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Research review

The thermal tolerance of photosynthetic tissues: a global systematic review and agenda for future research

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Summary

Understanding plant thermal tolerance is fundamental to predicting impacts of extreme temperature events that are increasing in frequency and intensity across the globe. Extremes, not averages, drive species evolution, determine survival, and increased crop performance. To better prioritise agricultural and natural system research, it is crucial to evaluate how researchers are assessing the capacity of plants to tolerate extreme events. We conducted a systematic review to determine how plant thermal tolerance research is distributed across wild and domesticated plants, growth forms and biomes, and identify crucial knowledge gaps. Our review shows that most thermal tolerance research examines cold tolerance of cultivated species; ~5% of articles consider both heat and cold tolerance. Plants of extreme environments are understudied, and techniques widely applied in cultivated systems are largely unused in natural systems. Lastly, we find that lack of standardised methods and metrics compromises the potential for mechanistic insight. Our review provides an entry point for those new to the methods used in plant thermal tolerance research and bridges often disparate ecological and agricultural perspectives for the more experienced. We present a considered agenda of thermal tolerance research priorities to stimulate efficient, reliable, and repeatable research across the spectrum of plant thermal tolerance.

Keywords: agriculture, climate change, extreme, temperature, thermal breadth, thermotolerance, warming.

Introduction

As the Earth's climate changes, our dependence on healthy vegetation systems is coming into sharp focus. Temperature is arguably the most important determinant of plant species adaptation and distribution across the planet (Nievola *et al.*, 2017). Researchers seek to understand plant species responses to temperature to breed crops for a growing population, gain fundamental insight into physiological, ecological, and evolutionary processes, and predict responses of wild species to the changing climate. There has been an ever-increasing number of publications over the last century in various specialist fields of plant thermal tolerance research, but the work is scattered across different fields and geographic regions. Thus, as a research community we cannot easily and objectively prioritise research effort or effectively summarise what the thousands of published studies tell us about plant thermal tolerance.

Many biological processes are fundamentally dependent on temperature: including growth, reproduction and, in plants, photosynthesis. Classic studies have established that thermal limits are key to establishing the distribution limits of land plants, constraining the survival of plant tissue between -60°C and +60°C, where species growing in the most extreme biomes exhibit a range of adaptations to function and persist (Osmond *et al.*, 1987). Importantly, it is extreme low and high temperatures that can impair physiological functions, growth, and determine survival by profoundly changing the structure and fluidity of cell membranes, altering enzyme function, and destroying proteins (Osmond *et al.*, 1987; Sung *et al.*, 2003; Hatfield & Prueger, 2015). Extreme temperature events that are increasing in frequency and severity (IPCC, 2018) can affect organisms profoundly and are a major driving force for selection, adaptation, and species persistence (Gutschick & BassiriRad, 2003; Buckley & Huey, 2016; Lancaster & Humphreys, 2020).

Studies have shown that plant cold tolerance varies depending on factors such as elevation, ontogeny (Marcante *et al.*, 2012; Sierra-Almeida & Cavieres, 2012), microsites (i.e. sheltered *vs* exposed) (Bannister *et al.*, 2005; Briceño *et al.*, 2014; Venn & Green, 2018), and water availability (Sierra-Almeida *et al.*, 2009; Venn *et al.*, 2013). For example, alpine plants can withstand very low temperatures and tolerate extracellular ice formation and the resulting dehydration (Sakai & Larcher, 1987; Larcher, 2003). Higher heat tolerance is found at lower absolute latitudes and is positively correlated with mean annual temperature (Lancaster &

Humphreys, 2020). For a given latitude, desert species have higher tolerance to heat relative to coastal congeneric species *in situ*, but these differences can diminish under common garden conditions (Knight & Ackerly, 2002; 2003). Recent studies of Australian desert species have found that within a single desert biome, species vary widely in their physiological response to high temperature (with critical temperatures ranging from 48-54°C). Further, critical damage thresholds are driven less by macro-scale climate or latitude, than by microhabitat variation, especially soil moisture variation (Curtis *et al.*, 2016).

Crops are susceptible to temperature extremes and exposure to sub- and supra-optimal temperatures can cause significant yield losses. The degree of susceptibility to temperature stress varies with species, duration, intensity, and developmental stage. Extreme heat after seedling establishment can scorch leaves, impair biochemical processes, and accelerate premature senescence. Cold or heat stress coinciding with reproductive development in major cereal crops (the most temperature-sensitive stage; Yoshida *et al.*, 1981) negatively affects reproductive processes and structures, which consequently reduces yield quantity and quality (Jagadish *et al.*, 2007; Coast *et al.*, 2016). If, and to what extent, crops acclimate to thermal stress is still being tested. However, research is increasingly showing that crop varieties can acclimate their physiology to both low (Yamori *et al.*, 2010) and high temperatures (Li *et al.*, 1991; Wang *et al.*, 2011) to varying extents, similar to that observed in wild species.

Our rapidly changing climate means that extreme events are having major impacts on wild and agricultural systems worldwide (Gitz *et al.*, 2016; Harris *et al.*, 2018); plant thermal tolerance research must be well directed, or risk floundering at such a critical time. At one extreme – high temperature – the frequency, intensity, and a-seasonality of heatwaves are breaking records annually (Hewitson *et al.*, 2014; Harris *et al.*, 2018). Although some species exhibit a high capacity to withstand higher temperatures and heatwaves than are currently experienced (Drake *et al.*, 2018; Aspinwall *et al.*, 2019), heatwaves are predicted to exceed the thermal tolerance limits of many species across a wide latitudinal range (O'Sullivan *et al.*, 2017). Shortened growing seasons, yield reductions, and crop losses have been occurring and are predicted to worsen (>40% by 2100 in some regions), primarily due to increasing heat stress (Jha *et al.*, 2014). Similarly, at the other extreme – low temperature – the frequency of cold snaps is increasing in some regions, both directly (e.g. through disruption of the polar vortex driving cold cells towards temperate regions; Kretschmer *et al.*, 2018) and indirectly (e.g. where warmer averages reduce snow cover and increase exposure to frost; Woldendorp *et al.*, 2008). If frosts occur during warmer conditions or if there is a substantial late-season frost event, such as the 2007 spring freeze in the USA, then this temperature backlash can cause substantial frost damage and widespread devastation to crops and natural species alike (Jönsson *et al.*, 2004; Gu *et al.*, 2008). Understanding cold tolerance limits may elucidate which species may be released from temperature limitation in future, for instance the expansion of subtropical and tropical plants into temperate zones due to reduced frequency or severity of cold snaps (Cavanaugh *et al.*, 2014).

Thermal tolerance in practice reflects a range of interacting elements. In many regions, plants may experience both hot and cold extremes, with events in each direction causing a shift in overall resource allocation from growth and reproduction to protection from physiological stress (Lortie *et al.*, 2004; Mitra & Bhatia, 2008). For example, heating events are common in alpine environments, where small stature plants track soil rather than air temperatures and thus heat to potentially damaging levels (Squeo *et al.*, 1991). The few studies examining heat tolerance for alpine species indicate that it can be surprisingly high (~48-50°C), with species living in warmer microhabitats having higher heat tolerance than species living in sheltered habitats (Buchner & Neuner, 2003; Larcher *et al.*, 2010).

Focusing on responses of a given species to only one of these extremes is therefore unlikely to provide a comprehensive understanding of thermal tolerance or to increase our predictive power in the face of climate change. Moreover, the potential for an extreme temperature event to become critically stressful to a plant may depend on a range of accompanying circumstances, such as water status, light conditions, or ambient temperatures prior to or following the event. Plants in cold climates may shift their thermal tolerance or alter their phenology in response to average warming conditions, but this may be at the cost of frost hardiness (Jönsson *et al.*, 2004). In addition, what constitutes an ‘extreme’ event for a given species or biome may be relatively benign in a different context. Thus, it is essential to consider abiotic factors and the dynamics of plant thermal tolerance.

Here, we present the results and synthesis of a large-scale systematic review focused on the tolerance of photosynthetic tissues of land plants to extreme heat and/or cold stress for both cultivated and wild species across life forms, biomes, and the world. We explore the many techniques that are used to measure thermal tolerance, the metrics derived from them, and the widely diverging experimental conditions under which thermal tolerance is assessed. We note that the concept of what constitutes 'thermal tolerance' is debatable. Some studies focus on reduced productivity under simulated future climates, others assess repairable damage after moderate chilling or heat stress, and others focus on the onset of irreparable damage following extreme freezing or heatwave events. For the purposes of this review we define thermal tolerance as the temperature (high or low) beyond which the plant exhibits substantial or lasting damage; we note that this temperature is often estimated from (and assumed to be correlated with) the temperature at which the plant invokes protective mechanisms.

