

## Tansley review

# Phenological physiology: seasonal patterns of plant stress tolerance in a changing climate

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## Summary

The physiological challenges posed by climate change for seasonal, perennial plants include increased risk of heat waves, postbuddbreak freezing ('false springs'), and droughts. Although considerable physiological work has shown that the traits conferring tolerance to these stressors – thermotolerance, cold hardiness, and water deficit stress, respectively – are not static in time, they are frequently treated as such. In this review, I synthesize the recent literature on predictable seasonal – and therefore, phenological – patterns of acclimation and deacclimation to heat, cold, and water-deficit stress in perennials, focusing on woody plants native to temperate climates. I highlight promising, high-throughput techniques for quantifying thermotolerance, cold hardiness, and drought tolerance. For each of these forms of stress tolerance, I summarize the current balance of evidence regarding temporal patterns over the course of a year and suggest a characteristic temporal scale in these responses to environmental stress. In doing so, I offer a synthetic framework of 'phenological physiology', in which understanding and leveraging seasonally recurring (phenological) patterns of physiological stress acclimation can facilitate climate change adaptation and mitigation.

## I. Introduction: phenological patterning in physiological stress tolerance

Fixed in place, land plants must withstand environmental stress to complete their life cycles and reproduce. This reality requires them to tolerate environmental stressors ranging across characteristic temporal scales: short-term disturbances such as fire; repeated seasonal challenges such as the onset of winter; and long-term

directional trends, including climate change. The second of these three characteristic temporal scales, repeated seasonal stressors, constitutes a particularly fascinating intermediate challenge, in which it is not enough either to survive occasional extreme events or to negotiate long-term, directional change. Instead, individual plants must be able to acclimate physiologically, meaning they must respond dynamically to the challenging environmental stressors likely to confront them repeatedly and at least somewhat

predictably over their life spans (Preston & Sandve, 2013; Varpe, 2017; Stotz *et al.*, 2021). This imperative for plastic acclimation to physiological stress is especially critical for plants:

(1) *distributed in temperate (seasonal) climates*, which, unlike tropical or polar climates experience marked periodic variation in temperature (hottest month > 10°C and coldest month between 0°C and 18°C; Peel *et al.*, 2007) and comprise *c.* 40% of angiosperms (Barlow *et al.*, 2018) and *c.* 50% of gymnosperms (Fragnière *et al.*, 2015), and/or

(2) with *life histories spanning multiple years (perennials)*, which entail seasonal fluctuations over life spans ranging from 2 to > 4000 yr (Thomas, 2013) and include representatives in the majority of angiosperm clades (Friedman, 2020) and all gymnosperms.

Perennial plants living in temperate climates (hereafter 'seasonal perennials'), then, provide an excellent case study for understanding the capacity of plants to acclimatise in response to repeated, physiologically challenging shifts in their environments, which I refer to as 'seasonal stress tolerance'.

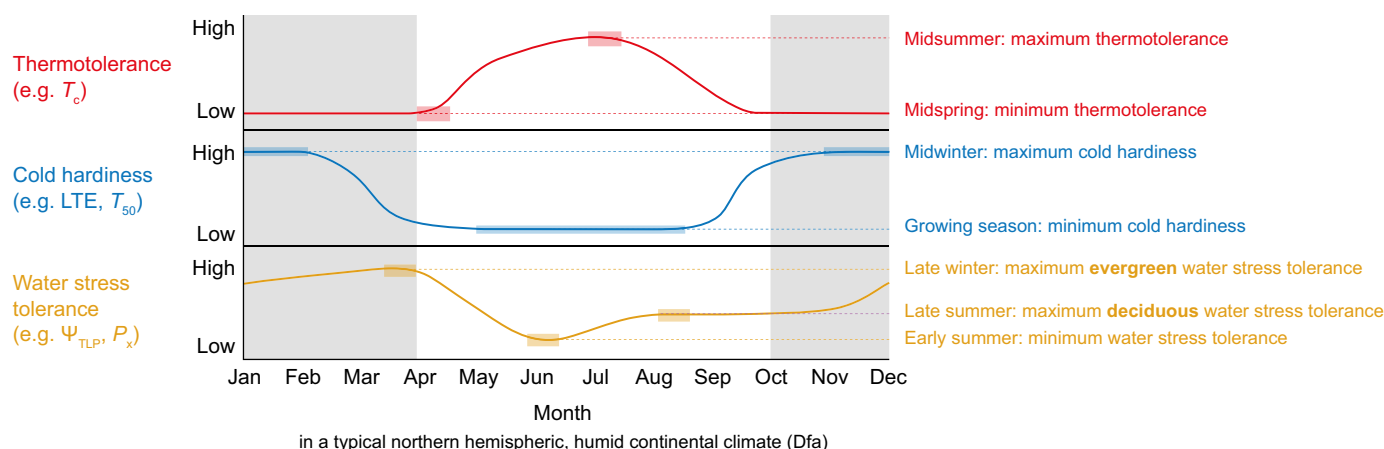
Understanding physiological acclimation to seasonal stress promises to shape our capacity to forecast and manage the ecological consequences of climate change. Given recent projections of the likely trajectory of the global climate over the next 80 yr, land plants will be confronted with between 1.8°C and 3.6°C of surface warming by the end of this century and an increased likelihood of dry soil conditions due to increased variability in precipitation timing and intensity (RCPs 4.5 and 6.0 per the IPCC, 2022). Taken together, these primary forms of climate change are projected to intensify heat and water-deficit stress during temperate summers and dry seasons (Anderegg *et al.*, 2019; Breshears *et al.*, 2021; IPCC, 2022) and increase the risk of false spring damage (freezing following early budbreak) for winter-deciduous species in some, but not all, regions (Allstadt *et al.*, 2015; Casson *et al.*, 2019; Chamberlain & Wolkovich, 2021). As such, climate change is the paramount threat to our planet's ecological integrity. Yet, the physiological challenges climate change poses for many plants, and especially seasonal perennials, differ from those posed by seasonal stress in degree rather than in kind. Perennial plants living in seasonal environments must be *a priori* adapted to acclimate plastically in response to the dynamic environmental conditions typical of their native ranges over the course of a year. Although climate change compounds and will continue to compound these challenges (Reyer *et al.*, 2013), the physiological mechanisms that allow seasonal perennials to survive a warmer, more drought-prone world will likely be those that already allow them to negotiate a typical year.

Although it is widely accepted that plant species vary in their physiological tolerance of a variety of stressors (Niinemets & Valladares, 2006; Valladares *et al.*, 2007; Neuner *et al.*, 2019) and that such stress tolerance can show *acclimation*, a plastic, beneficial in response to seasonal change (Vitasse *et al.*, 2014; Parmesan & Hanley, 2015; Hiron *et al.*, 2020), we lack an integrative framework for understanding how these shifts happen in natural environments and over repeated, predictable time frames. Within the comparative literature (e.g. Niinemets & Valladares, 2006; Choat *et al.*, 2012; Lancaster & Humphreys, 2020; Perez &

Feeley, 2020), physiological adaptations conferring stress tolerance are frequently represented as mechanistic traits (*sensu* Voltaire, 2018, those traits with a clearly defined physiological basis) typical of a species or population. For instance, Niinemets & Valladares' (2006) influential monograph, which ranks over 800 woody plant species by their tolerance to shade, drought, and waterlogging stress, offers a single tolerance value for each species considered and has been cited almost 1000 times to date. Similarly, the USDA-NRCS (2022) PLANTS database offers a single, categorical value for several environmental tolerances and has been cited thousands of times. Both the Niinemets and Valladares dataset and the PLANTS database are widely incorporated into studies addressing the ecological consequences of climate change for woody plants (Fei *et al.*, 2017; Danneyrolles *et al.*, 2019, among many others). It is a critical, implicit assumption in the literature citing these rankings that maximum stress tolerance is fixed temporally. For example, the average water-deficit stress tolerance of a given species is frequently treated as stationary, shaping species interactions, setting range limits, and providing a threshold of tolerance for either transient, seasonal, or long-term environmental stress. However, even a cursory consideration of the plant physiological literature shows this assumption to be inaccurate for a range of stressors relevant to climate change, including heat waves (Zhu *et al.*, 2018), false springs (Lenz *et al.*, 2013), and droughts (Hiron *et al.*, 2020). Although well-established, this intuitive understanding that physiological tolerance changes over time in response to exposure to seasonal stress has yet to be integrated fully into our comparative understanding of physiological tolerance as a trait or its use in forecasting and managing the response to climate change.

