Evans *et al.*, 2025). Without significant thermal acclimation, forest canopies may experience increasing surges of damage and dieback (Still *et al.*, 2023) that in turn could result in reductions in net uptake of atmospheric CO_2 by terrestrial vegetation and trigger a significant positive feedback loop of climate warming (Bonan, 2008). Significant leaf loss may also impact species interactions and potentially alter future thermal environments via changes in boundary layers and shade provision. Thus, understanding the mechanisms and extent of plant heat tolerance and acclimation is critical for predicting plant responses to extreme high temperatures induced by climate change (Evans *et al.*, 2025).

Photosystem II (PSII) is the protein complex responsible for initiating the light reactions of photosynthesis, and it plays a crucial role in the photosynthetic process. Among the components of photosynthetic machinery, PSII is recognized as one of the most heat-sensitive components of photosynthesis (Weis & Berry, 1987; Geange *et al.*, 2021), and its impairment is a key indicator of heat impacts on leaf function. Consequently, the critical temperatures at which PSII function becomes inhibited (T_{crit}) or reduced by 50% (T_{50}) (See Box 1, for terminology and definitions) are among the most widely adopted metrics for describing photosynthetic heat tolerance (Weis & Berry, 1987; Knight & Ackerly, 2003; Allakhverdiev *et al.*, 2008). Broad variation in PSII heat tolerance (including T_{crit} and T_{50}) has been established across plant taxa and biomes (O'Sullivan *et al.*, 2017; Bison & Michaletz, 2024). However, while there is some evidence that T_{crit} adjusts/acclimates to sustained changes in growth temperature over time periods of hours to days and weeks (e.g. Zhu *et al.*, 2018, 2024; Posch *et al.*, 2022), our wider understanding of how this trait varies temporally in response to phenological, biotic, and abiotic factors remains limited (Perez *et al.*, 2021; Grossman, 2023). Without accurate knowledge of the capacity of PSII to acclimate to high temperatures, we risk underestimating leaf thermal tolerance and thus introducing errors into models of global vegetative carbon cycles, water budgets, and species distributions (Evans *et al.*, 2025).

Here, we review the mechanisms, limits, and ecological significance of high-temperature acclimation of PSII – defined as temporal plasticity in PSII heat tolerance (and associated shifts in T_{crit} or T_{50} values) in response to sustained heat exposure. Given that T_{crit} can vary in response to diel changes in temperature (Posch *et al.*, 2022) as well as to sustained warming over days and weeks (Zhu *et al.*, 2024; Cox *et al.*, 2025), our review explores the extent to which acclimation occurs over both short (e.g. hours) and longer (days–weeks) timescales. We address three interrelated questions: can patterns of PSII high-temperature acclimation be predicted based on a suite of factors related to plant water availability, previous heat exposure, water use strategy, leaf construction costs, and leaf habit? Over what temporal scales does PSII high-temperature acclimation occur, and is the rate of PSII high-temperature acclimation influenced by environmental and leaf morphological factors? And what is the outlook for using spectroscopy to map PSII high-temperature acclimation across temporal and spatial dimensions? We introduce the overarching hypotheses that failing to account for acclimation may result in the underestimation of PSII heat tolerance and that the extent of acclimation can be predicted from leaf economic traits, leaf habit, plant water use strategies, photosynthetic pathway, and habitat (Fig. 1). In addressing these questions, we provide a comprehensive review of the current state of knowledge of PSII heat tolerance and acclimation, as well as highlighting the key areas in which we still require further advances. In doing so, we make the case that continued efforts to fill these knowledge gaps will enhance our understanding of the high-temperature limits of PSII by incorporating knowledge of plasticity into leaf function and thermal tolerance in ways that have yet to be broadly quantified among plant taxa.

II. The global range of photosystem II heat tolerance and its relationship to permanent leaf damage

Our knowledge of the general biogeographical patterns of PSII heat tolerance has been expanded by numerous studies over the last two decades, particularly regarding the relationship between PSII heat tolerance and mean annual temperature (MAT). A recent global meta-analysis estimated PSII heat tolerance to be *c.* 50°C (Lancaster & Humphreys, 2020), with clear latitudinal and functional type patterns in global variation. Heat tolerance tends to be higher in plants hardened by previous heat exposure than in nonheat-exposed plants and increases toward the equator (Lancaster & Humphreys, 2020). The latter observation is consistent with O'Sullivan *et al.* (2017), who used the F_o rise method (Box 1)

Box 1. Basic theory and definitions

Chl molecules – found within pigment protein complexes in photosynthetic tissues – absorb light energy from the sun that, in turn, either provides energy for photosynthesis, is dissipated as heat, or re-emitted as light (Maxwell & Johnson, 2000; Murchie & Lawson, 2013). These three processes are competitive such that an increase in the yield of one will lead to a decrease of at least one of the other two (Murchie & Lawson, 2013). For instance, both positive and negative correlations between photochemistry and fluorescence yields have been observed as nonphotochemical quenching yield varies in response to environmental stress (Magney *et al*., 2020; Pierrat *et al*., 2024). Measurements of Chl fluorescence can provide valuable information on the state of photosystem II (PSII) because these measurements estimate the relative yield of each of these processes and subsequent quantum efficiency of photochemistry. The quantum yield of PSII is defined as the proportion of absorbed light (in the red wave bands < 700 nm) that is harvested by PSII for photochemistry relative to the amount of light that is re-emitted. The maximum quantum yield of PSII can be calculated as the ratio F_v/F_m, where F_v is the variable fluorescence and F_m is the maximum fluorescence measured following a saturating light pulse, when the reactions centers of the thylakoid membranes are open. Variable fluorescence is determined from F_m–F_o, where F_o is the minimum Chl fluorescence measured while leaves are in a dark-adapted state and reaction centers are closed (Table B1).

Table B1 List of abbreviations with common units.

Abbreviation	Definition	Units
F_o	Minimum fluorescence yield	Unitless
F_m	Maximum fluorescence yield	Unitless
F_v	Variable fluorescence ($F_m - F_o$)	Unitless
T–F_o	Temperature-dependent rise in steady state F_o	°C
T_{crit}	Critical temperature of T–F_o or T–F_v/F_m; temperature at which PSII is initially disrupted	°C
T_{50}	Temperature at which F_o reaches 50% of its maximum, or 50% decline in F_v/F_m	°C
T_{max}	Temperature at which F_o reaches its maximum	°C
T_{leaf}	Leaf surface temperature	°C
TSM	Leaf thermal safety margin ($T_{crit}-T_{leaf}$, or $T_{50}-T_{leaf}$)	°C

Decades of Chl fluorescence studies have yielded important information on the temperature sensitivity of PSII (Weis & Berry, 1987; Knight & Ackerly, 2003; Geange *et al*., 2021). An increased F_o and subsequent decrease in F_v/F_m at high temperature indicate disruption of electron transport capacity, primarily due to conformational changes and potential denaturation of lipids and proteins comprising the thylakoid membranes (Hüve *et al*., 2011). As photosynthetic tissues are exposed to increasing temperatures, PSII disruption amplifies and is eventually nonreversible. Stages of PSII damage in response to high-temperature stress are illustrated with example fluorescence temperature–response curves in Fig. B1. The F_o temperature response typically follows a pattern whereby levels remain relatively constant with moderate heating before increasing sharply at higher temperatures, then declining sharply (Knight & Ackerly, 2002; O'Sullivan *et al*., 2013, 2017). The critical temperature at which electron transport of PSII is initially disrupted (T_{crit}) is determined at the point of intersection of two lines, representing the flat and steep parts of the F_o temperature–response curve (Fig. B1a). At higher temperatures, F_o rises to 50% (T_{50}) of its maximum value (T_{max}), reflecting a 50% reduction in functionality of PSII (Knight & Ackerly, 2002), before falling sharply at even higher temperatures. The temperature response of F_o is similar, yet inverse to the temperature response of F_v/F_m (Fig. B1b). Each defined point along the curve reflects different levels of membrane fluidity and the dissociation of membrane-bound proteins involved with the electron transport capacity of PSII (Schreiber & Berry, 1977; Hüve *et al*., 2011). Despite being inverse of one another, F_o rise and F_v/F_m decline approaches typically provide similar approximations of T_{crit} and T_{50} parameters and their responses to changes in air temperature. For instance, Slot *et al*. (2021) reported a 0.40°C increase in F_v/F_m-derived T_{50} per °C change in MAT, closely resembling the 0.38°C increase in T_{50} of F_o rise per °C change in the mean temperature of the warmest month reported by O'Sullivan *et al*. (2017). Similarities across these measurements could partly be related to the influence of heat-induced rise in F_o for both approaches. However, for the F_v/F_m method, the inhibition of PSII has been reported as more closely linked to decline in F_m than in F_o rise (Yamane *et al*., 1997). Thus, the extent to which T_{crit} and T_{50} are offset across methodologies and why remains unclear, as well as whether this offset is predictable. Given the frequent use of both methodological approaches to calculating T_{crit} and T_{50}, in addition to the increasing focus on PSII heat tolerance and acclimation, future studies that directly compare these methods under common conditions for the same individuals would help to address this uncertainty.