Our objective was to review the geographic and temporal distribution of research efforts, assess methodological approaches, and highlight the commonalities, ambiguities, and deficiencies in global plant thermal tolerance research. Our review provides a timely synthesis of research to date and bridges often disparate ecological and agricultural perspectives. We also present recommendations and an agenda to highlight thermal tolerance research priorities and provide a go-to reference to inform efficient and reliable research across the spectrum of plant thermal tolerance.

Our approach to the systematic review

A systematic review relies on synthesis of a comprehensive and repeatable literature search (Lowry *et al.*, 2013; Lortie, 2014; Gurevitch *et al.*, 2018). We employed the Preferred Reporting Items in Systematic Reviews and Meta-Analyses (PRISMA) framework (Moher *et al.*, 2009) to compile a database of articles that measured plant thermal tolerance (Fig. S1). Briefly, our literature search (December 2017) of the Institute for Scientific Information (ISI) Web of Knowledge used an extensive list of search terms (Supporting Information Notes S1) and yielded more than 21,000 articles. We first screened the titles and then the abstracts and at each step excluded articles that did not include investigations into tolerance of leaves or leaf-buds of angiosperms and gymnosperms exposed to potentially damaging high or low temperature events as distinct from growth conditions.

Each article was evaluated based on 15 criteria (Notes S1) relating to each thermal tolerance assay technique being reported, important elements of experimental design, focal species, and characteristics thereof. Experimental conditions for assessing thermal tolerance diverge widely and methods for imposing experimental thermal stress can include mild to severe temperatures that are either applied gradually (ramped), suddenly (shocked), as a sustained growth temperature, or as a combination of any of these three. There is good biological justification for considering different rates of exposure to change. Thus, our survey focused on characterising specific design elements of the studies we included. We documented the conditions with which thermal stress was imposed to determine how consistent and comparable they were.

Many articles reported multiple techniques to evaluate thermal tolerance. Henceforth we refer to scientific publications as 'articles' and uses of individual techniques within an article as 'studies'. After quality checks, the dataset contained data from 1,691 unique articles comprising 3,743 studies of thermal tolerance assays (Fig. S1). The dataset is publicly available through the figshare repository (10.6084/m9.figshare.13083662).

A brief history and description of plant thermal tolerance techniques

A broad array of techniques is used to assay thermal stress. Thermal tolerance research on both cultivated and wild species became more common in the 1990s, but the rate of increase was more dramatic in cultivated species, which has culminated in four-fold more thermal tolerance articles on cultivated (n = 1,358) than wild species (n = 339). The technologies used to measure thermal tolerance have evolved through time (Fig. 1a,b). Early studies assessed thermal tolerance simply by quantifying visual damage. Moving forward, researchers of cultivated species were consistently earlier adopters of emerging techniques, such as (epi)genetics and 'omics (e.g. metabolomics, proteomics, genomics), often 10-20 years in advance of use in wild species research (Fig. 1, Notes S1). Overall, the most widely used techniques for assaying plant thermal tolerance in the past 20 years have been chlorophyll fluorescence (487 studies), electrolyte leakage (468 studies), and a broad array of other biochemical assays (446 studies in total). In recent years, studies using (epi)genetics and 'omics, biochemical assays, and reactive oxygen species (ROS) and antioxidant techniques have been rapidly increasing. These specific techniques are expanded upon below and Notes S1 summarises these and the remaining thermal tolerance techniques and includes relevant indicators and references.

Fluorescence techniques measure changes in fluorescence re-emitted from chlorophyll in the photosystems in response to high or low (potentially stressful) temperature. A variety of measures have been applied in this context, including minimum fluorescence (F_0); maximum fluorescence (F_M); photosynthetic quantum efficiency (φPSII); maximum photosynthetic quantum efficiency (F_V/F_M); non-photochemical quenching (NPQ); and chlorophyll *a* fluorescence transients

(Maxwell & Johnson, 2000). Exemplary articles have used these methods to define thermal metrics such as LT_{50} (also T_{50}), the temperature at which F_V/F_M declines to 50% of the maximum F_V/F_M of unstressed photosystems (Curtis *et al.*, 2014) or T_{crit}, the inflection point between slow and fast rise phases of the temperature-dependent increase in F_0; (Knight & Ackerly, 2002). Others have measured R_{fd}: chlorophyll fluorescence decrease ratio or vitality index, calculated on the decline of F_M to the fluorescence steady-state level (F_S) (Perera-Castro *et al.*, 2018). Their popularity has increased in recent years as fluorescence techniques can be high throughput, but there has been little explicit comparison of how the various measures differ in their interpretation.

Measures of electrolyte leakage are another widely applied technique; these assess change in ion concentrations in response to thermal damage using electrical conductivity. These methods are highly conducive to determination of thermal metrics such as critical temperatures at which 50% (or other standard) change in tissue ionic conductance (*gTi*) or electrical conductivity (*EC*) is reached. From these, researchers have calculated LT_{50}, which is well correlated with frost damage (Kreyling *et al.*, 2015), and other damage indices (I_d) (Whitlow *et al.*, 1992). Tolerance metrics derived from electrolyte leakage are strongly related to the climate of origin of both native and non-native species (Kreyling *et al.*, 2015) and species that are cold-sensitive release electrolytes more rapidly than cold-resistant species (Patterson *et al.*, 1976). Electrolyte leakage measures the site of physiological injury at extreme temperatures and can be high-throughput, but it is potentially less sensitive than chlorophyll fluorescence or gas exchange, and is limited to laboratory assays (Xu *et al.*, 2014).

There is a wide array of biochemical measures employed in thermal tolerance research including heat shock proteins (HSPs) and studies of ROS. Heat shock proteins and factors are produced rapidly in response to abiotic stresses to alleviate cellular damage (Wang *et al.*, 2004). HSPs function as molecular chaperones, assist in protein folding, maintain signal transduction, and prevent protein aggregation (Chen *et al.*, 2018). Their relative abundance can be detected using western blotting or slot/dot blotting. In general, more tolerant individuals or species will induce a larger abundance of HSPs, or changes in gene expression associated with their production (Feder & Hofmann, 1999); however, this pattern is not universal or clear-cut (Barua & Heckathorn, 2004). An array of techniques including chromatography, quantitative real-time PCR, and *in vitro* chaperone-like activity assays are used to assess heat shock responses (Chen *et al.*, 2018). Although their name suggests a specificity for heat stress, HSPs can be upregulated in response to a wide range of other stresses that induce protein unfolding including cold, drought, salinity, and oxidative stress (Feder & Hofmann, 1999; Barua & Heckathorn, 2004; Wang *et al.*, 2004). However, patterns of protein synthesis during cold acclimation can differ substantially to those expressed during heat shock responses (Guy, 1999). Therefore, while HSP determination may aid mechanistic understanding of the stress response for a given species, we are far from using such techniques widely, especially for wild species.

ROS and antioxidants play important roles in maintaining the redox state in plant cells. ROS are natural by-products of metabolic processes that can affect gene expression and contribute to plant growth, signalling, development, cell cycles, programmed cell death, abiotic stress responses, pathogen defence, and adaptation (Gill & Tuteja, 2010; Mittler *et al.*, 2011). Like HSPs, ROS concentrations can increase rapidly in response to diverse stimuli, including temperature extremes. Increased ROS concentration following thermal stress leads to unfavourable modification of lipids, proteins, and nucleic acids, resulting in cell damage and metabolic dysfunction. These impairments inhibit growth, reduce fertility, and promote premature senescence. Plants produce antioxidants to scavenge or detoxify ROS or their precursors and prevent free radical formation to mitigate cellular damage caused by uncontrolled ROS accumulation. However, under extreme temperature stress, antioxidant production can lag ROS production, making ROS a major factor in crop yield loss. A wide variety of ROS and antioxidants can be assayed with various methods to assess concentration or expression patterns with thermal stress (Gill & Tuteja, 2010; Mittler *et al.*, 2011).

More recently, epigenetics, genomics, and other ‘omics (e.g. transcriptomics, metabolomics, phenomics) have been applied in thermal tolerance research. These approaches have revealed regulatory mechanisms, new gene variants and their expression and function, and have been instrumental in adaptive plant breeding for resistance to abiotic stressors (Jha *et al.*, 2014; 2017; Shah *et al.*, 2018). For example, identifying molecular mechanisms underlying heat stress responses *in silico* has led to the refinement of transgenic techniques to engineer the overexpression of HSPs and genes related to ROS activity and membrane stability to confer increased heat tolerance in various crop species (Grover *et al.*, 2013). However, assessing the success of these efforts is confounded by various research groups applying non-standardised methods, and limited field-scale phenomic capabilities (Grover *et al.*, 2013).

Often what determines the adoption of an approach to assessing thermal tolerance is a combination of context of the research question, conventional wisdom, and local practice. However, when bodies of work are produced in isolation, in a limited number of research laboratories, or focused on one biome or study organism, the potential for siloing and lack of comparability among research programs arises. Thus, our review considers when and where these various techniques have been applied.

What comprises the plant thermal tolerance literature?