I propose here that a phenological perspective can allow us to meet the urgent need to evaluate and compare environmental stress tolerance among species and predict how such traits will shift in a changing climate (Fig. 1). Phenology is the study of recurring events in the life cycle. Historically, phenological research has focused on the timing of particular morphological transitions in life history ('phenophases' such as leaf-out, flowering, etc.) and on the sensitivity of phenophase timing to environmental conditions (Cleland *et al.*, 2007). Physiological monitoring has largely played a role in phenological research insofar as physiological mechanisms are recognized as the underpinning of the gross morphological changes underlying the classic phenophase transitions (Lenz *et al.*, 2013; Savage & Chuine, 2021; Kovalski, 2022). Yet, this very observation implies that *physiology is phenological*, or, in other words, that a predictable series of physiological changes underlies seasonal responses to environmental drivers. Although the timing and extent of such plastic physiological change will vary from year to year, as does morphological phenophase timing (Panchen *et al.*, 2014, 2015; Gallinat *et al.*, 2015), it appears to follow predictable patterns and, critically, confer tolerance to seasonal environmental stressors in a predictable way. To the extent, then, that plants show seasonally varying and predictable physiological acclimation in order to tolerate seasonal, predictable environmental stressors, physiological tolerance to a given stressor should be treated as a phenological trait.

In the remainder of this review, I synthesize the existing physiological literature on the seasonal patterning (phenology) of



**Fig. 1** Physiological tolerance to heat waves (thermotolerance; red; top), freezing damage typical of false springs (cold hardiness; blue; middle), and droughts (water-deficit tolerance; orange; bottom) varies periodically over a year, producing a distinct phenological signal for each stress tolerance trait. Patterns here represent likely patterns of stress tolerance for a seasonal perennial plant growing in a typical Northern Hemispheric location with freezing winters (coldest month  $< 0^{\circ}\text{C}$ ), warm summers (hottest month  $> 22^{\circ}\text{C}$ ), and no pronounced dry season (humid continental climate, Dfa), such as that of Boston, MA, USA. The growing season (white) is bracketed by the dormant season (gray), during which leaves are either less physiologically active (evergreens) or have been shed (deciduous plants). Maximum water-deficit tolerance is shown for leaf tissue of both evergreen and deciduous plants. Curves synthesized from patterns reported in Hinckley *et al.* (1980), Seemann *et al.* (1986), Grossnickle (1992), Kolb & Sperry (1999), Froux *et al.* (2004), Lenz *et al.* (2013), Deacon *et al.* (2019), Kovalski & Grossman (2021), and Northing (2022).

three forms of physiological tolerance: thermotolerance, cold hardiness, and water-deficit (or drought) tolerance (Table 1). Each of these confers tolerance to a climate change-associated stressor for terrestrial plants: heat waves, false springs, and droughts, respectively. For each stressor and its associated form of physiological tolerance, I

- (1) survey the climate change stressor and relevant mechanisms of stress tolerance;
- (2) describe one or two accessible, high-throughput techniques for quantifying the relevant form of stress tolerance;
- (3) survey what is known about seasonal patterns for these metrics of physiological performance; and

**Table 1** Synthesis of phenological pattern in three climate change stressors and associated forms of physiological tolerance in seasonal perennials.

Climate change stressor	Physiological tolerance	Measured as	Temporal scale of acclimation		Phenological pattern (Fig. 1)	Key references
			Grain	Extent		
Heat waves	Thermotolerance	Critical temperature for chlorophyll fluorescence ( $T_c$ )	Minutes	Days	Fluctuations in tolerance ultimately producing a pattern of acclimation in the hot/dry season relative to the cool/wet season	Seemann <i>et al.</i> (1986); Havaux (1993); Ladjal <i>et al.</i> (2000); Ghouil <i>et al.</i> (2003); Froux <i>et al.</i> (2004); Duan <i>et al.</i> (2015); Teskey <i>et al.</i> (2015); Sastry & Barua (2017); Zhu <i>et al.</i> (2018)
False springs	Cold hardiness	Low-temperature exotherm (LTE) Critical electrolyte leakage ( $LT_{50}$ )	Days	Weeks to months	Hardening (acclimation) in autumn and dehardening (deacclimation) in spring, both occurring over weeks or months	Sakai (1960); Grossnickle (1992); Ögren (2001); Schaberg <i>et al.</i> (2005); Mills <i>et al.</i> (2006); Charrier <i>et al.</i> (2011); Lenz <i>et al.</i> (2013); Vitra <i>et al.</i> (2017); Kovalski (2022)
Droughts	Water stress tolerance	Turgor loss point ( $\Psi_{\text{TLP}}$ ) Xylem cavitation vulnerability ( $P_x$ )	Weeks	Months	Acclimation over the growing season for deciduous plants; evergreens may further acclimate during the dormant season	Meyer & Boyer (1972); Teskey <i>et al.</i> (1984); Wenhui (1998); Kolb & Sperry (1999); Awad <i>et al.</i> (2010); Sjöman <i>et al.</i> (2015); Cardoso <i>et al.</i> (2018); Hirons <i>et al.</i> (2020)

For each form of physiological tolerance, key high-throughput measurements, the characteristic temporal scale of acclimation, and a generalized phenological pattern (based on key references) are summarized.

(4) synthesize the current phenological understanding of the relevant stressor, including identification of key gaps in our understanding of phenological patterns in stress tolerance.

I provide key examples from the empirical literature with a focus on seasonal perennials (plants with distributions primarily in temperate climates and with multiyear life cycles), and particularly on woody plants, for which phenological patterns in physiological stress tolerance are likely to be most pronounced and relevant. Case studies highlighted here are representative of the contemporary physiological literature and focus on perennial plant taxa with predominantly temperate distributions. Within this set of criteria, I have included studies focusing on phylogenetically and functionally diverse systems, including both gymnosperms and angiosperms and deciduous and evergreen taxa. For this reason, an account of the natural history of taxa highlighted in this review is not provided, and readers are encouraged to seek out the reports reviewed here for further reading.

A key objective of this work is the development of a perspective that contextualizes resilience to climate change stress within the context of predictable seasonal patterns of environmental stress response (Fig. 1). This framework includes an exploration of the characteristic temporal scale (Clark, 1985) of acclimation and deacclimation in thermotolerance, cold hardiness, and water-deficit stress tolerance, including estimates, based on the current balance of research, of the temporal grain and extent (*sensu* Wu & Li, 2006) for each stress response (Table 1). I conclude this review by suggesting promising avenues for addressing them and linking new physiological measurements to the challenges posed by forecasting and adapting to the ecological consequences of climate change.

## II. Heat waves and thermotolerance

### 1. Heat waves in a warming climate

The most direct impact of climate change on terrestrial organisms will be an increase in ambient temperature across the Earth's surface, with extremely hot temperatures occurring in what are already the warmest parts of the year (IPCC, 2022). Such heat waves, periods in which ambient temperature exceeds that of the 90<sup>th</sup> percentile for a particular time and place for three or more consecutive days (Perkins & Alexander, 2013), already occur with greater frequency than in the recent past and will increase in frequency and intensity in the future (reviewed in Breshears *et al.*, 2021). A growing experimental literature indicates that exposure to heat waves – as a distinct driver relative to water-deficit stress – can disrupt plant physiological processes, leading to lower photosynthetic productivity, reduced vegetative and reproductive yield, and death (Marchand *et al.*, 2005; Bauweraerts *et al.*, 2014; Birami *et al.*, 2018, reviewed in Teskey *et al.*, 2015; Breshears *et al.*, 2021).

In recognition of the consequences of heat stress for plant performance, past work has included quantification of plant performance across a range of temperature conditions and calculation of both thermal optima and limits, which tend to be presented on a species- or cultivar-specific basis (Knight &

Ackerly, 2003; Froux *et al.*, 2004; O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018). Yet, thermotolerance, the capacity to tolerate and retain physiological function when exposed to high heat, not only confers resistance to heat waves but also plays a role in facilitating seasonal acclimation over the course of a growing season or during the transition from a hot/dry to a cold/wet season. At the organismal level, this tolerance trait is coordinated by diverse underlying molecular mechanisms (Yeh *et al.*, 2012), with heat-shock proteins (Hsps) playing a critical role. This highly diverse class of proteins confers tolerance of high temperatures by reducing protein aggregation following heat damage and facilitating the repair of heat-damaged cellular constituents (Vierling, 1991; Chen *et al.*, 2018). Diversity in Hsps and their cellular functionality may be one factor contributing to the high level of variability in thermotolerance observed within study populations (Downton *et al.*, 1984; Neuner & Buchner, 2012; Sastry & Barua, 2017; Zhu *et al.*, 2018). This variation speaks to the highly plastic nature of thermotolerance and the complex mechanistic underpinning of the heat stress response in plants (Chen *et al.*, 2018).