Both T_{50} and T_{crit} are frequently employed along with leaf temperature to define a leaf's thermal safety margin, a useful metric for quantifying how close maximum leaf temperature is to critical thresholds for physiological disruption and damage. In addition to the direct effect of heat on PSII, changes in the F_o signal in dark-adapted leaves may also reflect other sources of inhibition of the chloroplast electron transport chain further downstream. This can be addressed by incorporating the imposition of far-red light into F_o rise assays, as this relaxes electron acceptors downstream of PSII and thus helps to ensure that changes in the F_o signal are attributable to experimental heating (Bilger *et al*., 1984). The speed at which leaf samples are heated during a fluorescence assay may also have a significant effect on T_{crit} and T_{50}. Using the F_o rise approach, Arnold *et al*. (2021) examined heating rates from 0.1°C min^{-1} to 4°C min^{-1} and found T_{crit} generally declined with faster heating rates, although this effect was highly species-specific (Arnold *et al*., 2021). The authors ultimately recommended a ramp speed between 0.5 and 1.0°C min^{-1} to allow for induction of thermal protective mechanisms during the assay and thus achieve a more realistic T_{crit} value for cross-study comparison (Arnold *et al*., 2021).

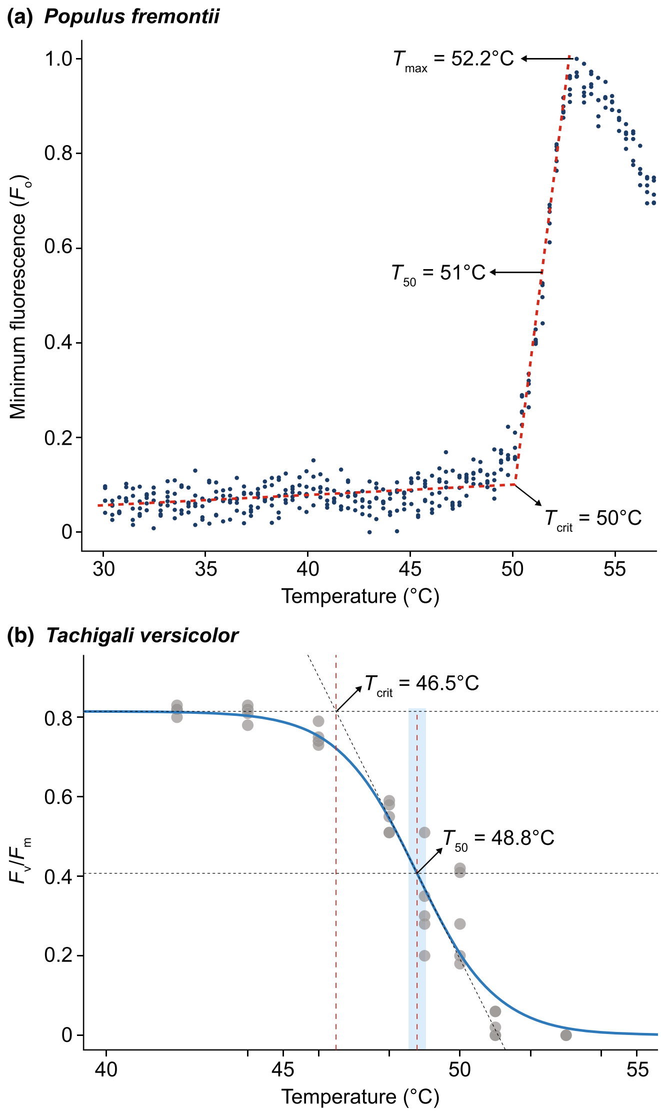

Fig. B1 Example (a) F_o – temperature response curve of *Populus fremontii*, and (b) F_v/F_m – temperature–response curve of *Tachigali versicolor*, with locations of T_{crit}, T_{50}, and T_{max} identified on the curves. T_{crit} is determined from the intersection of two lines (dashed lines) modeled on the slow, and fast rise of F_o, or decline in F_v/F_m. T_{50} is defined as the temperature at which F_o increases to 50% of T_{max} (Knight & Ackerly, 2002) or decreases to 50% of maximum F_v/F_m (Slot *et al*., 2021).

to determine the critical temperature at which PSII electron transport becomes disrupted by heat stress (T_{crit}; see Box 1, for definitions) for 218 plant species from seven distinct biomes. They found T_{crit} decreased by *c.* 0.11°C per degree latitude from 50.8°C in a Peruvian lowland tropical forest to 41.5°C in the Alaskan tundra. This variation in T_{crit} values aligns with the majority of PSII thermal limits reported in the literature, as well as identifying an upper bound up to 66°C (O'Sullivan *et al.*, 2017).

While there have been a growing number of efforts to quantify the relationship between PSII heat tolerance and growth temperature, the comparison of these studies reveals the extent to which these relationships range across species, habitats, and methodologies. The decrease in T_{crit} with latitude corresponded to an increase in T_{crit} of *c.* 0.26°C per °C increase in mean temperature of the warmest month (O'Sullivan *et al.*, 2017). Using the F_v/F_m decline method (Box 1), Feeley *et al.* (2020) found a 0.40°C decline in T_{50} for every 1000-m increase in elevation above sea level in 166 tropical Colombian tree species, equating to a 0.083°C increase in T_{50} per °C MAT. Slot *et al.* (2021) also used the F_v/F_m decline method to find both T_{crit} and T_{50} of tropical