Geographic spread

An examination of the geography of thermal tolerance research based on both the country of affiliation of the first author and the location where the experiments were conducted (when available), shows that plant thermal tolerance is researched all over the world but, unsurprisingly, the distribution of this research is not uniform. The volume of articles by authors based in the USA, China, and Europe, vastly outweighs contributions by other individual countries (Fig. 2; see Figs S2-S5 for more detailed global and regional distributions). The patchy network of research likely reflects institutional bias and availability of research funding, where most articles, even for ecological research in the tropics, for example, are led by authors from developed countries (Stocks *et al.*, 2008). Many of the thermal tolerance articles on cultivated species pre-date the more recent focus on climate change and trace back to developing domesticated species suited to a range of growing environments.

Overall, articles published on wild species represent a narrower portion of global distribution than do those on cultivated species (Fig. 2a,b). Wild species are understudied in many of the more thermally extreme regions on Earth (e.g. north-west Asia, Middle East, Africa, South and Central America, and India, Fig. 2a,c,e,g). These gaps in global coverage, particularly for heat tolerance (Fig. 2g,h), mean that thermal tolerance is understudied in exactly those developing countries where there is rising demand for increased crop yield and where some of the greatest climate change-induced yield losses are predicted to occur (Parry *et al.*, 2004; Tester & Langridge, 2010).

Comparative thermal tolerance studies

Delving deeper shows that our understanding of thermal tolerance is informed by an eclectic spread of research across growth forms, and that there is relatively little broad-scale comparative work. We have a far greater understanding of the thermal tolerance of species that we have bred and depend on for food, timber, and fibre (n = 1,358), than those that comprise the rest of Earth's terrestrial biosphere that perform essential ecosystem services (n = 339; Fig. 3). Within the literature, and for both cultivated and wild species, a greater proportion of articles investigate cold (59%) than heat tolerance (35%) and there are strikingly few articles that examine both heat and cold tolerance together (5%, Table 1).

In terms of taxonomic selection, research on cultivated species tended to focus on a single species (42%) or on differences among intraspecific varieties (41%), but less often across multiple species (17%; Fig. 4a). In contrast, studies on wild species were split evenly between focusing on single or multiple species (44%) but investigated intraspecific diversity far less often (12%; Fig. 4b). The representation of different life forms also varied between cultivated and wild systems. Studies on cultivated species contained a greater proportion of graminoids (e.g. Poaceae), forbs/herbs (e.g. vegetable species) and vines (e.g. viticulture), with fewer shrubs or trees (Fig. 4c). In contrast, studies on wild species were more evenly spread with relatively more focus on woody species (Fig. 4d).

The recent work of Lancaster and Humphreys (2020) demonstrates the potential for meta-analytic comparison of thermal tolerance, and there remains ample opportunity to build on the relatively few studies that apply a standard method of assessing thermal tolerance and take an explicitly broad comparative approach. In particular, extension of excellent comparative works such as O'Sullivan *et al.* (2017), Zhu *et al.* (2018), Sentinella *et al.* (2020), and Lancaster and Humphreys (2020) into extreme biomes, across a wider range of growth forms, and considering other experimental nuances is still warranted. Such efforts will lead to a better understanding of general rules in thermal tolerance and have potential to explore the underlying mechanistic differences in the various measures of tolerance.

Cold *vs* heat tolerance research

Studies on cultivated species covered both cold and heat tolerance across the different types of cultivation, but with more studies on cold tolerance overall (Table 1, Fig. 3a). Cold tolerance was more often assessed within viticulture, plantation forestry, horticultural and vegetable crops, *Arabidopsis*, and multiple or other types of cultivation (e.g. tobacco, plants for oil). In contrast, heat tolerance made up more than half of the studies within cereals, fibre crops, and pasture and turf grasses. Cereals and fibre crops had the lowest proportion of articles that considered both heat and cold tolerance simultaneously.

For wild species, the proportion of studies focusing on heat, cold, and both heat and cold tolerance varied across biomes, but cold tolerance research made up the majority for all biomes except for arid ones (Table 1; Fig. 3b). Plant responses to both cold and hot extremes may be linked at localised scales via processes such as early snowmelt (Körner, 2003) or microhabitat variability (Suggitt *et al.*, 2018), or across a species' distribution by large scale changes in global circulation patterns influence extreme events (Kretschmer *et al.*, 2018). In tropical/subtropical biomes, the proportion of studies on cold and heat tolerance was more equal and these had the highest number of articles that examined both heat and cold tolerance. Studies in temperate biomes made up 34% of the wild dataset and these were dominated by cold tolerance studies. Articles on boreal forests were focused entirely on cold tolerance, as were most articles on arctic/alpine/subalpine biomes. Remarkably, heat tolerance was assessed far less often than cold tolerance in wild species; the greatest proportion of heat tolerance research was conducted in the warmer biomes: arid/semi-arid/savannah and tropical/subtropical, but even here, cold tolerance research was as or more prevalent. Given consistent predictions of increasing frequency and intensity of heatwaves across the world together with average warming (Perkins-Kirkpatrick & Gibson, 2017; Harris *et al.*, 2018; IPCC, 2018), the relatively low coverage of studies on plant heat tolerance is concerning.

Considerations when designing thermal tolerance experiments

Application of techniques

Our assessment of the history of thermal tolerance research indicates that there were not gaping holes in coverage by cultivation type, biome, or life form in the application of techniques for evaluating thermal tolerance. However, there is clearly opportunity for expanding the application of many techniques into new areas and non-model systems. For example, it is perhaps not surprising that HSPs have not been examined in species from the world's coldest biomes.

Plant thermal tolerance arises from complex phenomena involving perception of thermal stress, transmission of the information (cascade signalling), genomic regulatory processes, and then physiological and biochemical changes (Urano *et al.*, 2010; Hasanuzzaman *et al.*, 2013). By integrating approaches across scales we can shed light on the molecular mechanisms and cellular pathways that lead to physiological changes and confer tolerance (comprehensively reviewed by Nievola *et al.*, 2017). Applying multidisciplinary and holistic approaches to diverse species will reveal new gene variants, products, and traits for crop-breeders to target for engineering or breeding programs to obtain new stress-tolerant varieties (Fragkostefanakis *et al.*, 2015; Jha *et al.*, 2017; Shah *et al.*, 2018). Our review found a range of techniques under the umbrella of biochemistry (including ROS, HSPs, and other biochemistry) and 'omics (metabolomics, transcriptomics) that are commonplace in cultivated studies but rare in wild studies. We see great potential to gain better mechanistic understanding in wild species by applying more of these biochemical techniques and aiming to scale to the whole phenotype (e.g. Aspinwall *et al.*, 2019).

The emergence of high-throughput techniques for proteomics and metabolomics (Zivy *et al.*, 2015) along with phenomics (Furbank *et al.*, 2019) allows thermal tolerance to be assessed in both controlled environments and field studies for cultivated and wild species alike. This presents the opportunity to scale from mechanism to emergent phenotype (Deshmukh *et al.*, 2014; Campbell *et al.*, 2018). Greater crosstalk among researchers studying thermal tolerance on cultivated and wild species and application of these approaches to high-throughput scales would be mutually beneficial.

Ours is an era of evidence synthesis and meta-analyses (Gurevitch *et al.*, 2018), in which new analytical tools are released frequently. The rise of open trait databases such as TRY (Kattge *et al.*, 2020) and GlobTherm (Bennett *et al.*, 2018) underpins efforts to consolidate knowledge and extend the application and utility of individual studies to a global context. Databases hold great promise to generate comparative analyses; for example, contrasting thermal metrics across species or biomes, or assessing different measurement techniques for given species (e.g. Lancaster & Humphreys, 2020). We caution that there remain many considerations and caveats to consider in such syntheses; for example, the differences in measurement conditions and the specific methods of application of thermal stress, techniques to measure tolerance, and other aspects of experimental design. Armed with new insights and databases, researchers can contribute improvements to the accuracy and dynamic capabilities of model predictions and decision-making tools for regional-scale suitability, growth, and yield of crop species as extreme events become more frequent and intense (Caubel *et al.*, 2015; Zampieri *et al.*, 2019).

Experimental design considerations

It is abundantly clear that experimental designs and techniques vary widely among studies, and most notably between wild and cultivated systems (Figs 5a, S6). We found that it was common for research on cultivated species to compare relative performance of many varieties under a set of controlled conditions, but rare to provide an explicit explanation for temperature treatment choices (see Zub *et al.*, 2012 for an exemplary exception). On the other hand, these studies also generally conducted several complementary assays to achieve broader mechanistic insights. In contrast, studies on wild species focused on identifying tolerance limits under natural conditions more than understanding tolerance mechanisms; however, they generally provided explanations for their chosen rates of temperature change and treatment temperatures (e.g. Sierra-Almeida & Cavieres, 2012). Our review demonstrated three areas that warrant careful consideration and explanation when designing thermal tolerance research: how temperature stress is applied, the importance of recognising thermal legacy, and accounting for interactions with other factors. These are presented in detail below and summarised in Box 1A.