### 2. Measuring thermotolerance

A variety of phenotypic measurements meant to index plant thermotolerance has been adopted in studies of land plants (Yeh *et al.*, 2012). Although estimates of thermotolerance based on range limits (e.g. Feeley *et al.*, 2020; Lancaster & Humphreys, 2020) or survival following experimental exposure to high heat (e.g. Allen *et al.*, 2010; Birami *et al.*, 2018) can be interpreted in terms of fitness, these metrics do not convey much about mechanisms of thermotolerance. They may also integrate information about the interacting consequences of heat and other environmental stressors (e.g. water-deficit stress) for fitness. For this reason, I focus here on a widely used, high-throughput, and mechanistically informative metric of thermotolerance:  $T_c$  (Box 1), the critical temperature at which chlorophyll fluorescence emitted by leaf tissue markedly increases (Fig. 2; Schreiber & Berry, 1977).

A wealth of past scholarship has documented several notable patterns that, taken together, provide context for  $T_c$  as a metric of thermotolerance. First, estimates of  $T_c$  generally surpass (by a wide margin) the maximum ambient temperature plants are likely to experience in their locality during the warmest part of the growing season, reflecting the widespread adaptive development of thermal safety margins (Curtis *et al.*, 2016; O'Sullivan *et al.*, 2017; Sastry & Barua, 2017), even when the contribution of solar radiation is taken into account (Perez *et al.*, 2020). Second, although  $T_c$  is generally higher in warmer environments than in cold ones (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018), reflecting broad biogeographic sorting of species into their native distributions, thermal tolerance is not closely tied to temperature-based climatic niche (Curtis *et al.*, 2016; Feeley *et al.*, 2020). For instance, O'Sullivan *et al.* (2017) surveyed 218 species distributed across a global temperature gradient ranging from a mean warmest temperature during the warmest season month of 36.6°C in Australia's Northern Territory to 16.7°C in the Alaskan tundra and found that  $T_c$  only varied from 41.5°C to 50.1°C across these sites. This is to say that local adaptation to a c. 20°C gradient in extreme warm



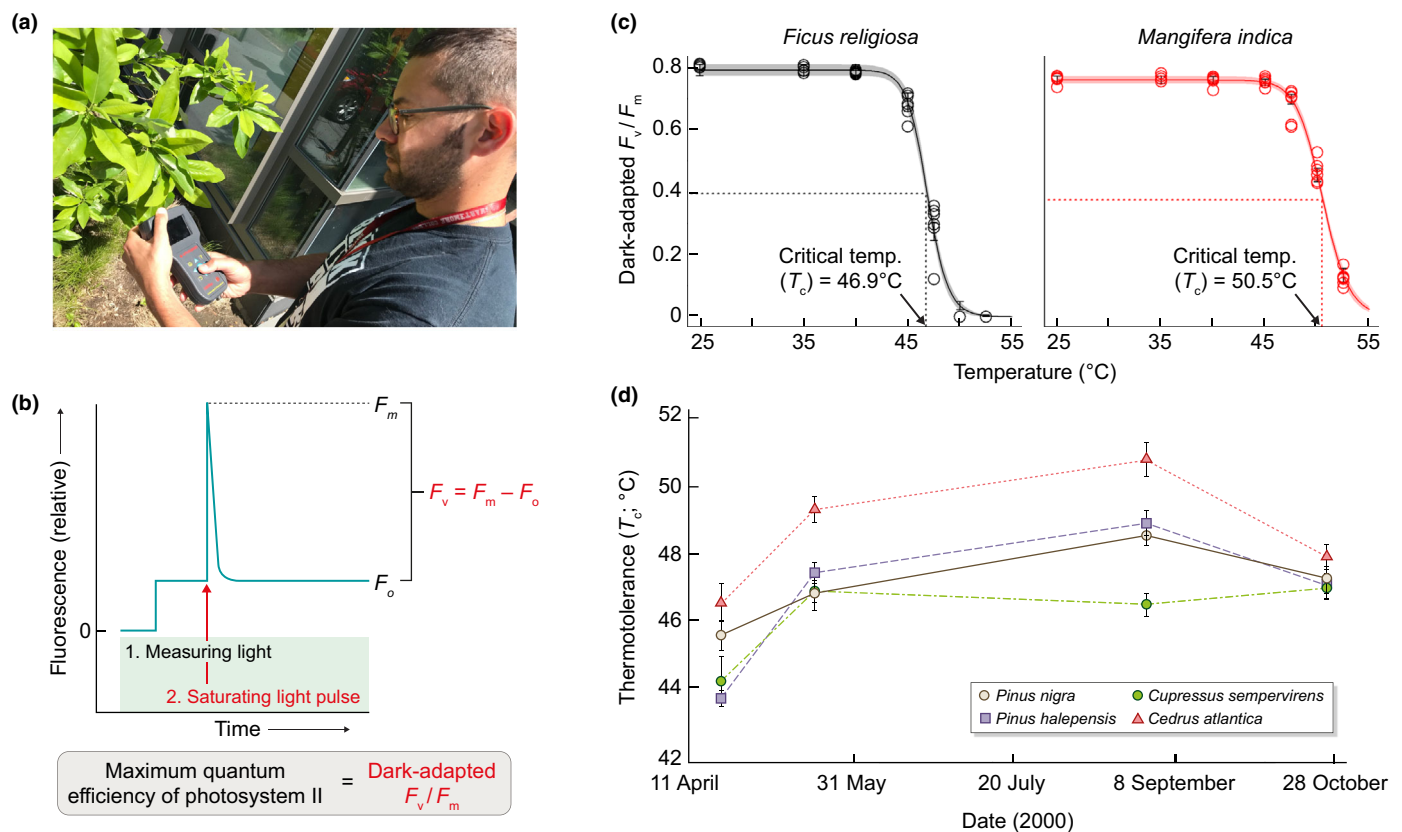
**Box 1** Critical temperature ( $T_c$ ) as a metric of thermotolerance.

In this work, I treat  $T_c$  ( $^{\circ}\text{C}$ ) the critical temperature for chlorophyll fluorescence emission, as a key indicator of thermotolerance. Solar energy absorbed by chlorophyll can be channeled into photochemistry (photosynthesis), quenched nonphotochemically, or reemitted as fluorescence (Fig. 2a; Maxwell & Johnson, 2000). Several stressors, including high heat, can inhibit chlorophyll (and in particular, Photosystem II), from absorbing incoming radiation. When chlorophyll fluorescence resulting from illumination with an intense light is measured in dark-adapted leaves – in which nonphotochemical quenching is downregulated – stressed plants will emit more fluorescence than nonstressed plants (Fig. 2b). Considerable work has further shown that plants incubated at progressively higher temperatures will show consistent levels of dark-adapted fluorescence until a critical point at which the photosynthetic machinery becomes dysfunctional and fluorescence increases (Fig. 2c; Schreiber & Berry, 1977; Knight & Ackerly, 2003; Sastry & Barua, 2017). Though a metric of cellular physiology, this index of critical temperature is associated with visual tissue-level damage inflicted by heat (Bilger *et al.*, 1984) and so constitutes an easily interpretable metric of both photosynthetic and whole-plant thermotolerance.

temperatures has only generated an apparent  $c. 8^{\circ}\text{C}$  gradient in thermotolerance (as measured by  $T_c$ ). Third, and of key importance in this review, the response of  $T_c$  to both naturally occurring and experimentally manipulated ambient temperature appears quite plastic, allowing plants to acclimate to the thermal stress posed by seasonal changes in temperature and heat waves (Fig. 2d).