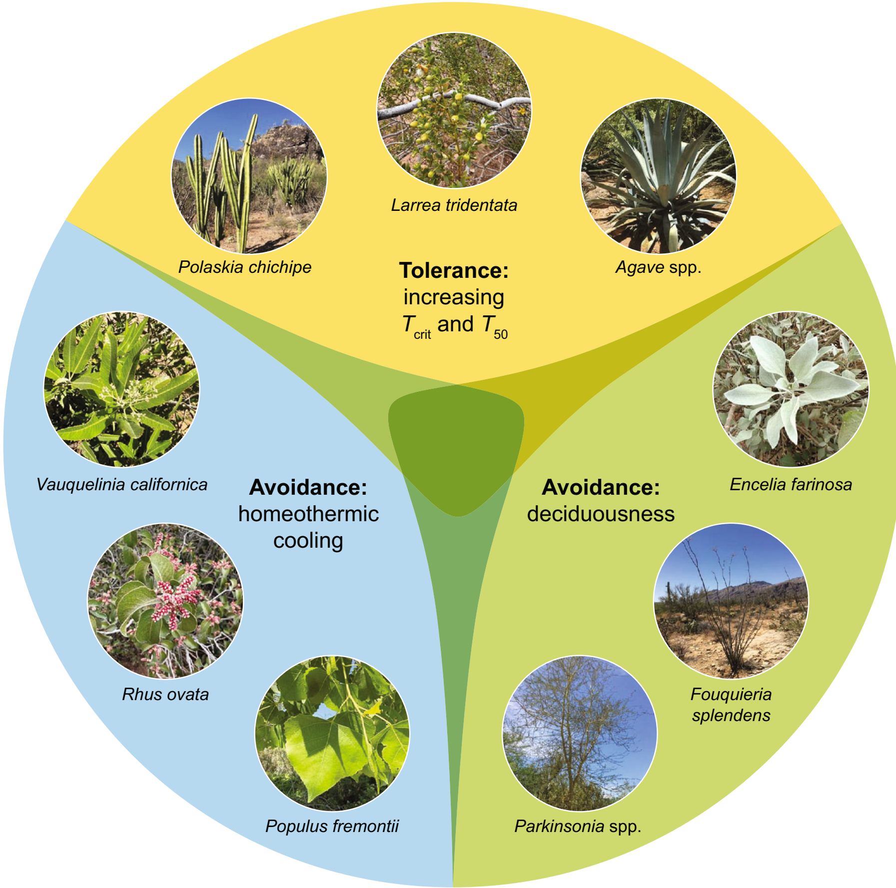

Fig. 1 Venn diagram of major strategies used to cope with heat stress with examples of species expected to specialize in heat tolerance/acclimation (yellow circles), heat avoidance through drought-deciduousness (green circles), and heat avoidance through homeothermic leaf cooling (blue circles). Species represented are *Polaskia chichipe* (Rol.-Goss) Backeb., cactus occurring in southern Mexico; *Larrea tridentata* (DC.) Coville, evergreen perennial shrub occurring in central Arizona, USA; *Agave* spp., occurring in central Arizona, USA; *Encelia farinosa* A. Gray ex Torr., drought-deciduous perennial shrub occurring in central Arizona, USA; *Fouquieria splendens* Engelm., drought-deciduous perennial shrub occurring in southeastern Arizona, USA; *Parkinsonia* spp., drought-deciduous tree occurring in central Arizona, USA; *Populus fremontii* S. Watson, riparian winter-deciduous tree occurring in southeastern Arizona, USA; *Rhus ovata* (Wats.), evergreen perennial shrub occurring in central Arizona, USA; and *Vauquelinia californica* (Torr.) Sarg. evergreen perennial shrub occurring in central Arizona, USA. Leaf homeothermic cooling during high-temperature exposure has been observed in *R. ovata*, *V. californica*, and *P. fremontii* (Aparecido *et al*., 2020; Moran *et al*., 2023; Posch *et al*., 2024).

forest species increased with MAT, but by a substantially larger rate of 0.60 and 0.40°C per °C MAT, respectively. Zhu *et al.* (2018) reported summer T_{crit} values of 48.5°C across 62 broadly distributed Australian species using the F_o rise method, corresponding to a 0.28°C increase per °C difference in the mean maximum temperature of the preceding 30 d. In a common garden of 177 species, T_{crit} ranged from *c.* 40 to 51°C and increased by 0.18°C per °C increase in the three-day average air temperature before sampling (Bison & Michaletz, 2024). Larger variation in T_{crit} has been reported within wheat alone (*Triticum aestivum* and *Triticum turgidum*; 35–52°C), based on compiled global data from 13 studies and 133 wheat genotypes (Posch *et al.*, 2022). Nonetheless, the global variation in wheat T_{crit} is only about two-thirds of the variation (33–67°C) reported for 225 species across globally distributed sites spanning 43°S to 68°N latitude (O'Sullivan *et al.*, 2017).

Measurements of T_{50}, particularly those derived from F_v/F_m temperature–response curves, are often used as proxies for irreversible damage to PSII and disrupted photosynthetic function. However, the relationships between fluorescence-based parameters – such as T_{50} and the other key parameters presented in Box 1 – and other measures of damage are typically correlative, with a detailed mechanistic understanding still lacking. Multiple studies of stress tolerance have found negative correlations between F_v/F_m and electrolyte leakage, indicative of irreversible membrane damage (Srinivasan *et al.*, 1996; Guadagno *et al.*, 2017). However, comparative studies further found that T_{50} based on electrolyte leakage was higher than T_{50} based on F_v/F_m decline for mature leaves of *Coffea arabica* L. (Marias *et al.*, 2017a) and Douglas fir (Marias *et al.*, 2017b), despite using 15-min incubations for the fluorescence protocol and 20-min incubations for the electrolyte protocol. A key factor contributing to these differences may be the timing of the measurements; specifically, Winter *et al.* (2025) found T_{50}-measured 14 d after heat imposition was a stronger predictor of necrotic damage than T_{50}-measured 24 h after heating in two tropical tree species. This indicates that typical approaches to measuring T_{crit} and T_{50} may be more reflective of reversible disruptions to leaf photosynthetic machinery (e.g. protein denaturation), rather than that of the irreversible damage represented by electrolyte leakage. Given these mixed results, further investigation involving a broader range of species and heating durations is needed to expand our understanding of the relationship between Chl fluorescence metrics and physiological damage.

The potential reversibility of Chl fluorescence following experimental heat treatment has motivated the use of a 24-h recovery period before measuring F_v/F_m in the F_v/F_m decline method (Krause *et al.*, 2010). Both T_{crit} and T_{50} estimated with the F_v/F_m decline method following 15-min incubations were higher after 24 h than directly following heat treatment, and T_{50} obtained after 24 h corresponded closely to the temperature at which 50% leaf necrosis occurred after 11 d (Krause *et al.*, 2010). However, in two other tropical tree species, T_{50}-measured 24 h after heat exposure was associated with visible leaf area damage substantially below 50% (Winter *et al.*, 2025), suggesting species-specific differences in the correlation between F_v/F_m decrease and leaf necrosis. Although Bilger *et al.* (1984) reported a strong correlation (r^2 = 0.76) between T_{crit} and the temperature at which 30-min heat exposure caused 50% leaf necrosis after 2–4 wk, their F_o rise method adopted a heating rate of 0.7°C min^{-1} instead of the 1.0°C min^{-1} that is now common (e.g. O'Sullivan *et al.*, 2017; Zhu *et al.*, 2024). These differences in protocols pose a challenge for comparisons and syntheses, particularly as the duration of heat exposure likely has a strong effect on estimates of T_{crit} (Arnold *et al.*, 2021; Perez *et al.*, 2021).

The duration of heat exposure is an important consideration when evaluating the thermal limits of PSII. Recently, Neuner & Buchner (2023) examined the impact of exposure duration for alpine plants, reporting a consistent, albeit species-specific, negative logarithmic relation between the estimated T_{50} and exposure duration; the longer the exposure duration (in their study from 1 to 512 min), the lower the temperature at which F_v/F_m decreased by 50%. They further assessed heat damage as the percent necrosis developed post incubation and established that, consistent with Bilger *et al.* (1984), functional impairment of PSII occurs at much lower temperatures than irreversible heat damage. This concept was expanded upon by Cook *et al.* (2024), who used a cumulative stress framework from concepts derived from microbiology research to determine thermal tolerance landscapes while also considering stress exposure duration. It is worth noting that all three studies used phenomenological approaches, lacking a mechanistic basis for the relationship between temperature, exposure time, and PSII inactivation. Thus, there remains a need for a mechanistic theory of PSII heat tolerance that accounts for both the magnitude and duration of heat exposure.

III. Heat acclimation of photosystem II: current evidence and unknowns

Physiological acclimation to temperature fluctuations has been recognized for decades by thermal biologists as a critically important coping mechanism for heat stress in plants, animals, and microbes (Bullock, 1955; Atkin & Tjoelker, 2003; Lagerspetz, 2006; Crowther & Bradford, 2013). Yet, there are few general models that describe patterns of thermal acclimation for any organism (for one example in *Drosophila*, see Rezende *et al.*, 2020). In plants, there is evidence for general global patterns of temperature acclimation of both leaf dark respiration (R_d) and Rubisco carboxylation capacity (V_{cmax}), independent of plant functional type or biome (Wang *et al.*, 2020; Zhu *et al.*, 2021). In turn, estimates of global temperature acclimation of R_d and V_{cmax} have improved parameterization of ecosystem and land-surface models (Wang *et al.*, 2020). However, thermal acclimation of PSII has yet to be well-characterized across broad scales, and thus, general models of PSII acclimation rates and magnitudes have yet to be established.

There are numerous published studies describing leaf PSII heat tolerance based on 'snapshot' measurements, yet far fewer descriptions of temporal patterns of PSII heat tolerance. Recently developed high-throughput methods have yielded evidence of acclimation occurring over timescales ranging from hours (Posch *et al.*, 2022; Zhu *et al.*, 2024), to days (Andrew *et al.*, 2022; Bison & Michaletz, 2024; Coast *et al.*, 2024), to months (Froux *et al.*, 2004; Coast *et al.*, 2022; Moran *et al.*, 2023), depending on the timing and magnitude of heat exposure (Fig. 2). These recent studies indicate that 'snapshot' measurements taken at a single time point may fail to capture temporal patterns of heat acclimation. Consequently, current approaches that do not consider acclimation risk misrepresenting the upper limits of PSII heat tolerance for many plant taxa (Grossman, 2023). To illustrate this point, we compared T_{crit} values collected from two species during a recent heating experiment (Posch *et al.*, in preparation) against a global dataset of T_{crit} values (O'Sullivan *et al.*, 2017) to determine the potential effect of acclimation on where a species might rank among snapshot measurements of T_{crit}. In this study, potted, well-watered individuals of a broad-leaf deciduous tree species, *Populus fremontii* S. Watson and a perennial desert shrub, *Encelia farinosa* A. Gray ex Torr., were exposed to a three-day heatwave treatment, during which the mean daily maximum temperature was 51.5°C. By the third day of the heatwave, *P. fremontii* trees had exhibited a 2.2°C increase in T_{crit} (Fig. 2a). The preheatwave T_{crit} of the *P. fremontii* trees was *c.* 51°C, which would place in the *c.* 85th percentile of global T_{crit} values reported in O'Sullivan *et al.* (2017). However, on the third day of the heatwave, *P. fremontii* T_{crit} increased to *c.* 54°C and thus would sit in the *c.* 95th percentile of the O'Sullivan *et al.*'s (2017) global range. By contrast, no change in T_{crit} was observed in *E. farinosa* during the heatwave (Fig. 2a). Furthermore, the mean afternoon leaf temperature during the heatwave was *c.* 34°C in *E. farinosa*, while in *P. fremontii*, it was *c.* 44°C, suggesting that variation in the PSII heat acclimation response may be inversely predicted by leaf temperature. These results highlight the potential effect of accounting for acclimation, as well as illustrating the considerable variation in heat capacity among plant taxa.