Application of temperature stress

Field, common-garden, glasshouse, and growth chambers each present different limitations, and the specific context of growth conditions can greatly influence plant responses (Passioura, 2006; Poorter *et al.*, 2016). Overall, we found that most articles (94%) imposed stress in an experimentally controlled manner, such as with a temperature-controlled growth chamber or water bath, as opposed to focusing on natural extreme events such as frosts or heatwaves (6%). In some experimentally controlled studies, thermal stress was imposed as a controlled ramp and in others as a sudden shock (Fig. 5b), each of which can induce different response mechanisms and pathways. In contrast to shocks, ramping temperature allows time for hardening processes to provide some thermal protection before reaching critically damaging temperatures. The application of ramp *vs* shock approaches differed between studies of cultivated and wild species.

Research on cultivated species applied thermal stress as shocks more often than on wild species (Fig. S7). Within wild species, most studies on cold tolerance ramped stress, whereas those researching heat tolerance applied a shock more often than ramping (Fig. S7). Biochemical assays and (epi)genetics and ’omics were most often conducted on plant tissue that was exposed to a temperature shock, whereas studies using electrolyte leakage, assays of visual damage, and thermometry were more often conducted on plant tissue that was exposed to a temperature ramp (Fig. 5b).

Cultivated species were assayed most often for periods of hours (1,322 studies) or longer (days = 785 studies and weeks = 431 studies), whereas for wild species, shorter timeframes were generally used: hours or less (415 studies). The exception was for HSPs, where stresses lasting <24h were common for both cultivated and wild species. Research on wild species that did apply stress over longer periods of days (89 studies) and weeks (72 studies) tended to focus on water potential, ROS/antioxidants, other biochemical factors, and gas exchange (Fig. 5a). In wild species, short stress intervals of 60 minutes or less were often used in association with gas exchange or chlorophyll fluorescence assays (Fig. 5a). A greater proportion of studies on cultivated species failed to clearly specify the maximum stress duration compared to those on wild species (Fig. 5a). In some cases, these differences reflect that the type of assay dictates the stress duration and cannot be consistent, but nonetheless such variation among studies hampers our ability to identify common responses.

In nature, the rate and frequency of exposure to extreme temperatures varies between cold and hot extremes. Leaf temperature can vary rapidly and repeatedly on a hot, calm day (Vogel, 2009), such that the frequency, duration, and magnitude of the heat stress are likely to affect the impact of and response to the stress. In contrast, exposure to extreme low temperatures tends to be more gradual and sustained over hours or even days (Sierra-Almeida & Cavieres, 2012). Thus, there is biological justification for using different rates to apply thermal stress when studying heat *vs* cold tolerance. However, we found that in many cases, studies elected to deliver their heat or cold treatments as a shock (e.g. moving a plant directly from a benign to a high or low temperature-controlled growth room) without providing the rationale behind that approach. The insect thermal tolerance literature is actively debating how moving to a dynamic delivery of extreme temperature (i.e. ramping temperature at biologically-relevant speeds, as opposed to a quick shock) would increase the relevance and impact of their research (Rezende *et al.*, 2014), and plant researchers could stand to benefit from considering a similar approach.

One limitation to adopting techniques used in animal thermal tolerance is the growth form of plants, which determine how we measure them. In the animal literature, it is standard to measure critical temperatures on small arthropods on which whole-organism tolerance can be assessed (e.g. Slatyer *et al.*, 2013; Hoffmann & Sgrò, 2018; MacLean *et al.*, 2019). Fundamentally, whole-organism measures on plants are more challenging due to their modularity, below-ground biomass, and growth form variation that contribute to a complex array of alternative mechanisms to escape or cope with thermal stress (Huey *et al.*, 2002). Modular organs such as leaves are therefore targeted for most thermal tolerance measurements in plants. However, this only determines limits to photosynthetic performance or organ survival, rather than higher-level or probabilistic measurements of whole-organism performance and survival that are more common in the animal thermal tolerance literature (Rezende & Bozinovic, 2019). Seedlings will be essential to exploring whether tolerance of leaves can be reasonable approximations for thermal tolerance measurements for whole plants or how these approaches could be developed.

Adopting more realistic regimes and justifying these with data from relevant natural settings, as well as providing better descriptions of the temperature ranges around set points would enable a more nuanced investigation of the differences between acute *vs* chronic stress responses, and between facultative protective responses *vs* signs of irreparable damage (Lai & He, 2016; Trapero-Mozos *et al.*, 2018). At present, the definition and use of 'stress' and 'stressful events' is somewhat *ad hoc* and impedes our ability to compare results or derive generalisations (Jansen & Potters, 2017). Differentiating damaging conditions from those that are suboptimal or induce protective mechanisms is essential contextual information; researchers need to attempt to explain how and why selected treatments and assays were conducted. By placing treatments in context with historical, realised, or projected climatic conditions, researchers provide an opportunity for others to assess the extremity of the treatments imposed relative to the biology of that species. For example, what may be an extremely high temperature for vegetative growth in broccoli (*Brassica oleracea* var. italica Plenck) is sub-optimal for maize (*Zea mays* L.), and sensitivity to thermal stress will also vary across life-stages and with environmental history (Hatfield & Prueger, 2015).

Understanding thermal legacy

Although warmer origin species often exhibit higher heat tolerances than cooler origin species under common conditions (Zhu *et al.*, 2018; Lancaster & Humphreys, 2020), it is important to note that the acclimation state of plants or tissue can substantially affect thermal tolerance and understanding the potential to acclimate will be important for predicting impacts of our changing climate. For example, geographic trends in thermal tolerance appear to be much stronger in acclimated (hardened) plants (Lancaster & Humphreys, 2020). While we did not directly assess acclimation, the term acclimation certainly frequents the literature we reviewed (Fig. S8). Thermal tolerance can shift in response to changes in both continuous growth temperature and exposure to extreme temperature events (Downton *et al.*, 1984; Hamilton *et al.*, 2008; Drake *et al.*, 2018) and changes can occur across the scale of minutes (e.g. heat shock) to months (e.g. seasonal change) (Havaux, 1993; Bannister *et al.*, 2005). Acclimation of thermal tolerance can be influenced by temperature alone (Strimbeck *et al.*, 2008), as well as other environmental conditions such as photoperiod (Bannister *et al.*, 2005) and water availability (Lu & Zhang, 1998). Thus, in addition to considering interactive effects on thermal tolerance, it is crucial for studies on thermal tolerance to be explicit about the thermal legacy of their study organisms.

Variability in background thermal regimes may have significant effects on plant responses to extreme conditions (Gutschick & BassiriRad, 2003; Bita & Gerats, 2013). Furthermore, plant thermal tolerance research seldom reports variability of ambient environmental factors in controlled growth environments (including temperature, light, and humidity) or differences between air and leaf temperatures, which can differ among species by up to 10°C in hot conditions (Wise *et al.*, 2004; Vogel, 2009). Comparisons among studies that differ in experimental designs, biomes, and species may be complicated by ambiguity at best and, more concerningly by legacy, if prior thermal exposure is not reported explicitly and terms to describe changes in thermal tolerance are not defined carefully.

Interactions with other environmental factors

Average temperatures are increasing alongside more intense and frequent extreme events, often with a backdrop of resource limitation. These factors will likely exacerbate the effect of thermal stress with potentially long-lasting or irreversible community-level effects (Harris *et al.*, 2018). Variation in other abiotic factors may include ordinary elements such as seasonal variation in temperature, light, or water availability. In many situations thermal stress from high temperatures will occur with or following onset of water limitation. Nonetheless, most studies in the literature focused on thermal tolerance in the absence of additional experimental variables (57%). Among the studies that included additional environmental factors, the most common was the effect of a controlled growth temperature prior to applying thermal stress (13%), e.g. to determine whether hardening alters the effect of extreme events. Given that heat stress events often co-occur with belowground resource limitations, it is concerning that an extremely small percentage of studies considered how availability of water (6%) or soil nutrients (2%) affected thermal responses. Likewise, we found few studies that considered the effects of light (3%), CO_2 (1%), or other non-climate factors (8%) on thermal stress responses. Indeed, such two- and three-way treatment interactions were investigated by just 10% of all studies. Given that our changing climate will bring shifts in both thermal and precipitation regimes and that drought and thermal acclimation have been shown to interact (Sierra-Almeida *et al.*, 2009; Hoover *et al.*, 2014), it seems pertinent to consider their combined impact on tissue damage, yield loss, or mortality. For studies of thermal tolerance to have real-world meaning, a greater understanding of how other factors limit responses to temperature is crucial.

Towards development of standard approaches and comparable thermal metrics

The more we can apply a set of standardised approaches across species, crop types or biomes, and different thermal regimes, the greater our potential to identify general patterns in the physiology, ecology, and evolution of thermal tolerance. Of course, the reality is that methods are regularly fine-tuned and refined for specific study organisms and contexts. Plant thermal tolerance research is most informative if the underlying premises regarding experimental conditions are well justified and experimental procedures are explained unambiguously.