### 3. Temporal patterns of thermotolerance

Rapid acclimation of  $T_c$  has been demonstrated in response to both naturally occurring and experimental ambient warming. In diverse natural systems, this pattern takes the form of higher  $T_c$  in the hot and/or dry season than in the cold and/or wet season (Seemann *et al.*, 1986; Froux *et al.*, 2004; Duan *et al.*, 2015; Zhu *et al.*, 2018, but see Marchand *et al.*, 2005). Seasonal acclimation in  $T_c$  is likely species-specific – Sastry & Barua (2017) document acclimation for 17 of 33 montane woody species observed. And its magnitude seems robust but limited in natural systems: Zhu *et al.* (2018) found an average increase in thermotolerance of between  $0.20^{\circ}\text{C}$  and  $0.34^{\circ}\text{C}$  per  $^{\circ}\text{C}$  of seasonal temperature gain from winter to summer across



**Fig. 2** Thermotolerance can be easily measured through (a) chlorophyll fluoremetry. (b) In this method, chlorophyll fluorescence in response to a low-intensity measuring light is compared with fluorescence emitted in response to a saturating light pulse. The ratio of the difference in fluorescence ( $F_v$ ) to the max fluorescence ( $F_m$ ) of dark-adapted plant tissue gives the maximum quantum efficiency of PSII ( $F_v/F_m$ ). (c) Thermotolerance is frequently represented as  $T_c$ , the critical temperature at which  $F_v/F_m$  drops precipitously, indicating a loss of photosynthetic function. (d) Limited evidence suggests that  $T_c$  increases (acclimates) in response to warmer temperatures over the growing season and declines (deacclimates) as temperatures drop in the autumn. Photograph is courtesy of author, (c) is adapted from Sastry & Barua (2017), and (d) is adapted from Froux *et al.* (2004). Both figure sources are licensed under CC-BY 4.0.



midwinter damage to highly acclimated plants will increase in likelihood under prevailing models of climate change (but see Comeau *et al.*, 2021). Instead, a combination of warmer and shorter winters and the earlier onset of warmer spring temperatures in conjunction with a stable photoperiod (which is not affected by climate change) are likely to increase the risk – in some areas and for some clades – of false springs (Allstadt *et al.*, 2015; Liu *et al.*, 2018; Richardson *et al.*, 2018; Chamberlain & Wolkovich, 2021).

## 2. Measuring cold hardiness

The extent to which a seasonal perennial exposed to a false spring will experience damage or death is determined by its cold hardiness (also, variously, ‘freezing tolerance’) – its physiological capacity to withstand near or subfreezing temperatures (Sakai & Weiser, 1973; Wisniewski *et al.*, 2003; Vitasse *et al.*, 2014). Cold hardiness is gained through acclimation in response to progressively lower temperatures and/or shorter days (hardening) and lost through exposure to higher temperatures and/or longer days (dehardening). As for thermotolerance, cold hardiness is estimated using diverse phenotypic measurements (Kovaleski & Grossman, 2021), including observation of tissue necrosis (Deans *et al.*, 1995; Lindén *et al.*, 2000). Here, I focus on two metrics that, like the calculation of  $T_c$  from chlorophyll fluorescence analysis, have been shown to predict plant performance and can be carried out across diverse clades in a high-throughput approach: differential thermal analysis (DTA), which yields estimates of low-temperature exotherms (LTE), and electrolyte leakage (EL), which yields estimates of critical leakage values ( $LT_{50}$ ). These cold hardiness metrics are further explored in Box 2 and Fig. 3.

## 3. Temporal patterns of cold hardiness

Likely due to long-standing human interest in cold de/acclimation in crops and other species of economic importance, there is a strong foundation of evidence regarding seasonal patterns in de/hardening in environments facing cold winters (Figs 1, 3e; Weiser, 1970; Kalberer *et al.*, 2006). Under field conditions of dropping temperatures and shorter days, diverse seasonal perennials have been shown to gain cold hardiness, whether measured as LTE (Mills *et al.*, 2006; Ferguson *et al.*, 2011; Londo & Kovaleski, 2017; Kovaleski, 2022) or  $LT_{50}$  (Grossnickle, 1992; Schaberg *et al.*, 2005; Charrier *et al.*, 2011). Both metrics, along with vulnerability to visually apparent necrosis, tend to plateau by midwinter, but, as noted above, can be manipulated further through artificial de/hardening (Sakai, 1960, 1966; Kobayashi, 1981; Ögren, 2001; Vitra *et al.*, 2017; Richardson *et al.*, 2018; Kovaleski *et al.*, 2019; Kovaleski, 2022). Regardless, starting in late winter or early spring, both LTE (Mills *et al.*, 2006; Ferguson *et al.*, 2011; Londo & Kovaleski, 2017; Kovaleski, 2022) and  $LT_{50}$  (Grossnickle, 1992; Schaberg *et al.*, 2005; Charrier *et al.*, 2011; Lenz *et al.*, 2013; Vitra *et al.*, 2017) have been shown to rise exponentially as days become warmer and longer. This phenological loss of cold hardiness in the spring co-occurs with – and may even coordinate (Vitasse *et al.*, 2014; Kovaleski, 2022) – morphological phenological transitions, such as budbreak.

### Box 2 Low temperature exotherms (LTE) and critical leakage ( $LT_{50}$ ) as metrics of cold hardiness.

Here, I focus on two complementary approaches to quantify cold hardiness of plant tissue. Differential thermal analysis (DTA; Quamme, 1986; Mills *et al.*, 2006) involves harvesting of plant organs, often buds or stems, and gradual freezing of this tissue at progressively lower temperatures in the presence of thermoelectric sensors, which can detect the release of energy (exotherm) associated with freezing. In DTA, the freezing of extracellular water at temperatures shortly below 0°C produces an uninformative high-temperature exotherm (HTE, °C) followed by an informative low-temperature exotherm (LTE, °C) when cellular water freezes (Fig. 3a, b). The temperature at which an LTE is emitted reflects the limit to which the tissue being measured can prevent freeze-induced damage and has been shown to reflect resistance to cold-induced necrosis (Mills *et al.*, 2006; Neuner & Buchner, 2012).

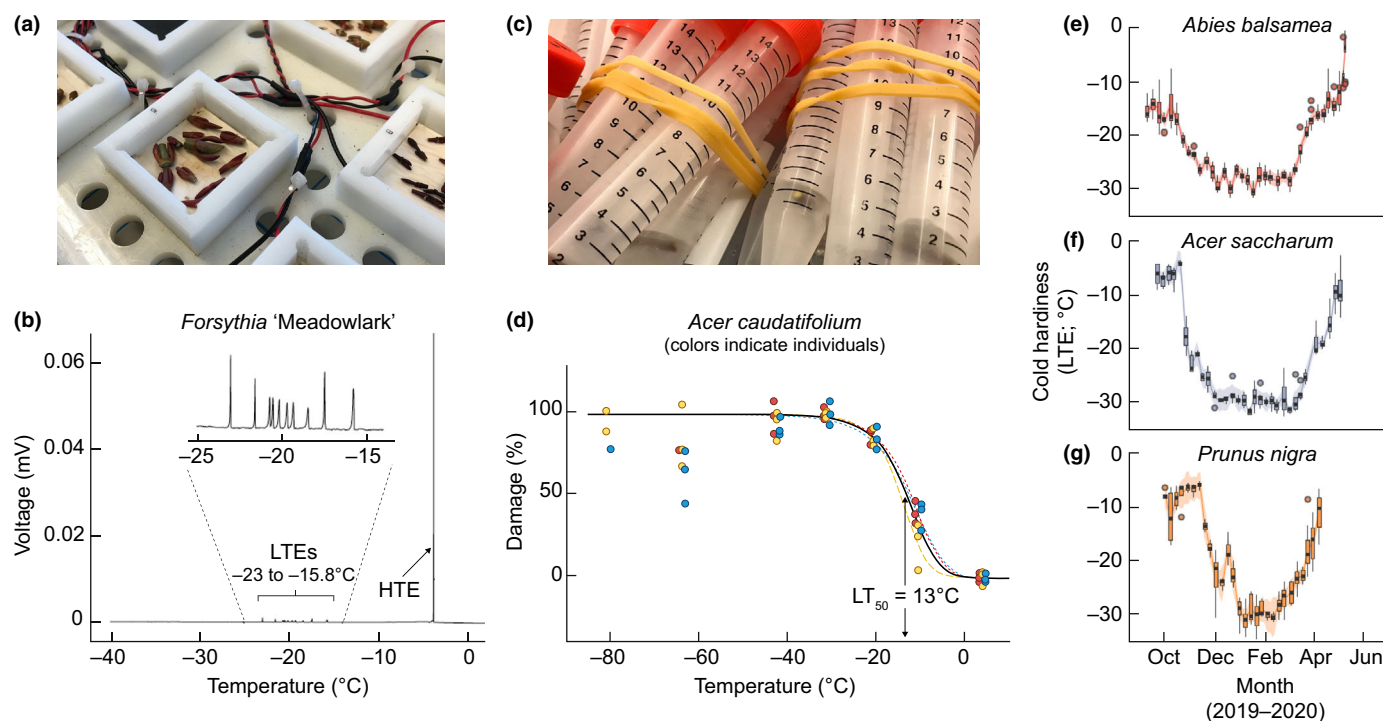
A second approach, electrolyte leakage (EL) analysis, complements DTA by elucidating the temperature threshold at which cellular membranes become unstable, allowing symplastic solutes to leave the cell (Fig. 3c,d; Flint *et al.*, 1967; Lim *et al.*, 1998; Kovaleski & Grossman, 2021). In an EL measurement, sampled tissue (buds, leaves, or stems are most common) is incubated in water to a range of temperatures, after which conductivity is measured. Higher conductivity indicates greater membrane instability and thus freezing damage. Typically, a critical leakage value ( $LT_{50}$ , °C) is extracted from EL analysis, reflecting the temperature at which leakage has reached 50% of the maximum estimated for a particular sample. Estimates of  $LT_{50}$  predict visually estimated tissue damage (Palonen & Lindén, 1999; Savage & Cavender-Bares, 2013) and generally align with LTEs (Aniško & Lindstrom, 1995; Mancuso & Fiorino, 2000), although the two metrics do capture different aspects of cold (Kovaleski & Grossman, 2021).