Recent evidence has highlighted how temporally dynamic PSII heat acclimation can be, with PSII heat tolerance not only increasing following heat exposure over multiple days but also rising and falling hourly with diurnal changes in temperature. For example, leaf T_{crit} in the rainforest tree *Polyscias elegans* (C. Moore & F. Muell.) Harms increased from 48°C to 52°C within 2 h of exposure to a 40°C temperature treatment (Fig. 2b; Zhu *et al.*, 2024). Similarly, Posch *et al.* (2022) reported a *c.* 2°C decrease in T_{crit} of dark-adapted leaves from noon to sunrise the following day in six *T. aestivum* genotypes grown under nonstressful temperatures (Fig. 2c). The same study also measured *T. aestivum* T_{crit} at consecutive intervals during a high-temperature treatment (36°C), finding that T_{crit} continued to rise 3–4 d following the onset of the heat treatment, but plateaued on Day 5, suggesting a potential upper limit to acclimation (Posch *et al.*, 2022). Acclimation of T_{crit} has also been observed over longer, seasonal timescales, as evidenced in Moran *et al.* (2023). In their study at an experimental common garden in southwestern AZ, USA, mean T_{crit} of 48.9°C was reported in *P. fremontii* in early May when air temperatures peaked at *c.* 37°C (Fig. 2d). In late August, mean T_{crit} of the same trees was significantly higher, reaching 50.1°C after air temperatures peaked at *c.* 45°C (Fig. 2d). Interestingly, the magnitude of thermal acclimation was no higher in *P. fremontii* genotypes sourced from the lower Colorado River Basin, one of the

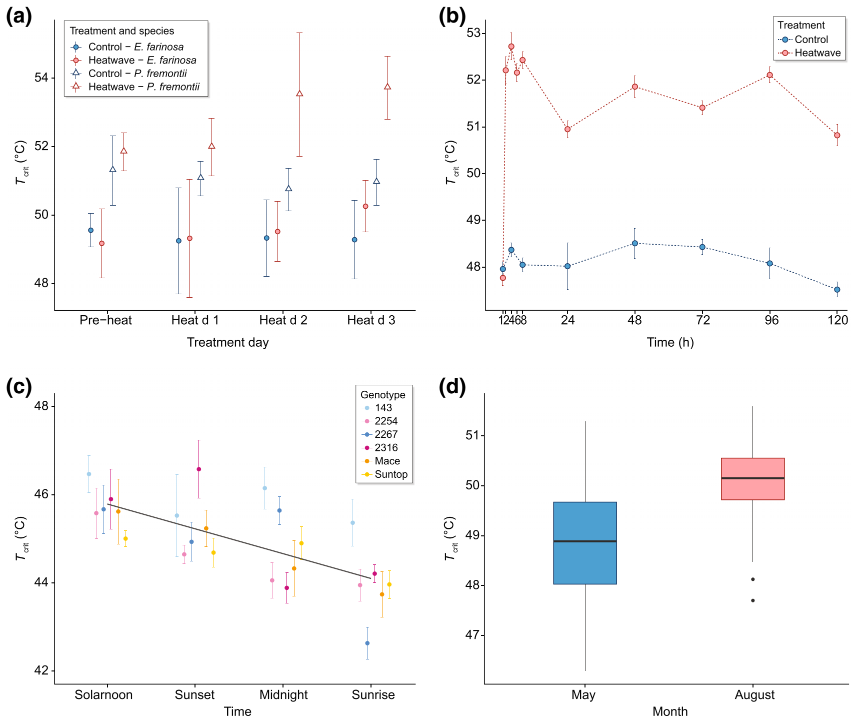

Fig. 2 Examples of photosystem II (PSII) heat acclimation across various timescales from hours to weeks. In all examples, T_{crit} was measured according to the high-throughput F_o rise method first described in Posch *et al*. (2022); (a) T_{crit} (mean ± SE) in response to experimental heatwave treatment in the desert shrub, *Encelia farinosa* (circles) and the deciduous tree, *Populus fremontii* (triangles). A subset of plants was moved from a control shade house after the first measurement day (*Pre-heat*) to a passively heated glasshouse for 3 d (*Heat d 1–3*; mean daily maximum temperature 51.5°C; red points). Remaining plants were kept in the control treatment (mean daily maximum temperature 45.8°C; blue points). Data are previously unpublished. (b) Temporal pattern of T_{crit} (mean ± SE) over 5 d in the rainforest tree *Polyscias elegans* after plants were moved into a 40°C : 35°C (day : night °C) heatwave glasshouse (red points). T_{crit} of *P. elegans* trees in control 25°C : 20°C glasshouse is shown for comparison (blue points). Data are from Zhu *et al*. (2024). (c) Variation in leaf T_{crit} (mean ± SE) of field-grown *Triticum aestivum* genotypes over the course of 18 h. Data are from Posch *et al*. (2022). (d) Box and whisker plot showing patterns of leaf T_{crit} in *P. fremontii* genotypes measured in a common garden in Yuma, Arizona. Leaves were collected in May 2021, when maximum air temperature to date was 37°C, and August 2021, when maximum air temperature reached 45°C. Boxes reflect interquartile range and whiskers show 10th and 90th percentiles and are 1.5 times the interquartile range. Data are from Moran *et al*. (2023).

hottest regions of North America, than in genotypes sourced from cooler areas (Moran *et al*., 2023). These results indicate that plasticity in heat tolerance is an adaptive trait among *P. fremontii* genotypes throughout its range. It is worth noting that the aforementioned reports of PSII acclimation encompass both the controlled imposition of a heat treatment (Froux *et al*., 2004; Coast *et al*., 2022; Posch *et al*., 2022; Zhu *et al*., 2024) and the response to natural heating (Moran *et al*., 2023; Posch *et al*., 2024). As pointed out by Grossman (2023), field-based measurements of acclimation may reflect both increases and decreases in heat tolerance following seasonal temperature change; thus, experimentally applied heat treatments may be preferable for providing mechanistic insight into PSII heat acclimation.

IV. Exploring the environmental, morphological, and evolutionary factors that shape photosystem II heat acclimation

Disentangling the disparate patterns of thermal acclimation across plant taxa, timescales, and habitats (Sections II and III) requires evaluation of factors influencing plant responses to thermal gradients, including the environment, morphology, and life history. In this section, we focus on how patterns of PSII high-temperature acclimation may be impacted by both key environmental factors, specifically water availability and previous heat exposure, and biotic factors, including plant water use strategies, leaf habit, and leaf construction costs.

Physiological acclimation to temperature can occur rapidly (Yamori *et al*., 2014; Bison & Michaletz, 2024; Zhu *et al*., 2024). While many studies quantify PSII heat tolerance and acclimation in response to air temperature, the most relevant temperature for PSII is leaf temperature. Leaf temperature is determined by interactions between climate and leaf energy budget traits, including latent heat flux via evapotranspiration (Michaletz *et al*., 2015, 2016). High rates of transpiration have been observed in both experimental and natural heatwaves and can decouple leaf temperatures from ambient air temperatures (Drake *et al*., 2018; Aparecido *et al*., 2020; Xu *et al*., 2020; Posch *et al*., 2024). Given its direct influence on stomatal conductance and transpiration, water availability plays an important role in regulating leaf temperature (Blonder & Michaletz, 2018). However, traits related to leaf energy budget are not the only determinant of leaf temperature.

Environmental factors, particularly ambient air temperature, also highly influence leaf temperature and thus are key drivers of PSII high-temperature acclimation.