Thermal tolerance metrics are a valuable tool to support comparative research to identify general patterns across species or biomes. For example, T_{crit} and T_{50} of F_V/F_M, often generated via measuring chlorophyll fluorescence, have been measured for hundreds of species (Notes S1; e.g. Knight & Ackerly, 2002; Zhu *et al.*, 2018; Lancaster & Humphreys, 2020). However, we found that only 23% of studies across both cultivated (49%) and wild (17%) species either reported a metric or provided information from which such a metric might be obtainable. Thus, where possible, we advocate adoption of techniques that generate a thermal tolerance metric that can be used for global comparative analyses.

The many different and nuanced approaches to researching plant thermal tolerance have propagated various metrics and terms. For example, plant thermal tolerance metrics frequently do not specify whether they reflect a heat or cold response (e.g. T_{crit} could refer to either hot or cold critical temperature). Further, measures of the same name, but derived from different thermal tolerance assays will vary in their functional significance depending on the underlying physiological processes that are being quantified. While measures and metrics from different tolerance assays (e.g. LT_{50} from F_V/F_M and LT_{50} from visual damage) yield interesting intra-assay comparisons, they do not always provide equivalent information, correlate well with each other, or represent biologically sensible comparisons (e.g. Neuner & Pramsohler, 2006; Curtis *et al.*, 2016). Ideally, streamlining metrics and terms would allow for greater comparability across experimental approaches and techniques, as is currently more commonplace in animal ecophysiology (Rezende *et al.*, 2014; Rezende & Bozinovic, 2019; Sunday *et al.*, 2019). Exploring how different assays correlate is a further vital step toward standardising approaches to evaluate thermal tolerance but also for understanding the mechanistic links among patterns of response in different measures.

We advocate a multidisciplinary approach to assessing plant thermal tolerance. For example, measure the thermal tolerance of photosynthesis directly using a method that produces a tolerance metric, such as chlorophyll fluorescence or electrolyte leakage. Biochemical responses to thermal extremes, particularly ROS and HSP, could then be measured to probe underlying mechanisms. To better understand the impact of thermal tolerance, a holistic view to growth and seed production is always useful, though we appreciate often logistically intractable. However, we note that until there are more studies that investigate the thermal tolerance responses of plants to extreme events using multiple approaches, we cannot infer which method generates the most reliable information or metric for predictive models.

An agenda for future thermal tolerance research

The primary objective of this synthesis was to determine the state of knowledge in the field of plant thermal tolerance research and to identify commonalities, ambiguities, and deficiencies in the global literature of plant thermal tolerance measurement. By mapping topics by article titles and author keywords, we can visualise the general siloing with respect to thermal tolerance assays, species selection, and geography (Fig. S8). After decades of research, there are still remarkable holes in our knowledge base, punctuated by large divides among specific sub-fields of thermal tolerance research. Our systematic review found little equivalency among techniques and study designs, let alone thermal metrics, indicating that cross-species comparisons remain far from straightforward. Addressing these issues will be crucial as trait databases become key sources for understanding plant responses to increased temperature means and extremes as the climate changes.

Our review has demonstrated the need to explicitly revisit not only how we study thermal tolerance, but also what our priorities are while studying it. The ‘how’ has been covered above. Below, we outline four broad areas that we see as priorities for empirical thermal tolerance research, for which our recommendations are summarised in Box 1B. This agenda seeks to provoke discussion and improve efficiency, repeatability, and comparative power in our research to catalyse fundamental advances and applied outcomes.

1. The comparative ecology of thermal tolerance in the ecological and evolutionary strategy spaces

Plant ecologists have made great advances in understanding how traits are related to distribution of species across the globe (O'Sullivan *et al.*, 2017; Lancaster & Humphreys, 2020; Sentinella *et al.*, 2020), but we have less understanding of how thermal ecology links to other elements of plant strategy space (Vasseur *et al.*, 2018). If we are to assess which ecosystems are most at risk under climate change accurately, a greater understanding of how thermal tolerance of species scales to the community level is essential. Multi-species comparative projects were under-represented within our dataset and these were not comparisons of within or between community variation in most cases. In the stand-out exemplary studies, there remains relatively low representation of non-woody growth forms. Undoubtedly, factors such as competition, facilitation, differential resource utilisation, and population demographics all modify the thermal response profiles of individual species and have flow-on effects to the functioning of communities and ecosystems. For example, the variation in thermal tolerance of species, growth forms, or functional types has the potential to change relative survival and dominance within communities, thereby leading to shifts in the distribution of species and communities (Ackerly, 2003). Such changes may then alter ecosystem function at small catchment and large landscape scales. Thus, improved understanding of how such variation affects community thermal tolerance in natural systems is warranted.

2. Understanding the geography and drivers of thermal tolerance breadth

Published research on wild plants in alpine biomes around the world has primarily focused on cold tolerance (e.g. Bannister, 2007; Briceño *et al.*, 2014) while in desert plants, research on heat tolerance dominates (e.g. Knight & Ackerly, 2002; Curtis *et al.*, 2014; 2016). Yet mountain plants can reach extreme high temperatures in summer (Larcher *et al.*, 2010) and desert plants are exposed to extreme cold (Lazarus *et al.*, 2019). Little is known about thermal tolerance breadth, including whether specialising for one extreme is antagonistic to the other. While responses to heat and cold shock may differ or have different kinetics, some share signalling and metabolic pathways (Kaplan *et al.*, 2004) and so fundamental insight about the mechanistic determinants of thermal tolerance could be revealed by comparing heat and cold tolerance. Further, thermal tolerance breadth may vary with climatic affiliation; for example, being broader in widespread species or species from variable or more extreme climates (Sheth & Angert, 2014).

Biodiversity models often assume that realised distributions reflect species' fundamental climatic tolerances, however, by underestimating thermal tolerances these models may underestimate the breadth of a species' niche (Bush *et al.*, 2018). Thus, we propose that the thermal tolerance breadth could be a better indicator of species' fundamental climatic tolerance, and thus adaptive capacity: important considerations to better predict species distributions or extinction risk under climate change. Thermal tolerance breadth could also be indicative of a crop's suitability for particular agro-ecological zones and potentially a desirable trait to target in crop breeding in growing regions that have both cold and hot extremes (Varshney *et al.*, 2011). Cultivars or species with narrow thermal tolerance breadth may be particularly vulnerable to changing climatic conditions, especially if that narrow tolerance is associated with low genetic diversity and narrow range sizes (Slatyer *et al.*, 2013). Conversely, cultivars selected for their tolerance to temperature extremes or natural species that have evolved with frequent extremes in temperature may have high thermal tolerance breadth and be buffered against crop failure and extinction (Buckley & Huey, 2016). Thus, thermal tolerance breadth has potential to yield insight with relevance to both wild and cultivated species. Such hypotheses have been tested in animals, but rarely in plants (Sheth & Angert, 2014).

3. Influences of other factors on thermal tolerance and the potential for shared mechanistic and evolutionary underpinnings

Few studies examine how thermal tolerance interacts with other abiotic factors that could enhance or reduce susceptibility to thermal extremes. Although research that has focused on thermal tolerances has yielded important information we cannot infer from these studies how plants would respond to combinations of temperature and one or more other stresses (Mittler, 2006; Suzuki *et al.*, 2014). In agricultural fields and natural habitats, plants are often exposed to multiple simultaneous environmental stresses. For example, heat stress frequently occurs in combination with drought. Interactions between water limitation and thermal response are ripe for investigation (Jagadish *et al.*, 2011; Fahad *et al.*, 2017), given that both temperature and precipitation regimes are changing across much of the globe. There is growing evidence that plant thermal tolerances are underpinned by molecular and metabolic processes that are both distinct to temperature stress (Rizhsky *et al.*, 2004) and common to other stresses (e.g. tricarboxylic acid-cycle intermediates increase in response to temperature and drought stress; Kaplan *et al.*, 2004). For combinations of thermal tolerance with tolerance to one or more other stresses, plants require unique metabolic and signalling responses (Zandalinas *et al.*, 2018). There remains much to be learnt about the drivers of these unique processes. Addressing this gap is essential for improving model parameterisation for the prediction of plant responses to climate change, identification of key traits for climate-resilient crop breeding programs, and the development of better adaptation strategies for managed agricultural settings and natural habitats.

4. Understanding the sensing of and response to thermal stress along the continuum from protective mechanisms to acquired damage

There is a complex continuum between temperatures that induce protective mechanisms and those that cause irreparable damage and impact survival (Nievola *et al.*, 2017). The relative impact of a single large *vs* repeated small exposures outside optimal temperatures remains poorly understood, and the mechanisms underlying priming or memory responses and recovery from thermal stress are complex and still an active area of investigation (Bruce *et al.*, 2007; Lämke & Bäurle, 2017; Hüve *et al.*, 2019). The extent of and mechanisms underlying the plasticity of thermal tolerance are thus another area needing attention and improved analysis (Arnold *et al.*, 2019).