## 4. Phenology of cold hardiness

The existing consensus on seasonal acclimation in cold hardiness suggests that, in climates with cold winters, tolerance to freezing is acquired or lost over a period of days to weeks or months, with some species de/acclimating faster than others (Table 1; Kovaleski, 2022) and climate of origin contributing to variation in the timing of budbreak among populations of the same species (Papper & Ackerly, 2021; Zeng *et al.*, 2022). Plants reach maximal acclimation during midwinter, when even unusually cold temperatures are unlikely to generate freeze-induced damage (Sakai, 1960; Sakai & Weiser, 1973; Kobayashi, 1981; Ferguson *et al.*, 2011; Strimbeck *et al.*, 2015). Conversely, a state of maximal deacclimation is generally attained during the growing season (Leinonen, 1996; Bower & Aitken, 2006; Arias *et al.*, 2015; Deacon *et al.*, 2019), a period during which freezing spells are already uncommon and will only become less so given climate change. Freezing damage during periods of extreme deacclimation is therefore also unlikely. These observations, along with the apparently widespread acquisition of autumn cold hardiness in advance of freezing temperature (Schreiber *et al.*, 2013; Vitasse *et al.*, 2014), frame spring budbreak as a period of vulnerability for plants growing in environments with cold winters (Figs 1, 3).

Given ongoing climate change, false spring damage may be especially onerous for particular species based on their sensitivity to





**Fig. 3** Two techniques for quantifying cold hardiness are reviewed here. (a) Differential thermal analysis of field-collected buds yields (b) an uninformative high-temperature exotherm and informative low-temperature exotherms over the range of temperatures at which apoplastic water freezes. (c) Electrolyte leakage analysis of field-collected stem segments yields (d) a curve showing increasing membrane leakage over declining temperatures, allowing calculation of a critical point ( $LT_{50}$ ) at which 50% of damage has occurred. (e–g) Both indices of cold hardiness show a characteristic pattern of acclimation (hardening) in the autumn followed by a plateau in midwinter and deacclimation (dehardening) in the spring. Photographs are courtesy of the author, (b, e–g) are adapted from Kovalski (2022; CC-BY-NC-ND 4.0 with permission), and (d) is adapted from Kovalski & Grossman (2021; CC-BY 4.0).

climate cues and/or cold temperatures. Those climate ‘responsive’ species that rely heavily on climate cues to coordinate loss of cold hardiness and budbreak are particularly likely to experience false spring damage as budbreak increasingly precedes the last hard frost of the season (Ma *et al.*, 2019; Chamberlain & Wolkovich, 2021). Similarly, species such as yellow-cedar (*Callitropsis nootkatensis*) that do not attain a high level of cold hardiness and instead rely on snowpack insulation to protect them low air temperatures may experience a higher degree of damage from chronic exposure to freezing temperatures and, potentially, from false springs (Oakes *et al.*, 2014; Comeau *et al.*, 2021).

## IV. Droughts and water-deficit (drought) tolerance

### 1. Water deficit and drought

Seasonal perennials require year-round access to soil water, with demand peaking during periods of active growth (e.g. Klein & Niu, 2014). On timescales of seconds to days, plants respond to water restriction through a variety of short-term regulatory behaviors, including reducing stomatal conductance and down-regulating photosynthesis (Hsiao, 1973). Over longer timescales, though, both acute and chronic water restriction lead to organ mortality and whole-plant death through multiple interacting pathways (McDowell *et al.*, 2008; Sevanto *et al.*, 2014; Choat *et al.*, 2018). Chief among these is cavitation, which occurs when

xylem, the vascular element that conducts water throughout the plant, experiences high tension under conditions of low water access and becomes filled with air rather than liquid water (‘embolized’; Tyree & Sperry, 1989). Progressive embolism leads to loss of physiological function and, if widespread within a plant, death. Even in less extreme cases, chronic water restriction limits plants’ capacity to fix carbon and attain nutrients, slowing growth, and increasing vulnerability to other stressors (Anderegg, 2015; Kolb *et al.*, 2016; Kannenberg *et al.*, 2020).

Ongoing climate change will continue to increase the intensity and decrease the frequency of precipitation in many regions. In conjunction with rising temperatures, changes in precipitation regimes will elevate the intensity and frequency of droughts (IPCC, 2022). Although the term ‘drought’ is defined variably in the ecological literature (Slette *et al.*, 2019 provide a helpful review), I use it here to indicate a period of reduced growing-season rainfall relative to the historical mean, leading to low soil moisture and attendant plant water deficits. Droughts can vary from local occurrences lasting for months to regional or continental events lasting for years to decades (Spinoni *et al.*, 2014). In this review, I focus on droughts as phenomena that limit plant access to water in ways that cannot be managed through transitory downregulation of stomatal conductance or plant metabolism. Although droughts have been defined historically in terms of their infrequency, they may become the ‘new normal’ in many seasonal environments (Balting *et al.*, 2021). And because seasonal perennials must be able



to survive occasional periods of dry (if not droughted) soil conditions, adaptive traits allowing acclimation to both dry soil conditions and drought are widespread across the plant phylogeny and vary considerably both within and across biogeographic regions (Niinemets & Valladares, 2006; Bartlett *et al.*, 2012b; Choat *et al.*, 2012; Skelton *et al.*, 2015). For simplicity, I follow precedent and discuss these adaptations as 'drought tolerance' or 'water deficit tolerance' traits below.

## 2. Measuring drought tolerance

As plant water-use physiology integrates across soil water availability, temperature, irradiance, vapor pressure deficit, and other environment cues (Hsiao, 1973; McCulloh *et al.*, 2019), drought tolerance as a syndrome resists measurement with any single cellular, organ-level, or organismal metric. Frequently used metrics of drought tolerance include estimates of whole-plant water-use efficiency (Cernusak, 2020) and observations of mortality following exposure to naturally occurring (Anderegg *et al.*, 2019) or experimentally induced (Binks *et al.*, 2016) water deficits. However, the current balance of evidence suggests that xylem vulnerability to cavitation forms the linchpin in plant responses to water deficits. Plants with limited access to water tend to die directly either due to hydraulic failure or due to carbon (McDowell *et al.*, 2008; Adams *et al.*, 2017) and/or nutrient (Salazar-Tortosa *et al.*, 2018) starvation resulting from a hydraulically conservative strategy. For this reason, I focus here on two widely used high-throughput approaches to quantify the vulnerability of vegetative organs to drying (Box 3; Fig. 4): turgor loss point ( $\Psi_{\text{TLP}}$ ) and vulnerability to xylem cavitation ( $P_x$ ). Both metrics have been measured across diverse clades and shape species' native ranges with respect to climatic gradients in water availability (Bartlett *et al.*, 2012b, 2014; Choat *et al.*, 2012; Oliveira *et al.*, 2019). Whereas patterns of seasonal acclimation in thermotolerance and cold hardiness have been well-documented, the gradual and sporadic accumulation of evidence for seasonal plasticity in  $\Psi_{\text{TLP}}$  (Hsiao, 1973; Begg & Turner, 1976; Hinckley *et al.*, 1980; Teskey *et al.*, 1984; Sanders & Arndt, 2012; Turner, 2018) and  $P_x$  (Jacobsen *et al.*, 2007; Jacobsen & Pratt, 2014; Anderegg, 2015) has received, to date, relatively limited synthetic treatment. But recent work, including some unpublished findings from my own group, has filled in gaps in our understanding of seasonal patterns in drought tolerance.