Although elevated growth temperature (typically expressed as MAT) has been associated with high PSII heat tolerance, especially when water for transpirational cooling is limited, its impact on the plasticity of leaf heat acclimation is less straightforward. Evidence to date has been mixed; while some studies initially suggested that elevated growth temperatures could increase T_{crit} acclimation, subsequent work highlighted examples in which elevated growth temperature has no effect for some plant species (Drake *et al.*, 2018; Ahrens *et al.*, 2021) or even a negative effect on acclimation capacity for others. For example, *Eucalyptus* trees grown under experimental warming had reduced T_{crit} plasticity during an experimental heatwave compared with cool-grown *Eucalyptus* trees (Aspinwall *et al.*, 2019). The authors suggested that the reduced T_{crit} acclimation capacity of the warm-grown trees may have been due to their lower concentration of stress response proteins and enzymes. Conversely, Posch *et al.* (2022) found that *T. aestivum* grown under warmer conditions showed greater acclimation of T_{crit} than *T. aestivum* grown in cooler temperatures. Overall, these patterns suggest that in at least some taxa, acclimation capacity may vary seasonally, with lower protein concentrations in warmer seasons increasing vulnerability to high temperatures, as well as at the evolutionary timescale if high-temperature episodes are rare and unpredictable.

Adaptation to climate of origin may also play a role in shaping PSII acclimation. While some work has reported weak phylogenetic signal in T_{crit} across environmental gradients (Lancaster & Humphreys, 2020; Hernández *et al.*, 2022), a recent common garden study of 177 species from 154 families, designed to maximize phylogenetic breadth, found no phylogenetic signal in T_{crit} (Bison & Michaletz, 2024). Likewise, previous studies have found that macroecological variation in heat tolerance was only very weakly phylogenetically structured and showed little to no evidence of adaptation to previous heat exposure on longer (evolutionary) timescales (Perez & Feeley, 2021; Slot *et al.*, 2021). Rather, variation was best explained by mean air temperatures averaged over the 3 d preceding the sampling date, which provided the highest explanatory power compared with other possible averaging windows (Bison & Michaletz, 2024). This result is consistent with other reports of relatively rapid acclimation of PSII heat tolerance to changes in the environment, from hours (Havaux, 1993; Posch *et al.*, 2022; Zhu *et al.*, 2024) to days and weeks (Coast *et al.*, 2022; Bison & Michaletz, 2024), to seasons (Moran *et al.*, 2023), as illustrated in Fig. 2 and covered in detail in Section III.

The extent to which environmental factors, such as water availability and growth temperature, impact PSII high-temperature acclimation likely depends on plant life history and strategy. These factors in turn influence morphological traits that govern leaf economics, energy balance, and phenology. The leaf economics spectrum characterizes evolutionary convergence on strong and consistent relationships between the form, function, chemistry, and longevity of leaves from diverse biomes and climates, collapsing this diversity onto a single axis of variation (Wright *et al.*, 2004). It has been proposed that PSII heat tolerance should also be coordinated along this axis, increasing with leaf mass per area (LMA) due to the positive effect of PSII high-temperature acclimation on leaf life span (Knight & Ackerly, 2003; Zhang *et al.*, 2012; Sastry *et al.*, 2018). Energy balance theory, by contrast, hypothesizes the opposite relationship between LMA and heat tolerance (Vogel, 2009; Slot *et al.*, 2021). This theory holds that, since LMA increases thermal buffering capacity, leaves with higher LMA will generally have lower maximum leaf temperatures in a variable environment and thus require less acclimation of T_{crit} (Michaletz *et al.*, 2015, 2016). Indeed, studies of native tropical trees in both India and Panama each reported significant relationships between T_{crit} and LMA (Sastry *et al.*, 2018; Slot *et al.*, 2021). However, the macroevolutionary patterns in carbon economics, energy balance, and heat tolerance from a more recent common garden study of 177 species from 154 families revealed no coordination between PSII heat tolerance and LMA (Bison & Michaletz, 2024). Phylogenetic comparative analyses instead revealed a mismatch in the timescales over which carbon economics and energy balance traits respond to environmental variation. Morphological traits appear to evolve, at least in part, via selection on carbon economics traits over longer timescales. Morphology then influences leaf energy balance and temperature, to which physiology must then acclimate over shorter timescales. Therefore, it is possible that the capacity for rapid acclimation of heat tolerance evolved in response to energy balance constraints imposed by selection on leaf carbon economics (Bison & Michaletz, 2024).

Although many potential biotic and abiotic constraints on plant thermal regulation may influence PSII high-temperature acclimation, we propose a framework for predicting patterns of PSII high-temperature acclimation independent of biogeography and phylogenetic relations among plant taxa (Fig. 1). Specifically, we describe three general strategies as predictors of PSII high-temperature acclimation capacity, based on existing literature: high heat tolerance of photosynthetic tissues; high water availability/homeothermic evaporative leaf cooling; and plant deciduousness (Fig. 1). Importantly, these strategies are not always mutually exclusive, and thus, some plants may have overlapping coping strategies for heat stress (i.e. overlapping circles in the Venn diagram of Fig. 1). For example, *P. fremontii* has a high capacity for evaporative cooling in its native environment (Blasini *et al.*, 2022; Moran *et al.*, 2023) yet has also demonstrated capacity for thermal acclimation of PSII heat tolerance during heatwaves (Fig. 2a). Likewise, under well-watered conditions, *E. farinosa* can maintain remarkably high transpiration rates and subsequent homeothermic leaf cooling at high air temperatures (Sandquist & Ehleringer, 1997; Aparecido *et al.*, 2020) but rapidly becomes drought-deciduous during dry summer months (Ehleringer & Sandquist, 2018). Given that *E. farinosa* falls squarely into both heat avoidance strategies (as well as having a high leaf albedo that aids in reflecting sunlight), it is perhaps not surprising that this species shows limited capacity for PSII high-temperature acclimation (Fig. 2a).

Alternatively, succulents that use crassulacean acid metaboloism (CAM) photosynthesis such as cacti and agave species have a limited capacity for either evaporative cooling, given their constitutive CAM expression, or drought-deciduousness.

Additionally, the high thermal time constants (Michaletz *et al.*, 2015; Bison & Michaletz, 2024) and thermal buffering capacity of succulents relative to nonsucculents can slow heat dissipation, particularly overnight (Griffiths & Males, 2017). Thus, succulents growing in hot climates require a high degree of heat tolerance and presumably PSII high-temperature acclimation to thrive in many of the arid habitats they occur in. Although temporal patterns of PSII high-temperature acclimation have not been well-established in CAM succulents, these plants have some of the highest reported heat tolerance values of photosynthetic tissues. Downton *et al.* (1984) found a mean T_{crit} of 54.7°C among a subset of cacti species in the Mojave and Sonoran Deserts. Additionally, Shivhare & Mueller-Cajar (2017) found the Rubisco activase of CAM species *Agave tequiliana* was *c.* 10°C more thermostable than that of *Oryza sativa* (rice). Considering that photosynthetic performance under high temperature is linked closely to both the light and dark reactions (Scafaro *et al.*, 2023), highly heat-tolerant Rubisco activase may also be indicative of PSII heat tolerance. In general, warm-adapted plants with high construction and maintenance costs of photosynthetic tissues and limited capacity for homeothermic evaporative cooling (e.g. CAM succulents) are predicted to express among the highest levels of PSII high-temperature acclimation.

We acknowledge that PSII high-temperature acclimation may be influenced by other factors not illustrated in Fig. 1, such as heat exposure over evolutionary timescales, canopy position (i.e. microhabitat preference for shade), leaf morphology and display angle, emission of volatile organic compounds, developmental stage, and leaf lifespan. The latter, for example, varies greatly across species and has been observed to be positively correlated with PSII heat tolerance (Zhang *et al.*, 2012). Nevertheless, heat avoidance (or lack thereof) via evaporative cooling or leaf shedding during heatwaves (Evans *et al.*, 2025) should serve as strong predictors of thermal acclimation of PSII across a broad range of plant taxa and habitats.