Timeframes over which thermal tolerance acclimates in response to realistic temperature fluctuations on diurnal and seasonal bases are yet to be explored in depth. Such studies will provide more comprehensive insight into capacity for stress priming, recovery, and memory (Crisp *et al.*, 2016; Hilker & Schmülling, 2019). Thermal tolerance is highly responsive to changes in climate, growing environment, and interactive abiotic factors and stressors, but not all observed responses will be equally important. On macroscales, general trends in plant thermal tolerance can be observed at a coarse resolution across a range of techniques (Lancaster & Humphreys, 2020), and there is evidence that thermal tolerance plasticity is consistent across different growing environments (Zhu *et al.*, 2018). Much like determining that extreme events have greater impact on selection pressure and population persistence than average warming (Buckley & Huey, 2016), it will be critical to determine the relative importance of the sensitivity and variability of thermal tolerance responses in dynamic environments.

Conclusions

A comprehensive understanding of the thermal tolerance of land plants is crucial. Our rapidly changing climate demands that we pay increased attention to the importance of thermal tolerance for agricultural production and efficiency, ecosystem services, and persistence of wild species. Our systematic review documents geographic and temporal distributions of research efforts and methodological approaches in plant thermal tolerance to date. It shows that there are substantial gaps in our knowledge, and we argue that these are hindering new insights into plant thermal tolerance. The lack of standardised research methods, limited transdisciplinary communication, ambiguous use of terminology and metrics, and unrepresentative global coverage are methodological issues that can be addressed. Conceptual advances will arise from a focus on understanding how thermal tolerance varies in ecological and evolutionary strategy space, studying the importance of thermal breadth, and delimiting mechanisms that underlie acclimation potential and thus the ability to induce protection *vs* accumulate damage. Finally, we crucially need more insight into how thermal tolerance interacts with and its relative importance in

comparison to other abiotic factors such as drought. To these ends, we have identified key design elements for effective thermal tolerance research and outlined an agenda to instigate both fundamental advances and applied outcomes.

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Author contributions

SRG, PAA, and ABN led the systematic review, data curation and analyses, and led manuscript writing with substantial input on drafts from all authors. All authors contributed significantly to the immense effort that was screening and evaluating articles in the systematic review.

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Figure Legends

Fig. 1. (a) Thermal tolerance techniques are presented in order of appearance within the literature for cultivated (left) and wild systems (right). (b) The uptake of techniques since the 1960s; a given article may use multiple techniques (studies) represented exceeds the total articles identified in the systematic review. Numbers to the right of each plotted line refer to the numbered techniques described in (c). (c) Definitions for each of the 10 techniques within the scope of this review. Techniques displayed with an adjacent circle indicate the capacity for a thermal metric to be generated. Additional information on the techniques and references are provided in Supplementary Notes S1.

Fig. 2. Global distribution of plant thermal tolerance research. The choropleth map is coloured by the number of articles in the country of the first author's affiliation. Total articles on (a) cultivated and (b) wild species; cold tolerance studies on (c) cultivated and (d) wild species; studies on cold and heat tolerance together (termed both) on (e) cultivated and (f) wild species; heat tolerance studies on (g) cultivated and (h) wild species. The number of studies varies considerably, hence each panel has a different scale for the colour gradient scale bars. The colour gradients are log-transformed. Regional maps of articles from USA, China, Europe, and wild studies by experiment location instead of author location are presented in Figs S2–S5.

Fig. 3. The number of studies of thermal tolerance measures on (a) cultivated species across types of cultivation and (b) wild species across different biomes that focus on either cold tolerance, heat tolerance, or both heat and cold tolerance. Inset figures highlight the relative uptake of heat, cold, or both heat and cold tolerance approaches through time for articles on (c) cultivated and (d) wild species.

Fig. 4. The proportion (and numbers) of intraspecific, single species or multiple species studies on (a) cultivated and (b) wild species. The variation in life form of the focal study organisms (forb/herb, graminoid, shrub, tree, vine, or multiple forms (for studies on multiple species)) for studies on (c) cultivated and (d) wild species.

Fig. 5. (a) The maximum duration of the thermal stress imposed and (b) the type of stress (ramp, shock, not specified), expressed proportionally within each assay technique for cultivated and wild systems. Maximum durations listed in order from the longest duration on the left to shortest on the right: months, weeks, days, hours, minutes, or unspecified. For (b) we defined ramp as a rate of

change in temperature less than 1^{o}C per minute and shock as a rate of change exceeding 1^{o}C per minute. Numbers of studies are shown to the right of the proportion bars.

Table

Table 1. Summary of the number of studies (and percentage of articles in parentheses) for thermal tolerance research on cultivated species of each type of cultivation and for wild species of each biome category investigating cold, heat, or both heat and cold tolerance.

Cultivated – type of cultivation	**Cold**		**Heat**		**Heat and cold**		**Total**
Arabidopsis	201	(61.5)	106	(30.8)	21	(7.7)	328
Cereals	339	(49.6)	388	(47.3)	22	(3.4)	749
Fibre	36	(39.4)	43	(54.5)	2	(6.1)	81
Horticulture and vegetables	523	(60.4)	334	(32.4)	61	(7.1)	918
Legumes	117	(51.3)	117	(38.3)	24	(10.4)	258
Pasture and turf grasses	71	(46.1)	111	(48.3)	9	(5.6)	191
Plantation forestry	71	(66.2)	44	(25.0)	14	(8.8)	129
Viticulture	45	(63.8)	38	(27.7)	5	(8.5)	88
Other crops	146	(64.3)	70	(29.7)	16	(4.0)	232
Multiple	33	(61.3)	19	(29.0)	7	(9.7)	59
Not specified	0	(0.0)	3	(100.0)	0	(0.0)	3
Cultivated – subtotal	**1,582**	**(56.7)**	**1,273**	**(37.7)**	**181**	**(5.6)**	**3,036**
Wild – biome							
Alpine/Arctic tundra/Subalpine	79	(74.5)	29	(21.3)	4	(4.2)	112
Arid/Semi-arid/Savannah	27	(45.0)	20	(55.0)	0	(0.0)	47
Boreal forest	45	(100.0)	0	(0.0)	0	(0.0)	45
Mediterranean	29	(52.0)	17	(40.0)	5	(8.0)	51
Temperate	179	(76.2)	54	(21.3)	5	(2.5)	238
Tropical/Subtropical	32	(61.8)	26	(23.5)	16	(14.7)	74
Multiple	65	(64.7)	39	(29.4)	9	(7.9)	113
Not specified	12	(42.9)	15	(57.1)	0	(0.0)	27
Wild – subtotal	**468**	**(69.7)**	**200**	**(25.9)**	**39**	**(4.4)**	**707**
All species total	**2,050**	**(59.3)**	**1,473**	**(35.3)**	**220**	**(5.4)**	**3,743**

Note that multiple individual uses of thermal tolerance techniques (studies) can occur in a single article; therefore, we reported both the number of studies along with percentages of articles in parentheses for each subcategory (row).

Box

Box 1. Key considerations and recommendations for future research.

A. ***Methodological and design considerations:***

1. **Application of techniques:** Greater crosstalk among researchers studying thermal tolerance of cultivated and wild species would be mutually beneficial to compare and apply different techniques and develop high-throughput approaches.
2. **Experimental design considerations:** Careful consideration when designing thermal tolerance research, particularly on how temperature stress is applied, thermal legacy effects, and interactions with other environmental factors.
3. **Development of standard approaches and comparable metrics:** Test comparability of methods and metrics and use multidisciplinary approaches to generate stronger insights into both mechanisms and patterns of thermal tolerance.

B. ***Research priority agenda:***

1. **The comparative ecology of thermal tolerance in the ecological and evolutionary strategy spaces:** Trait-based approaches in plant ecology should be linked to thermal tolerance to scale-up to higher-level ecosystem processes. Broad-scale comparative studies across a wider range of growth forms, biomes, and that can account for methodological differences will generate greater understanding of biogeographic patterns of tolerance.
2. **Understanding the geography and drivers of thermal tolerance breadth:** Prioritise measuring thermal tolerance breadth, both heat and cold tolerance, particularly in wild species in thermally extreme regions or regions where snowmelt dynamics are changing, and crop species in regions where climate vulnerability is high.
3. **Influences of other factors on thermal tolerance and the potential for shared mechanistic and evolutionary underpinnings:** Multi-factorial experiments are key

to identifying molecular and metabolic responses and for determining which are distinct to temperature stress or common to other sources of stress.

4. **Understanding the sensing of and response to thermal stress along the continuum from protective mechanisms to acquired damage:** Conduct detailed investigations into the time-sensitive aspects of recovery and damage dynamics, the role of plasticity, and effects of various thermal stresses, including means, extremes, variability, and microhabitats, on plant thermal tolerance.