## 3. Temporal patterns of drought tolerance

In most studies conducted in field conditions, plastic shifts in drought tolerance reflect acclimation (dropping  $\Psi_{\text{TLP}}$  and  $P_x$ ) over the course of the growing season (Teskey *et al.*, 1984; Kolb & Sperry, 1999; Sjöman *et al.*, 2015, 2018; Liu *et al.*, 2018; Hirons *et al.*, 2020; Herrera *et al.*, 2022), from the wet to the dry season (Wenhui, 1998; Jacobsen *et al.*, 2007; Jacobsen & Pratt, 2014), or in response to drought conditions (Bartlett *et al.*, 2014; Turner, 2018). For example, in Wenhui's (1998) study of 30 diverse perennial species in the Brazilian Cerrado (a neotropical savanna),  $\Psi_{\text{TLP}}$  declined an average of 0.34 MPa between the wet

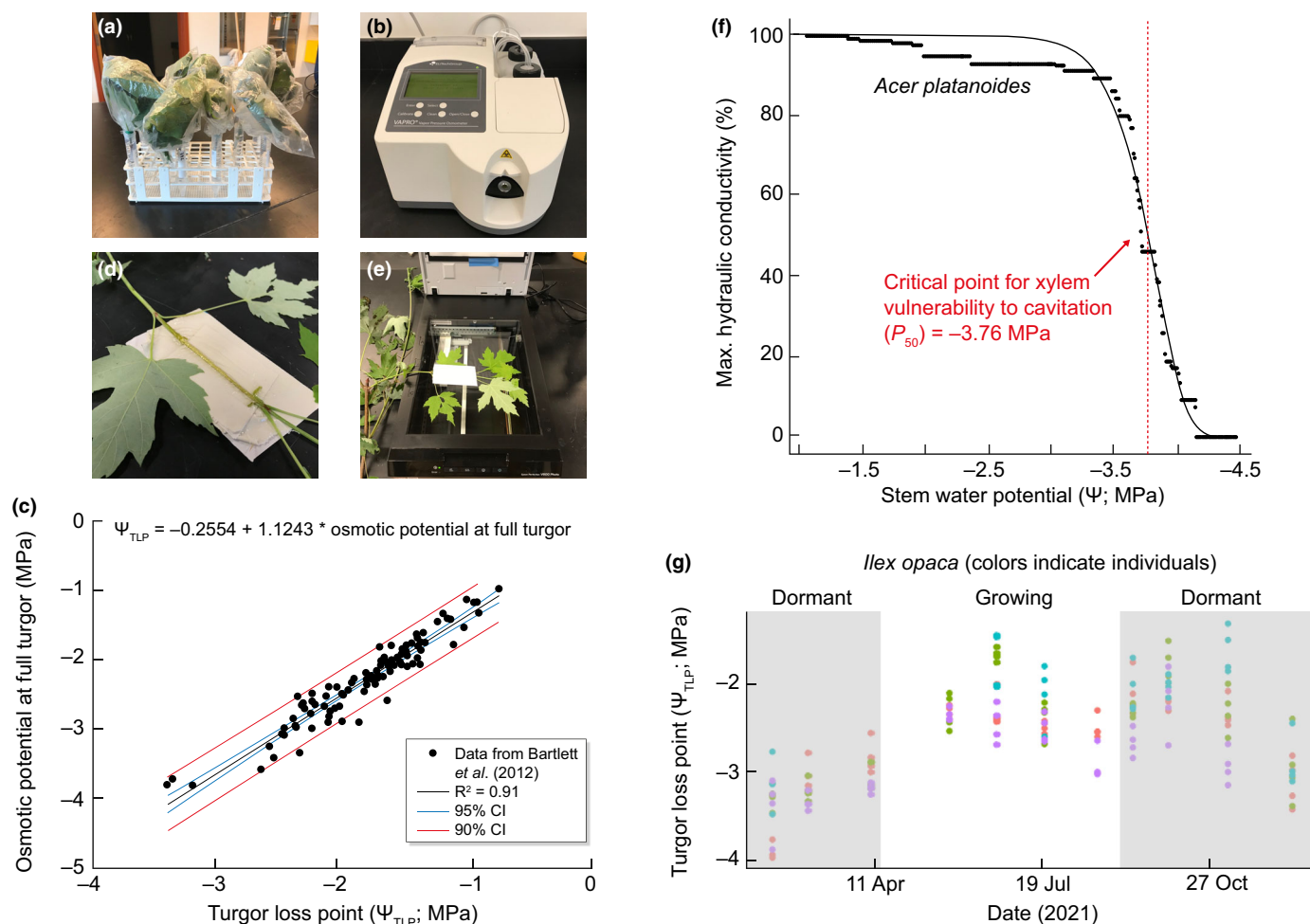
### Box 3 Turgor loss point ( $\Psi_{\text{TLP}}$ ) and vulnerability to xylem cavitation ( $P_x$ ) as metrics of drought tolerance.

Both phenotypic measures of drought considered here are presented in terms of the water potential ( $\Psi$ ) at which a critical degree of physiological function is lost (Lambers & Oliveira, 2019). Living plant tissue always experiences tension ( $\Psi < 0$ ), with water tending to migrate from tissues of higher (less negative) to lower (more negative)  $\Psi$ . Generally, for water uptake from the soil and maintenance of normal physiological function to occur,  $\Psi_{\text{plant}}$  must be lower than  $\Psi_{\text{soil}}$ . Under dry or droughted soil conditions,  $\Psi_{\text{soil}}$  and  $\Psi_{\text{plant}}$  decline. Both turgor loss point ( $\Psi_{\text{TLP}}$ ) and vulnerability to xylem cavitation ( $P_x$ ) reflect  $\Psi$  of a particular vegetative organ (e.g. stem or leaf) below which physiological dysfunction is likely to occur.

Measured at the level of a leaf or branchlet, but generally interpreted as a metric of whole-plant drought tolerance,  $\Psi_{\text{TLP}}$  reflects the water potential below which a leaf loses turgor (turgor potential [ $\Psi_p$ ] = 0) and any changes to  $\Psi$  are entirely the result of the osmotic potential ( $\Psi_o$ ) of the leaf or leaves (Fig. 4a–c; Bartlett *et al.*, 2012b). Two methods predominate in the calculation of  $\Psi_{\text{TLP}}$ : the traditional pressure–volume method (Kubiske & Abrams, 1991; Sack *et al.*, 2003) and a higher throughput, indirect, vapor pressure osmometry approach (Fig. 4a–c; Bartlett *et al.*, 2012a). Both are carried out on fully rehydrated, field- or glasshouse-collected leaves. By contrast,  $P_x$  can be assessed for any xylem-bearing organ (usually stem tissue, but also leaves, roots, etc.) and reflects the  $\Psi$  below which some critical percentage (x in the notation used here) of xylem conductivity is lost due to cavitation (Fig. 4d–f; Tyree & Sperry, 1989; Choat *et al.*, 2012). Historically, xylem embolism has most frequently been measured on stem tissue removed from field-grown plants and manipulated to reach increasingly negative  $\Psi$  (Venturas *et al.*, 2017). Conductivity at each  $\Psi$  is then measured, allowing construction of a tension–conductivity curve and extraction of, for instance  $P_{50}$ , the  $\Psi$  at which 50% of stem conductivity is lost (Fig. 4f). Recent modifications to these methods have included visualization of embolized tissue using x-ray computer microtomography (microCT; Choat *et al.*, 2016) or optical scanning (Fig. 4d,e; Brodribb *et al.*, 2016), indirect capture of embolism through measurement of pressure change kinetics (Pereira *et al.*, 2020), and increased interest in more extreme critical points (e.g.  $P_{88}$ ; Choat *et al.*, 2018) as ecologically informative indicators of drought tolerance.

and dry seasons, reflecting up to a 52% gain in drought tolerance. Hirons *et al.* (2020) report an even more substantial pattern of osmotic adjustment over the course of the growing season in temperate trees measured in a variety of North American and European public gardens. From spring (postbuddbreak) to mid-summer, the 115 species they surveyed showed a mean decline in  $\Psi_{\text{TLP}}$  of 0.68 MPa, with maximum osmotic adjustment again exceeding 50% of springtime (unacclimated)  $\Psi_{\text{TLP}}$  in some cases. Observational studies of seasonal change in  $P_x$  are much less common in the literature. Key reports from Jacobsen *et al.* (2007) and Jacobsen & Pratt (2014) document evidence of acclimation (declining  $P_x$ ) in some, but not all, species of Californian Chaparral shrub species, with most species showing a 1–2 MPa decline from the wet to the dry season.