V. Mechanisms underpinning photosystem II heat tolerance and acclimation, and potential limits to acclimation

To gain an improved understanding of PSII heat tolerance and acclimation, it is important to consider the biochemical mechanisms most likely underpinning tolerance and acclimation. The oxygen-evolving complex (OEC) is one of the most thermally vulnerable components of PSII, as it is easily damaged through loss of bound manganese (Mn) ions, leading to an over-reduced reaction center, the production of reactive oxygen species (ROS), and damage to the PSII reaction center (Takahashi & Badger, 2011). Due to the tenuous nature by which Mn ions are held in the OEC, the OEC subunits – particularly the D1 protein – continuously require stabilization and refolding, especially when stressors such as photoinhibition or heat are applied (Yoshioka *et al.*, 2006; Takahashi & Murata, 2008). Therefore, it is unsurprising that proteomic studies have found OEC proteins to be one of the notable protein profiles to change in abundance following heat exposure (Li *et al.*, 2013; Wang *et al.*, 2015). This emphasizes the strong relationship between the OEC and stress-induced loss of functionality of the PSII complex. Indeed, *in vitro* assays demonstrate a clear relationship between the continuation of oxygen evolution from the PSII complex at temperatures up to 50°C and the retained binding of OEC proteins and Mn abundance (Enami *et al.*, 1994). The importance of the D1 protein for PSII heat tolerance was evident in a study by Chen *et al.* (2020) that overexpressed and induced the expression of *psbA* (a chloroplast-located gene that encodes the D1 protein) in the nucleus of *Arabidopsis thaliana*, *Nicotiana tabacum* (tobacco), and *O. sativa* (rice). Overexpression of *psbA* in heat-treated plants was associated with maintaining F_v/F_m at similar levels to unstressed plants, as well as improved photosynthetic performance and biomass accumulation. This overexpression directly supports the importance of the D1 protein, and the OEC more generally, for providing heat stability to PSII, as well as the central role of PSII functionality in defining leaf heat tolerance and acclimation.

The speed at which PSII heat tolerance acclimates to high leaf temperature provides insight into what biochemical mechanisms underpin PSII protection. In the rainforest tree species *P. elegans*, T_{crit} increased rapidly by 4°C within 1 h of heat exposure (Fig. 2b; Zhu *et al.*, 2024). The increase in T_{crit} was concomitant with increases in heat-shock proteins (HSPs) of both large (HSP70 and HSP90) and small (Class I and II sHSPs) molecular weights. HSPs are associated with protein heat protection by contributing to the stabilization, synthesis, and refolding of damaged proteins (Wang *et al.*, 2004). Given their sensitivity to high temperature, susceptible subunits of the OEC are likely an example of a protein complex that is protected by HSPs. The link between HSPs and OEC heat tolerance is evident in the interaction between sHSP26 and the oxygen-evolving enhancer (OEE) protein 1 of the OEC in *Zea mays* (maize), determined by co-immunoprecipitation and yeast two-hybrid methodology (Hu *et al.*, 2015). Specifically, RNAi of the sHSP26 led to reduced abundance of OEE protein 1 under heat stress, suggesting sHSP26 is important for synthesis and/or stability of the PSII protein, and that PSII stability is bolstered by HSP protein stabilization of the OEC.

In the time-course study by Zhu *et al.* (2024), T_{crit} was observed to rise just 2 h after heat exposure began. However, it took more than 24 h for membrane fatty acid composition to shift to a more saturated, heat-stable form. Increasing the proportion of saturated fatty acids within lipid membranes enhances stability at higher temperatures and has been associated with increased PSII heat tolerance in plants adapted to warmer environments, as well as long-term seasonal adjustments to warmer weather (Larkindale *et al.*, 2005; Allakhverdiev *et al.*, 2008; Zhu *et al.*, 2018). The slow downregulation to preheatwave levels of specific HSPs and membrane fatty acids (Zhu *et al.*, 2024) may also be related to the persistence of elevated PSII heat tolerance following heat exposure (Zhu *et al.*, 2024; Alvarez *et al.*, 2025), although the extent to which PSII acclimation is maintained postheat remains largely unresolved. The delay between the increase in T_{crit} and the shift in membrane composition in Zhu *et al.* (2024) indicates that changes in membrane fatty acid composition do not contribute to the short-term thermal acclimation of PSII. Instead, there are indications that sugar solutes may provide a more rapid provision of membrane heat protection. Many soluble metabolites significantly increase in abundance within hours of heat exposure, closely mirroring increases in T_{crit} (Zhu *et al.*, 2024). Greater concentrations of sugar solutes surrounding membranes may provide thermal protection by binding within the phospholipid surface of membranes or at the lipid–water interface, altering their melting temperature, and potentially stabilizing them during heat exposure (Andersen *et al.*, 2011). Given the common co-occurrence of heat stress with drought, it is also worth noting that drought-stressed leaves, as well as leaves experiencing combined drought and heat stress, also often increase leaf nonstructural carbohydrate concentrations (Blessing *et al.*, 2015; Liu *et al.*, 2017). A recent study on *T. aestivum* further supports the link between leaf sugars and heat stress; leaves that developed under warmer nights had an increase in the abundance of multiple monosaccharides, including hexose, fructose, mannose, and tagatose (Coast *et al.*, 2024). The increases in soluble sugars were associated with an increase in chloroplast electron transport capacity and PSII stability at hotter temperatures in *T. aestivum* grown under warm nights. Indeed, allocation of sucrose exogenously to leaves has been observed to enhance heat tolerance of chloroplast electron transport in *Populus tremula* (Hüve *et al.*, 2006). Additionally, more targeted approaches are required to deepen our understanding of which sugars provide protection for membranes during high-temperature exposure, and the mechanisms by which they do this.

Despite the effectiveness of protective mechanisms that drive the dynamic rises in PSII stability following heat exposure, there is inevitably an upper limit at which protection is no longer adequate to prevent damage and repair denatured proteins and leaky membranes. As functional damage to PSII is sustained, the photosystem becomes over-reduced and ROS are accumulated. Excessive ROS production damages biological molecules to the point of total loss of function and irreversible damage through inhibition of respiration (Scafaro *et al.*, 2021). A mechanism that may alleviate PSII over-reduction and limit the accumulation of ROS is cyclic electron transport, in which electrons are cycled through PSI and bypass PSII. Cyclic electron flow maintains the transthylakoid proton gradient and generates ATP while also downregulating the cytochrome b_6/f complex (Yamori & Shikanai, 2016). Dissipation of excess reductant by cyclic electron transport can only provide temporary protection as, ultimately, loss of membrane integrity through fusion will occur at a certain temperature threshold (Hazel, 1995), negating thermal protection mechanisms and leading to cell lysis and necrosis. The role of cyclic electron transport during heat stress also highlights the broader point that processes downstream of PSII may also contribute to increases in F_o. As outlined in Schreiber *et al.* (1975), a rise in F_o can occur due to the reduction in Q_A, the primary electron acceptor of PSII. If, during high-temperature episodes, there is any inhibition of the electron acceptors Q_A and Q_B, or of any other key downstream components of the chloroplast electron transport chain such as cytochrome b_6f or PSI, this could lead to increases in the F_o signal before the heat-induced T_{crit}. Thus, care should be taken (e.g. provision of far-red light during assay to ensure relaxation of electron acceptors downstream of PSII; Bilger *et al.*, 1984) when attributing changes in the Chl fluorescence temperature–response curve to the thermal sensitivity of PSII.

While there has been growing attention given to quantifying the functional benefits of plant PSII heat acclimation and describing the mechanisms underpinning these benefits, the potential costs (metabolic, resource-based, or ecological) remain poorly understood. For instance, it remains unclear what the energy costs of acclimation are, or whether acclimation of PSII heat tolerance increases vulnerability to subsequent stress events (heat-related or otherwise). Evidence from coral systems shows that thermal priming can enhance short-term tolerance, but often with an energetic trade-off of reduced resilience to chronic or repeated heat stress (Humanes *et al.*, 2024; Li *et al.*, 2024). While analogous studies on PSII thermal acclimation in terrestrial plants are scarce, similar trade-offs are plausible. The induction and maintenance of acclimatory responses, such as the synthesis of HSPs, accumulation of osmolytes, or remodeling of membrane lipids, are likely to incur metabolic costs, potentially diverting energy and carbon from growth, reproduction, or other stress responses. Furthermore, repeated induction of acclimation pathways may lead to desensitization or reduced responsiveness to future stress, akin to patterns observed in corals. These considerations highlight a critical research gap for understanding not only the mechanisms and benefits of PSII thermal acclimation but also its limitations and potential costs in dynamic and increasingly extreme climates.

VI. Predicting photosystem II thermal tolerance and acclimation with spectroscopy

Spectroscopy, whether measured manually at the leaf level or via remote sensing, could potentially help reduce uncertainties in thermal tolerance estimates through space (snapshot thermal tolerance) and time (acclimation). The success of this approach would also offer a marked increase in capacity to quantify photosynthetic heat tolerance and acclimation, allowing for scaling up of data collection to a level otherwise impossible with other methodologies. The increased throughput offered by this approach may be similar in scale to that achieved via similar uses of solar-induced fluorescence and satellite-based remote sensing of canopy temperatures, although in-depth discussion of these methodologies is beyond the scope of our review (see Mohammed *et al.*, 2019 and Neinavaz *et al.*, 2021, for reviews of these topics). Here, we present an overview of the nascent efforts to employ spectroscopy methods to measure PSII heat tolerance, including the reported successes and difficulties inherent in using this approach to describe PSII heat acclimation.