Supporting Information

Fig. S1 PRISMA (Preferred Reporting Items in Systematic Reviews and Meta-Analyses) diagram illustrating the number of articles identified through database searching, title and abstract screening, and full-text searching.

Fig. S2 Choropleth map of the distribution of plant thermal tolerance research within the People's Republic of China.

Fig. S3 Choropleth map of the distribution of plant thermal tolerance research within the United States of America.

Fig. S4 Choropleth map of the distribution of plant thermal tolerance research within Europe.

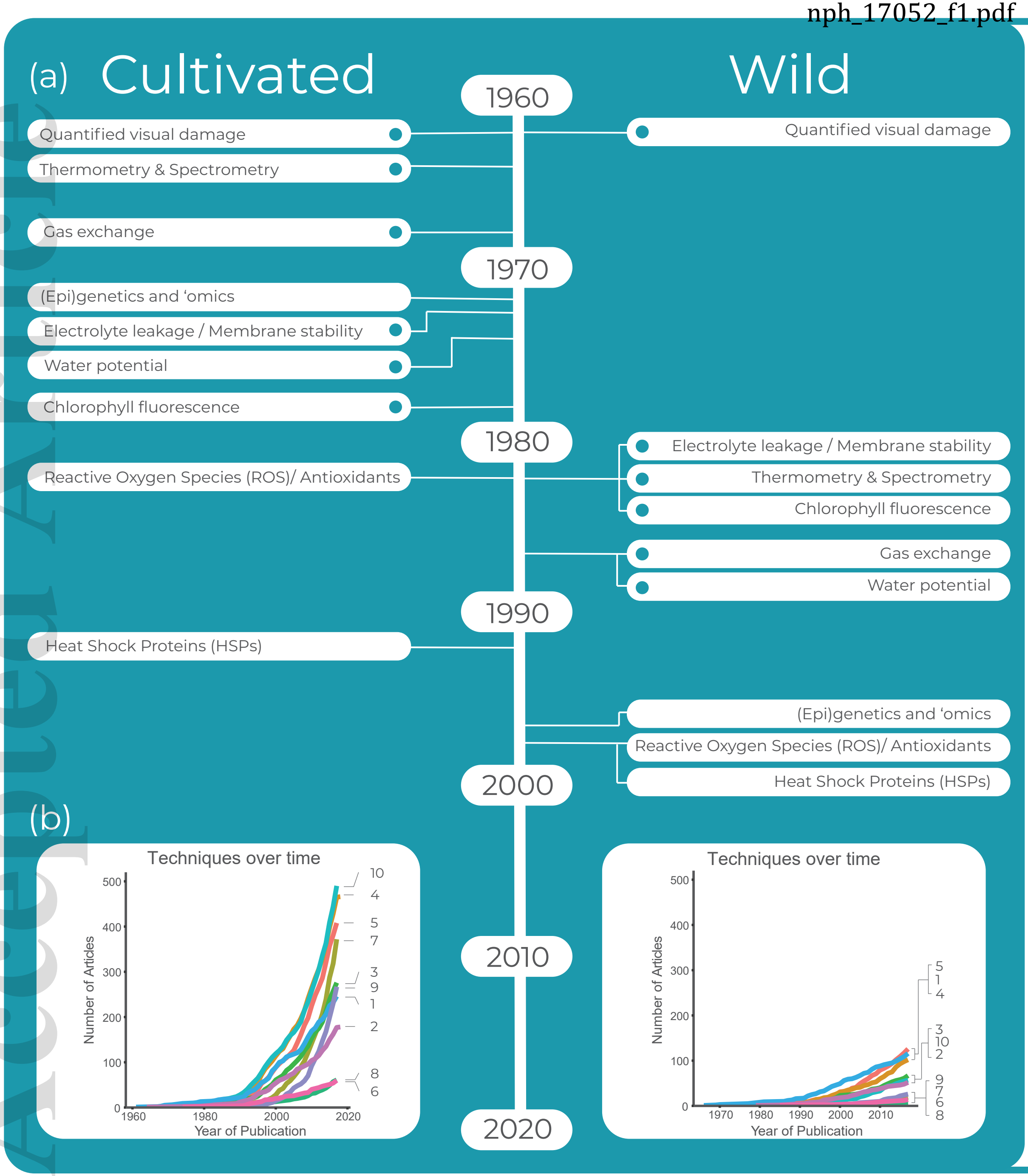
Fig. S5 Choropleth map of the global distribution of plant thermal tolerance research on wild plants coloured by where the experiment was conducted, rather than the country of origin of the first author's affiliation.

Fig. S6 Number of times a thermal tolerance technique was used within types of cultivation within cultivated systems and biomes within wild systems.

Fig. S7 Proportion (and number) of studies for cultivated and wild systems that employed a ramp or shock approach to initiating thermal stress, when considering cold tolerance, heat tolerance or both cold and heat tolerance.

Fig. S8 Topic mapping of thermal tolerance articles using title and author keywords.

Notes S1. Systematic review methods, options and justifications for reviewer screening of articles, and extended version of Fig. 1 glossary of common tools and techniques for measuring thermal tolerance in land plants.



(a) Cultivated

1960

Quantified visual damage

Thermometry & Spectrometry

Gas exchange

1970

(Epi)genetics and ‘omics

Electrolyte leakage / Membrane stability

Water potential

Chlorophyll fluorescence

1980

Reactive Oxygen Species (ROS)/ Antioxidants

1990

Heat Shock Proteins (HSPs)

2000

2010

2020

Wild

Quantified visual damage

Electrolyte leakage / Membrane stability

Thermometry & Spectrometry

Chlorophyll fluorescence

Gas exchange

Water potential

(Epi)genetics and ‘omics

Reactive Oxygen Species (ROS)/ Antioxidants

Heat Shock Proteins (HSPs)

(b)

(c) Techniques

1. Quantified visual damage ○

Measures the percentage of damaged (discolored/brown) leaves or leaf area (e.g. proportion of cell death; leaf area).

2. Thermometry & Spectrometry ○

Used to identify temperature-induced changes in plant tissue and can indicate functional parameters (e.g. leaf ice nucleation point and its progression through the plant and indices of photochemical health).

3. Gas exchange ○

Determines the ability of a leaf to recover photosynthethic capacity, or change its rate of respiration, after exposure to stressful temperature through examination of the time stability of rate of CO_2 uptake or O_2 evolution.

4. Electrolyte leakage / Membrane stability ○

Measures electrical conductivity to determine cell membrane damage/ leakiness in response to stress.

5. Chlorophyll fluorescence ○

Refers to light re-emitted from chlorophyll and provides a sensitive indicator of temperature stress.

6. Water potential ○

Quantifies the potential for water to move between one area of a plant to another through osmosis, gravity, mechanical pressure or matrix effects such as capillary action.

7. (Epi)genetics and ‘omics

Broadly, ‘omics refers to the fields of molecular biology that are specifically associated with whole-genome detection of: genes (genomics), gene expression (transcriptomics), proteins (proteomics), and metabolites (metabolomics). Epigenomics specifically refers to the molecular mechanisms that alter gene expression and function without changes in DNA sequence (e.g. through chemical modification of DNA (methylation) and histones, incorporation of histone variants and long or small non-coding RNAs).

8. Heat Shock Proteins (HSPs)

Rapidly induced in response to abiotic stresses and alleviates damage. HSPs function as molecular chaperones, assist in protein folding, maintain signal transduction and prevent protein aggregation.

9. Reactive Oxygen Species (ROS)/ Antioxidants

ROS are oxygen radicals and non-radical oxidizing agents that can be converted into radicals. They are by-products of a plant’s metabolic processes which can impact upon a plant’s growth, signalling, development, cell cycle, programmed cell death, abiotic stress responses and pathogen defence and can increase rapidly in response to temperature stress. Antioxidants mitigate the cellular damage that ROS cause.

10. Other biochemistry

Techniques can be used to assess thermal tolerance by the presence or absence of certain biochemicals.