The observational studies reviewed above have been complemented by experimental work in the field and laboratory, implicating sublethal water deficit as a mechanistic driver of



**Fig. 4** Two techniques for quantifying water-deficit (or drought) tolerance are quantified here. (a) Rehydration of field-collected leaves followed by (b) vapor pressure osmometry yields (c) turgor loss point ( $\Psi_{TLP}$ ) through an allometric equation (Bartlett *et al.*, 2012a; Sjöman *et al.*, 2015). Additionally, (d, e) visual scanning of progressive loss of hydraulic conductivity under declining stem water potential ( $\Psi$ ) yields (f) the critical point for xylem vulnerability to cavitation ( $P_{50}$ ). (g) Emerging evidence suggests that  $\Psi_{TLP}$  (and  $P_{50}$ ; not shown) decline over the course of the growing season, acclimating to drier conditions. Evergreens such as *Ilex opaca* show additional acclimation over the dormant season, potentially in response to cold and dry conditions. Photo courtesy of the author, (c) is adapted from Sjöman *et al.* (2015), and (f) is adapted from unpublished research from the Grossman laboratory group, and (g) is adapted from Northing (2022).

acclimation in drought tolerance (Meyer & Boyer, 1972; Jones & Turner, 1978; Premachandra *et al.*, 1992; Stiller, 2009; Awad *et al.*, 2010; Sanders & Arndt, 2012; Martorell *et al.*, 2015; Binks *et al.*, 2016; Cardoso *et al.*, 2018; Sorek *et al.*, 2021). The finding that observed declines in  $\Psi_{TLP}$  and  $P_x$  can be induced by experimental application of drought conditions provides evidence that observed seasonal patterns in drought tolerance are likely the result of acclimation to dry soil conditions and not merely leaf or branch ontogeny (Martorell *et al.*, 2015; Sorek *et al.*, 2021; Herrera *et al.*, 2022).

#### 4. Phenology of drought tolerance

Taken together, these observations suggest a pattern – one supported by evidence from relatively sparse continuous time series studies (Teskey *et al.*, 1984; Grossnickle, 1992) – that drought tolerance follows a roughly sinusoidal pattern of acclimation and deacclimation reminiscent of that found for cold hardiness (Figs 1, 4). Newly

expanded leaf tissue (emerging at the beginning of the growing or wet season) would be expected to be highly vulnerable to drought, with drought tolerance increasing with time until either (1) reaching a plateau in response to stabilizing conditions or (2) senescence of leaves and cessation of the active growing period. The evergreen habit of some seasonal perennials and the potential role of osmotic adjustment in protecting against freezing damage (Grossnickle, 1992; Arias *et al.*, 2015) also complicate this picture. For example, in preliminary year-round monitoring of the evergreen angiosperm tree American holly (*Ilex opaca*), my laboratory group found that a  $-0.36$  MPa per month acclimatory decline in  $\Psi_{TLP}$  over the course of the growing season was followed by a shallower but sustained decline ( $-0.21$ /month MPa) beginning in autumn and continuing until late spring, when  $\Psi_{TLP}$  started to increase again (Northing, 2022). The extent to which this apparent increase in evergreen drought tolerance during the relatively wet dormant season is linked to water-deficit stress, cold acclimation, or both remains to be determined.

Furthermore, the temporal scale of drought acclimation in seasonal perennials also remains unresolved (Table 1). Drought itself occurs over longer timescales (months to years or decades) than heat waves and false springs (days to weeks). Concomitantly, plant responses to natural or imposed soil drying – as opposed to transient fluctuations in stomatal conductance or plant water potential – have tended to be measured over seasonal timescales ranging from weeks to months (Kolb & Sperry, 1999; Awad *et al.*, 2010; Jacobsen & Pratt, 2014; Hirons *et al.*, 2020; Sorek *et al.*, 2021). On the one hand, this weekly to monthly scale for measurements may be justified. Structural acclimations to drought stress (e.g. structural vessel properties that determine  $P_x$ ; Lenz *et al.*, 2011), in particular, might be expected to occur slowly or be restricted to newly developed vegetative tissue. Yet, other forms of acclimation (e.g. osmotic adjustment in  $\Psi_{TLP}$ ) may be expected to occur more rapidly. None of the published work documented here has explored the capacity of droughted plants to adjust their drought tolerance over short timescales (days to weeks), leaving the grain of the temporal scale of physiological acclimation to drought (as measured by  $\Psi_{TLP}$  and  $P_x$ ) relatively unexplored. As such, the temporal scale of drought acclimation remains an unresolved topic of ecophysiological research.

## V. Toward a deeper integration of phenology and ecophysiology

Synthesis of existing evidence for phenological patterns of acclimation in thermotolerance, cold hardiness, and drought tolerance in seasonal perennials suggests four priorities for ongoing scholarship and integration in plant ecology and global change ecology.

### 1. Make physiology more phenological

The proliferation of publicly available plant trait databases (e.g. TRY, Kattge *et al.*, 2020; PLANTS, USDA, 2022) and datasets (e.g. Niinemets & Valladares, 2006; Choat *et al.*, 2012; Lancaster & Humphreys, 2020; Perez & Feeley, 2020) has provided novel opportunities for synthesis, allowing biologists to tackle previously inaccessible macroecological and evolutionary questions. Unfortunately, when temporally plastic physiological traits are included in these collections, they are generally presented as static values for a particular taxon or are not accompanied with explicit data about the timing of the measurements taken. But, as the long-standing evidence for temporal shifts in physiological traits reviewed above makes clear, temporal referencing should join georeferencing as a best practice for inclusion of observations in large databases or datasets (Bloom *et al.*, 2018). For samples measured in the field or collected and then assessed in the laboratory (e.g. Lenz *et al.*, 2013; O'Sullivan *et al.*, 2017; Hirons *et al.*, 2020), the inclusion of a field-collection time stamp along with latitude and longitude allows for physiological data to be interpreted in a phenological context. It is somewhat less obvious what the best practice should be for observations resulting from experimental manipulation (e.g. Ghoul *et al.*, 2003; Awad *et al.*, 2010; Kovaleski *et al.*, 2019). Metadata should at the least include when observations were made relative to key phenological events, such as experimentally forced

budbreak. Regardless, it is highly recommended that, going forward, trait datasets and databases be curated as to make it either recommended or required for all datapoints to include reference to the timing – as well as location – associated with an observation.

### 2. Make phenology more physiological

By calling for physiology to be interpreted in a phenological light, I build on others' treatment of repeated patterns of physiological change as phenological in nature (Lapointe, 2001; Vitasse *et al.*, 2014; Tang *et al.*, 2016; Savage & Chuine, 2021). As noted above, the classic phenophases have tended to be easily observable morphological transitions: leaf or flower budbreak and expansion, fruiting, leaf senescence and loss, etc. Yet critically, these morphological phenophases both coordinate and are coordinated by underlying physiological transitions, such as springtime bud, twig, and stem cambial reactivation (Savage & Chuine, 2021) and loss of cold hardiness (Kovaleski, 2022) in seasonal, perennial taxa. Morphological phenophase observations should still take center stage in phenological reporting, but the increased accessibility of high-throughput physiological measurements, such as those reviewed here (Boxes 1–3), now makes it feasible to monitor 'physiophenophases'. For instance, across diverse clades, measurements of temperate woody perennials have continued to demonstrate support for a late-spring or early-summer nadir in drought tolerance ( $\Psi_{TLP}$ ; Figs 1, 4e; Grossnickle, 1992; Hirons *et al.*, 2020, among others). This point of minimal drought tolerance could be treated as a critical phenophase – likely coordinated with leaf-out – particularly in phenological studies addressing themselves to climate change impacts or vulnerability. So, although such 'invisible' phenophases are likely unsuitable for inclusion in phenological monitoring conducted by citizen scientists (Fitzpatrick *et al.*, 2021) or through remote sensing (Richardson *et al.*, 2018), they promise to contribute key perspectives to new and ongoing monitoring campaigns.