Spectroscopy has been a useful method for predicting chemical, structural, and physiological traits at the leaf and canopy scales using partial least squares regression (PLSR) and other machine learning models (Fig. 3a; Asner & Martin, 2008; Doughty *et al.*, 2011; Haynes *et al.*, 2024). Given that heat tolerance of PSII is linked to shifts in leaf chemical composition (Zhu *et al.*, 2024), this raises the possibility that spectroscopy may be able to detect thermal tolerance of PSII within species growing in a common location (Fig. 3b), within species across multiple locations (Fig. 3c), across multiple species with disparate leaf morphologies

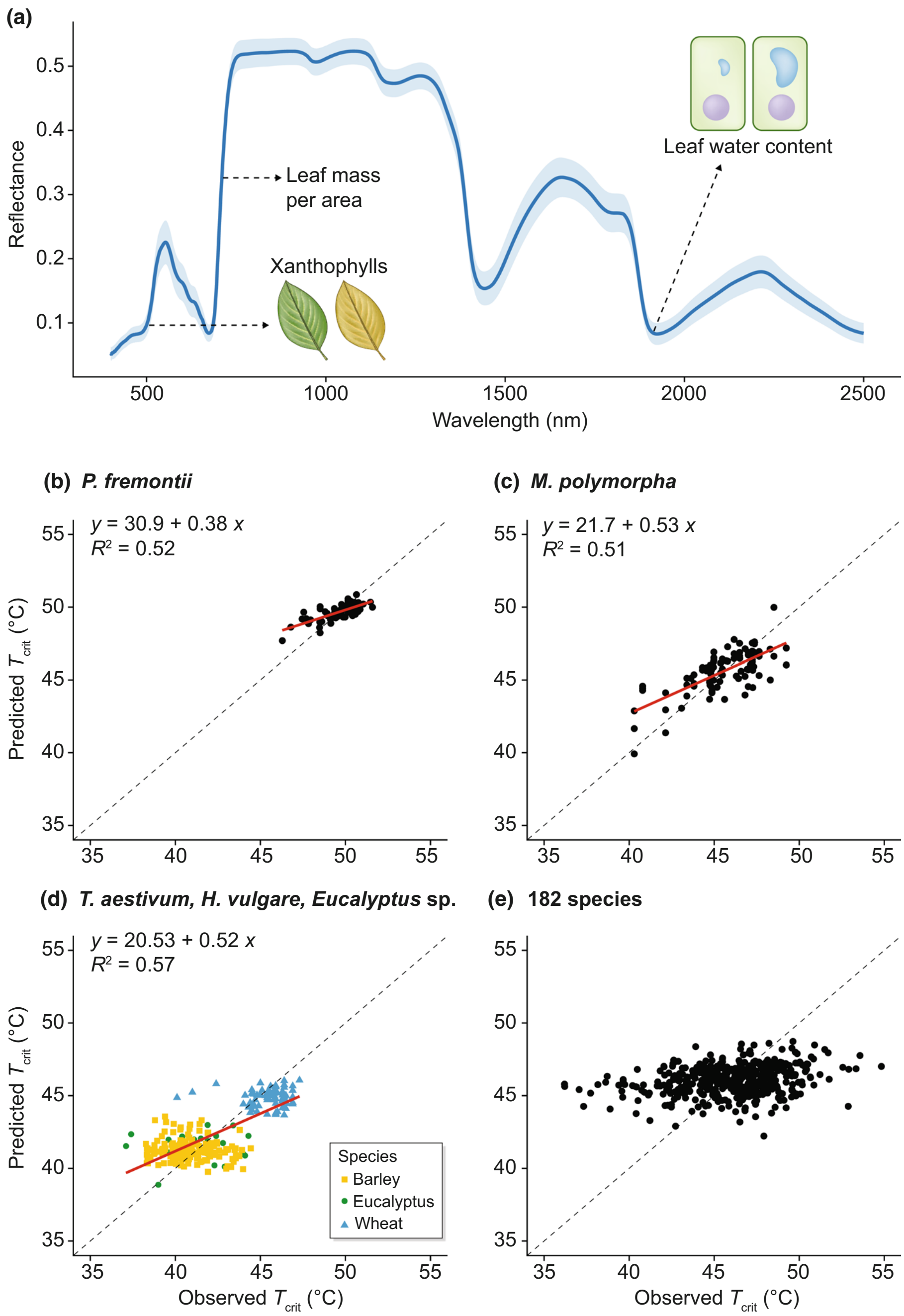

Fig. 3 Conceptual representation of leaf reflectance signature and examples of predicted vs observed leaf T_{crit}. (a) Typical leaf reflectance spectra (mean ± SD) across wavelengths of 400–2500 nm. Illustrations highlight possible mechanistic connections between leaf thermal tolerance and specific leaf spectra: carotenoid concentration and xanthophyll epoxidation state (*c.* 531 nm; Gamon *et al*., 1992, 1997); leaf structural components (e.g. red-edge transition between visible and near-infrared regions; Serbin *et al*., 2019); and leaf water content (e.g. shortwave infrared region; Seelig *et al*., 2008). The spectral regions most important for full-spectrum partial least squares regression (PLSR) model T_{crit} predictions were the visible (especially green) and red-edge regions. Predicted vs observed leaf T_{crit} using a PLSR model on full-spectrum (400–2500 nm) leaf reflectance of (b) *Populus fremontii* (n = 74) grown in a common garden in Yuma, Arizona, (c) *Metrosideros polymorpha* (n = 288), a foundation tree species, grown in a common garden in Volcano, Hawaii, (d) *Triticum aestivum* (50 genotypes, n = 300), *Hordeum vulgare* (213 genotypes, n = 639), and *Eucalyptus viminalis* (64 accessions, n = 191) grown in fields or glasshouses in Narrabri, Wagga Wagga, and Armidale, Australia, respectively, and (e) 85 species (164 families, n = 361) from two botanical gardens in Vancouver, BC, Canada. The PLSR models were developed in the R statistical environment (R Core Team, 2024) using the packages PLS (Mevik *et al*., 2016). Model predictions were based on datasets split 80 : 20 training: test/validation. Models were assessed by a single metric (R^2), which is indicated in each plot. The red line represents the linear regression fit, and the dashed line indicates the 1 : 1 line. The models predicted photosystem II heat tolerance with moderate accuracy (R^2 = 0.51–0.57) for species growing in a common environment (b), within species across locations (c), and across multiple species with differing leaf morphologies and habits (d). However, systematic residual patterns were evident in (b) and (c), with overprediction at low and underprediction at high observed T_{crit}. In (d), the apparent predictive strength was primarily driven by broad interspecific differences, particularly between *T. aestivum* and *H. vulgare*, rather than fine-scale resolution. These results should therefore be interpreted as exploratory proof-of-concept demonstrations rather than evidence of robust predictive performance.

and habits (Fig. 3d), and through time within individual plants. Predictive models have been used to accurately estimate leaf traits in test datasets by exploiting linkages between the traits of interest and leaf optical signatures. Indeed, models can produce mixed results for predicting PSII thermal tolerance within individual species (Fig. 3d) and among different species growing in similar habitats (Fig. 3e). A general limitation of current models is their failure to accurately predict traits when presented with novel spectra (from different species and environments). There may be an opportunity to increase model accuracy when extrapolating to new species and environments by incorporating information from mechanistic connections between thermal tolerance and spectra.