### 3. Define the characteristic temporal scale of physiological acclimation

The literature surveyed here provides a foundation for understanding the characteristic temporal scale of physiological acclimation in thermotolerance, cold hardiness, and drought tolerance (Table 1). Yet, some notable gaps in this understanding remain; future physiological measurements of both untreated and experimentally manipulated plants should target these areas. To this end, I summarize existing gaps in our understanding of the temporal scale of change in thermotolerance, cold hardiness, and drought tolerance and present key research priorities below.

**Thermotolerance** It has been demonstrated that leaf tissue can acclimate rapidly to experimental application of high heat and that this acquired thermotolerance is lost over time (Koppelaar *et al.*, 1991; Havaux, 1993). Furthermore, measurements of field-grown plants suggest a modest acclimation – at least for many taxa – when exposed to progressively warmer temperatures across the growing season or across seasons within a year (Downton



*et al.*, 1984; Seemann *et al.*, 1986; Froux *et al.*, 2004; Sastry & Barua, 2017). Missing is an understanding of the extent to which short-term (hours to days) fluctuations in both leaf ( $T_c$ ) and whole-plant thermotolerance translate to seasonal patterns such as those observed by Froux *et al.* (2004). Further growing season- and year-long time series observations of thermotolerance should also clarify the extent to which these patterns predominate across clades, functional groups (e.g. evergreen vs deciduous), and seasonal environments (e.g. continental vs Mediterranean climates).

**Key Thermotolerance research questions:**

- (1) How quickly is thermotolerance lost? Does short-term (hours, days) acquisition of thermotolerance protect plants from high heat over longer timescales (weeks, months)?
- (2) Does increased thermotolerance as measured by leaf  $T_c$  predict whole-plant resilience under elevated temperatures?
- (3) Does the seasonal attainment and loss of thermotolerance occur only in particular clades, functional groups, or floras of particular biogeographic regions?

**Cold hardiness** We currently have a better sense of the underlying temporal scale of seasonal changes in cold hardiness than we do for thermotolerance or drought tolerance (Table 1). Pressing research priorities pertaining to freezing de/acclimation instead deal with the underlying mechanisms driving gain and loss of hardiness. This is to say that, although it is well-established that cold-hardy plants exposed to dropping temperatures gain cold hardiness and then lose it when exposed to warming temperatures, it is unclear what molecular and cellular processes are driving these changes, how best to model or forecast them, or what their consequences will be in a changing climate. Recent progress uncovering the molecular and cellular underpinnings of cold hardiness has demonstrated their intimate linkage to patterns of growth and dormancy in woody plants (Wisniewski *et al.*, 2003; Cooke *et al.*, 2012), underscoring the inherently phenological nature of cold hardiness. Given this, it is unsurprising that, in addition to the widely recognized role of cold temperatures (chilling) and warm temperatures (forcing) in determining when dormancy is attained and lost, cold hardiness itself may be a key indicator of phenological timing (Kovaleski, 2022). Improvements in our capacity to predict cold hardiness and its role in structuring dormancy across the year will aid in forecasting the impacts of climate change, including the potential impacts of reduced winter snowpack (Comeau *et al.*, 2021) and false springs (Chamberlain *et al.*, 2019).

**Key Cold hardiness research questions:**

- (1) What are the critical molecular and cellular mechanisms conferring cold hardiness in seasonal perennials?
- (2) What are the freezing de/acclimation potentials for ecologically and economically important species and does this trait vary predictably across the phylogeny?
- (3) How will warmer winter temperatures (less chilling) interact with warmer spring temperatures (more forcing) to alter the attainment and loss of cold hardiness?

**Drought tolerance** As is the case for thermotolerance, the existing evidence suggesting phenological patterns in drought tolerance remains incomplete. In surveys of diverse temperate perennials, it

appears that osmotic adjustment (a drop in  $\Psi_{TLP}$ ) is relatively common, though not universal, over the course of the growing season, between wet and dry seasons, and in response to drought (Wenhui, 1998; Bartlett *et al.*, 2014; Hirons *et al.*, 2020). Yet, there is considerable variation in this response, with some taxa showing no pattern of acclimation and others declining in  $\Psi_{TLP}$  by 1–2 MPa or more. Future work may shed light on the extent to which acclimation or failure to acclimate depends on inherent differences among taxa or the external environment.

Furthermore, acclimation to restricted water conditions is presently better understood for winter-deciduous perennials, with less focus to date on evergreens (but see Grossnickle, 1992; Northing, 2022), which may show considerable acclimation not only during the growing season but also throughout the year (Fig. 4g). Exploration of dormant-season changes in drought tolerance may help disentangle the relative consequences of cold temperatures vs dry conditions in coordinating plant tolerance of drought. The interaction between cold hardiness and drought tolerance is of particular interest given that the two phenomena are each, in part, dependent on shared molecular pathways related to symplastic osmotic adjustment (Yamaguchi-Shinozaki & Shinozaki, 2006; Chen *et al.*, 2021) and that soil moisture deficits can trigger leaf turnover in drought-deciduous taxa (e.g. Fallon & Cavender-Bares, 2018). Finally, exploration of phenological patterns in the hydraulic vulnerability of xylem to embolism ( $P_x$ ) remains preliminary (Jacobsen *et al.*, 2007; Jacobsen & Pratt, 2014; Anderegg, 2015), despite strong evidence that this trait is highly plastic, varying within an individual tree's canopy (Cochard *et al.*, 1997) and across sites (Wortemann *et al.*, 2011; Anderegg, 2015) independently of genotypic or species identity. Further investigation of the temporal grain and extent of phenological acclimation in  $P_x$  will improve our capacity to detect points of particular seasonal vulnerability in this critical trait.

**Key Drought tolerance research questions:**

- (1) How widespread is the capacity to acclimate to water-deficit stress (both in terms of  $\Psi_{TLP}$  and  $P_x$ ) among seasonal perennials? Are there phylogenetic patterns in this capacity?
- (2) What is the relative contribution of local environmental factors (e.g. soil water and light availability) vs intrinsic capacity to phenological acclimation to water deficits?
- (3) How does the phenology of drought tolerance vary across taxa with differing leaf habits?
- (4) Is acclimation—measured as a decline in  $P_x$ —reversible within a growing season? How widespread is acclimation in this trait and what is its mechanistic basis?

#### 4. Integrate temporal physiological acclimation into climate change adaptation and mitigation

The identification and characterization of phenological patterns in physiological stress tolerance can provide managers with a critical tool for use in both climate change adaptation and mitigation. Societal adaptation to ongoing and future climate change will require iterative modification of species and genotype selection in our food production (van Etten *et al.*, 2019), silviculture (Brang *et al.*, 2014), and urban forest (Sjöman *et al.*, 2015) systems and



development of plans for assisted migration for species of conservation concern (Etterson *et al.*, 2020). And although reducing combustion of fossil fuels and other anthropogenic greenhouse gas emissions must play a primary role in mitigating future climate change, reforestation and restoration are already recognized as critical tools for reducing the size of the atmospheric CO<sub>2</sub> pool (Bastin *et al.*, 2019; Taillardat *et al.*, 2020).

Yet, successful planting and management for both climate change adaptation and mitigation ultimately rely on the selection of species that can tolerate a rapidly changing and often physically stressful environment (Andivia *et al.*, 2021, but see Kozłowski & Palardy, 2002, for some beneficial consequences of physiological stress). In the particular case of seasonal perennials in silvicultural, reforestation, and restoration projects, species selection optimally must incorporate tolerance of long-term environmental stress. Breeding for climate resilience (Ziska *et al.*, 2012), though promising, cannot provide immediate solutions, and the likely prevalence of novel climate conditions (Ordóñez *et al.*, 2016) suggests the limitations of wholesale translocation of species or assemblages poleward or to higher elevations. Instead, the assessment of physiological acclimatory potential in existing plant lineages, populations, and cultivars may provide our best bet of predicting and managing their responses to ongoing climate change.

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