Optical signals related to the xanthophyll cycle are promising candidates for spectral prediction of PSII thermal tolerance and acclimation, as they can indicate both oxidative stress and thermal protection of thylakoid membranes. Xanthophyll pigments undergo rapid interconversion between epoxidation states and resulting conjugation and play a critical role in nonphotochemical quenching (Demmig-Adams & Adams, 1992; Frank *et al.*, 1994). Multiple studies have found that antheraxanthin and zeaxanthin, which both increase in relative abundance in response to oxidative stressors such as high irradiance and temperature, significantly increase the thermostability of the thylakoid membrane and delay the resulting temperature-dependent changes in dark-adapted Chl fluorescence (Havaux & Tardy, 1996; Behnke *et al.*, 2007). The photochemical reflectance index (PRI) is a narrow-spectral-band index that can track xanthophyll epoxidation state, overall carotenoid pool concentration, and carotenoid/Chl ratios across species and functional types, thanks to varying absorption patterns of the pigments involved (Gamon *et al.*, 1992, 1997). The PRI is calculated as the normalized difference between spectral reflectance at 531 and 570 nanometers (Gamon *et al.*, 1997). PRI might be useful for predicting thermal tolerance in two main ways: differences in thermal tolerance snapshots might correlate with PRI, especially in hot environments where correlations in PSII thermal tolerance translate into variations in oxidative stress. Plants experiencing high-temperature-related oxidative stress have shown upregulation of the xanthophyll cycle (Behnke *et al.*, 2007). Diurnal, seasonal, and temperature-dependent variations in PRI might correlate with PSII thermal acclimation. In addition to zeaxanthin helping to stabilize thylakoid membranes, PRI has been shown to correlate with diurnal variation in isoprene emission in some plants, representing another mechanism of thermal tolerance acclimation correlating with an optical signal (Sharkey *et al.*, 2001; Siwko *et al.*, 2007; Penuelas *et al.*, 2013). While PRI is a promising avenue for future work assessing spectral relationships with both PSII thermal tolerance and acclimation, more research is required to determine whether these relationships exist across plant functional types, or when comparing between species in addition to within them.

Relationships between thermal tolerance and leaf structural traits may help mediate the predictive capacity of leaf thermal tolerance with spectroscopy. Several studies have suggested that thermal tolerance is related to the leaf economics spectrum and leaf construction costs (Slot *et al.*, 2021; Bison & Michaletz, 2024). Leaves with high construction costs may benefit from maintaining higher PSII thermal tolerance (Section IV), as plants would be expected to protect their increased investment into leaves, and these leaves tend to have a higher lifespan and are therefore more likely to experience high temperatures (Wright *et al.*, 2004; Slot *et al.*, 2021). Indeed, a positive correlation between leaf lifespan and PSII heat tolerance has been observed in 24 woody savanna species (Zhang *et al.*, 2012). Some studies have found correlations between PSII thermal tolerance and leaf construction costs, determined from LMA and leaf dry matter content in both highly seasonal (Knight & Ackerly, 2003; Sastry & Barua, 2017) and more thermally stable ecosystems (Slot *et al.*, 2021). Sastry *et al.* (2018) found that T_{50} varied along the 'fast – slow' resource acquisition spectrum, increasing with LMA and decreasing with photosynthetic rates in 12 dry tropical forest trees. More recently, Bison & Michaletz (2024) found thermal tolerance to be orthogonal to both leaf carbon economics and energy balance traits in 177 species, suggesting that this relationship is not universal, especially when considering plants from diverse phylogenies, functional groups, and source temperature exposure. There is considerable variability in LMA among functional groups regardless of temperature exposure, likely confounding 'universal' relationships between PSII high-temperature tolerance and leaf construction costs (Poorter *et al.*, 2009). More research is needed to determine under which contexts (e.g. plant functional types, air temperatures, leaf lifespan, temporal scale over which acclimation occurs) the relationship between leaf construction costs and PSII high-temperature acclimation exists. Imaging spectroscopy has been shown to be able to accurately predict LMA, leaf N, and other traits along the leaf economic spectrum (Doughty *et al.*, 2011; Serbin *et al.*, 2012; Asner *et al.*, 2016); thus, spectroscopy can likely improve estimates of PSII high-temperature acclimation in instances in which these traits correlate with PSII thermal tolerance.

While we expect there to be optical relationships with thermal tolerance in some species and contexts, it remains unclear the degree to which spectroscopy might help reduce uncertainties at broader spatial and temporal scales. Various research groups assessing spectral relationships with leaf thermal tolerance have yielded mixed results thus far. For instance, Wiebe *et al.* (in review) found that T_{crit} was associated with consistent visible and near-infrared spectral signatures in two highly variable foundation tree species, *P. fremontii* (Fig. 3b) and *M. polymorpha* (Fig. 3c), each grown in a common environment. PLSR models using leaf spectra were able to predict T_{crit} within *c.* 1°C (root mean square error of prediction for *P. fremontii* = 0.85°C ± 0.03; for *M. polymorpha* = 1.09°C ± 0.09), although in comparison with using the average T_{crit} of the training dataset, this was an improvement in accuracy of only 0.3°C for *P. fremontii* and 0.98°C for *M. polymorpha*. Amoanimaa-Dede (unpublished) found predictive capacity between (but not within) species in similar environments (Fig. 3d). By contrast, the PLSR model presented in Bison & Michaletz (in preparation) did not find predictive capacity for T_{crit} in a dataset of hundreds of species in a common garden (Fig. 3e). We suggest that sufficient variability in PSII heat tolerance and leaf spectra is necessary to build successful PLSR models within individual species, as has been suggested for other traits (Burnett *et al.*, 2021). Additionally, spectral signatures may become more pronounced under extreme heat, when variation in thermal tolerance more strongly influences physiological stress and its associated spectral expression. This pattern would be consistent with the improved model performance observed in *P. fremontii* under high-temperature conditions. While trait and spectral variability is important, however, variability in leaf structure and chemistry, or even differences in how samples are collected, will likely obfuscate more subtle spectral signals corresponding to changes in PSII heat tolerance. This could explain the lack of predictive capacity apparent in Fig. 3(e) when training models on hundreds of species. Ultimately, despite limited published evidence to date, multiple preliminary datasets suggest that further effort is warranted in the pursuit of scaling up PSII heat tolerance and acclimation through spectroscopy-based approaches.

VII. Conclusions and future perspectives

Despite mounting evidence that PSII high-temperature acclimation is prevalent across an extensive range of plant species, biomes, and functional types, attempts to account for acclimation when assessing the upper limits of leaf heat tolerance remain limited. Consequently, we risk underestimating the capacity of many plants to cope with novel heat stress. We advocate for increased efforts to quantify and catalog the extent to which PSII acclimates to high temperatures, and to determine the many molecular mechanisms and environmental factors that appear most promising for predicting patterns of acclimation to high temperature. Whereas individual studies each demonstrate specific timescales (from hours to months) over which PSII thermal acclimation occurs, taking these studies together confirms that acclimation is highly dynamic in many species, and thus, the duration of heat exposure is at least as important for determining the nature of acclimation as the intensity of exposure. Finally, we acknowledge the prospect of modeling PSII heat tolerance and acclimation from leaf and canopy spectral data, an approach that has begun to show promising signs of generating the capacity to predict acclimation more rapidly and at larger scales than ever before. However, current modeling approaches still have significant methodological obstacles to overcome.

We have put forward the hypothesis that heat acclimation of PSII is likely related to the inability to buffer photosynthetic tissue from high-temperature exposure via homeothermic cooling, phenology, or other factors (Fig. 1). However, there are many unanswered questions that need to be addressed in future studies related to phylogenetic patterns of acclimation, biogeography, legacy effects of previous heat exposure, correlations with functional trait syndromes, underlying mechanisms of heat acclimation, and among others. Addressing these questions will provide improved insight into the ability of plants to cope with heat stress, and thus, the extent to which forest canopies can limit leaf damage and loss during heatwaves. In turn, this could reduce feedback effects of increased light penetration (and radiant temperature increases) on canopy energy balance, and subsequent carbon and water cycling at scales ranging from small patches to entire forest regions. To ensure progress in this field, we have five recommendations for future studies: the continued accumulation of PSII heat acclimation data for new species and environments (including in understudied regions of Asia and Africa), collected according to a standardized and well-documented methodology; experimental approaches that directly test the relationship between heat exposure and PSII acclimation, including the interaction between duration, frequency, and intensity of heat; development of models that use physiological and/or morphological data to accurately predict high-temperature acclimation of PSII, and integrate PSII heat acclimation data into trait-based representations of terrestrial vegetation in Earth System Models; continuation of ongoing work to refine predictive modeling of PSII acclimation using remotely sensed leaf and canopy hyperspectral data; and research that advances our understanding of how PSII heat tolerance and acclimation relate to irreversible tissue damage, and the extent to which the accumulation of such damage ultimately influences plant heat tolerance and survival.

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Competing interests

None declared.

Author contributions

BCP and KRH conceived of and planned the manuscript. HAD, NNB, STM, OC, CED and BW contributed leaf reflectance and T_{crit} data and analysis. BCP, HA-D, LMTA, OKA, NNB, BWB, OC, CED, JSG, JVH, STM, MEM, APS, MS, BCW, KW, LZ, BBZ, and KRH contributed to manuscript writing and editing.

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