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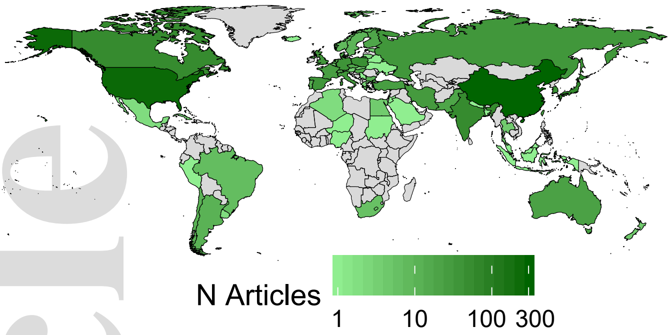
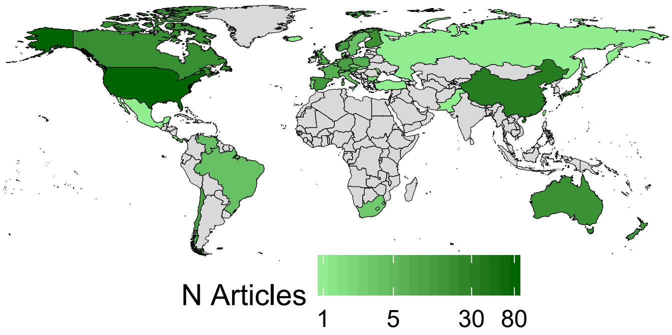
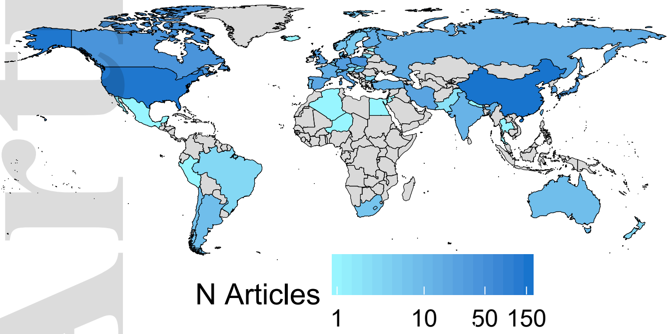
(a) Cultivated: total



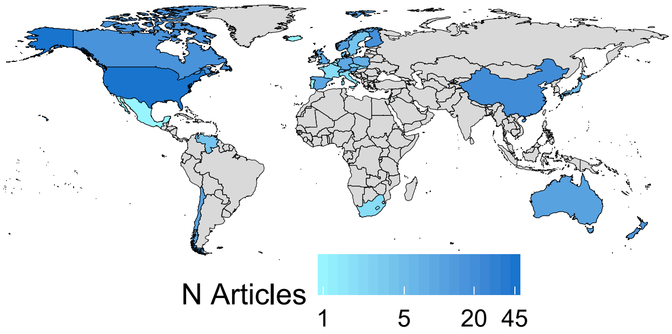
(b) Wild: total



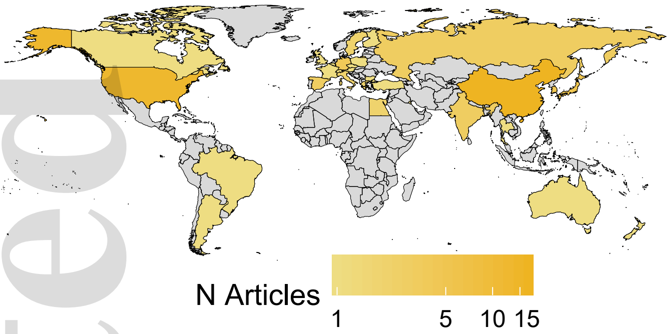
(c) Cultivated: cold



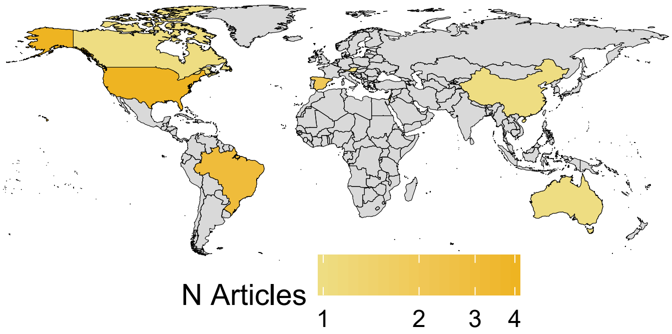
(d) Wild: cold



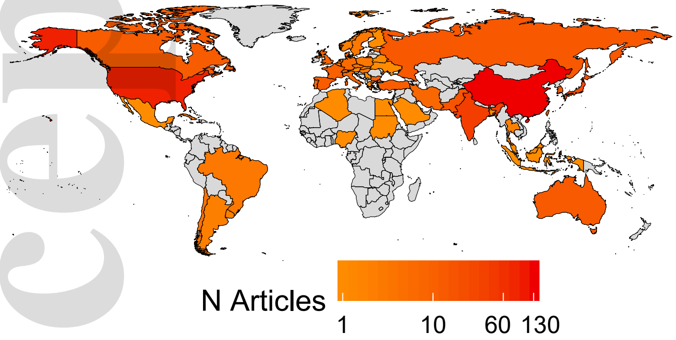
(e) Cultivated: both



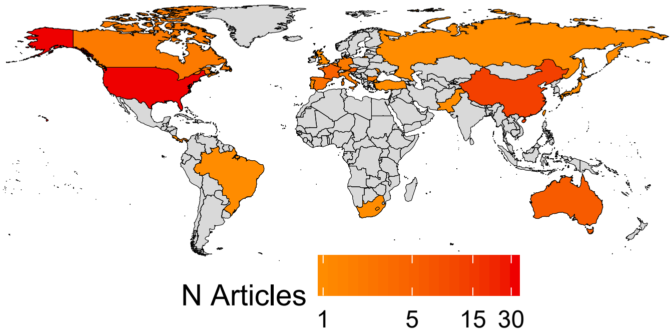
(f) Wild: both

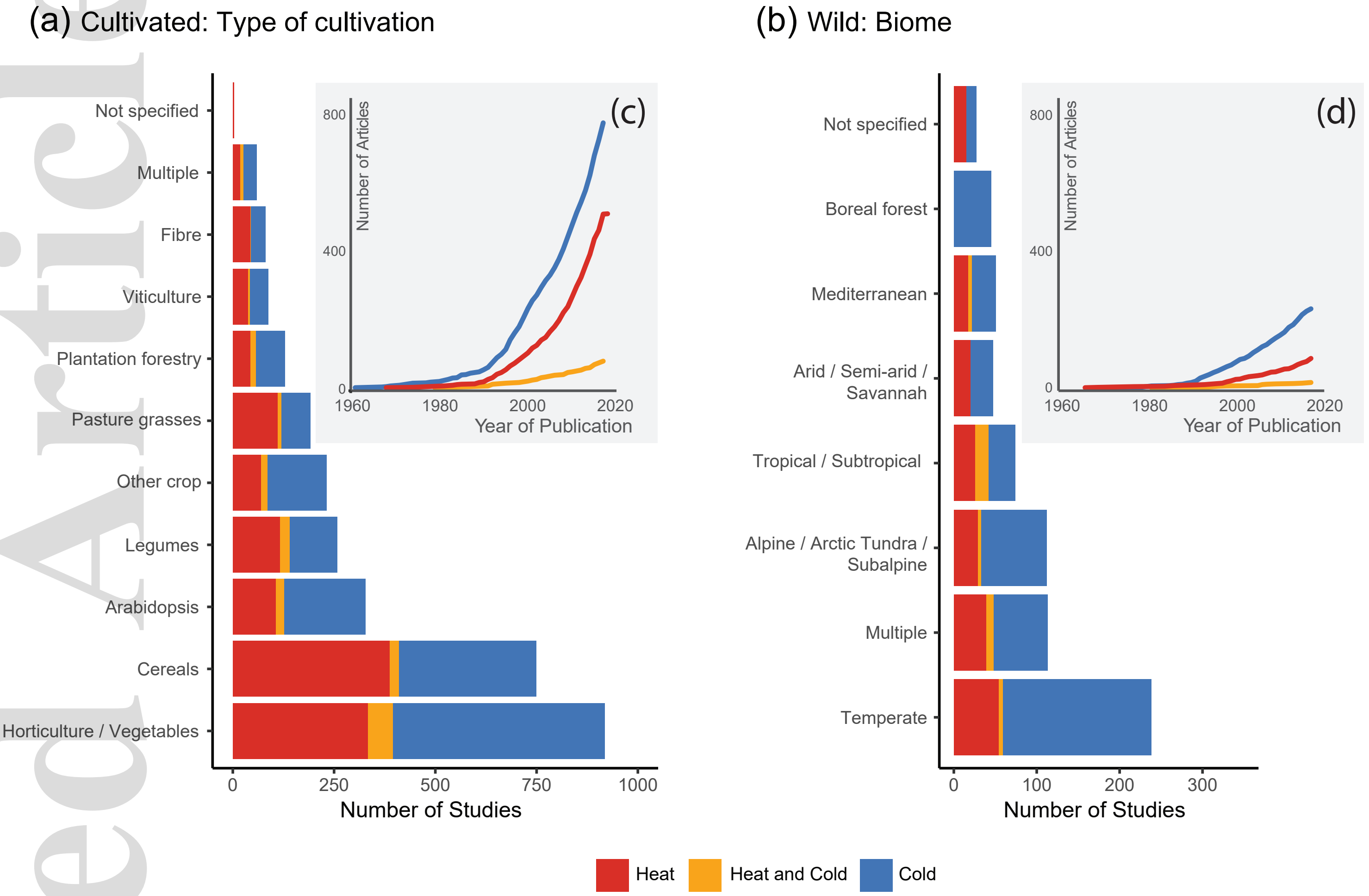


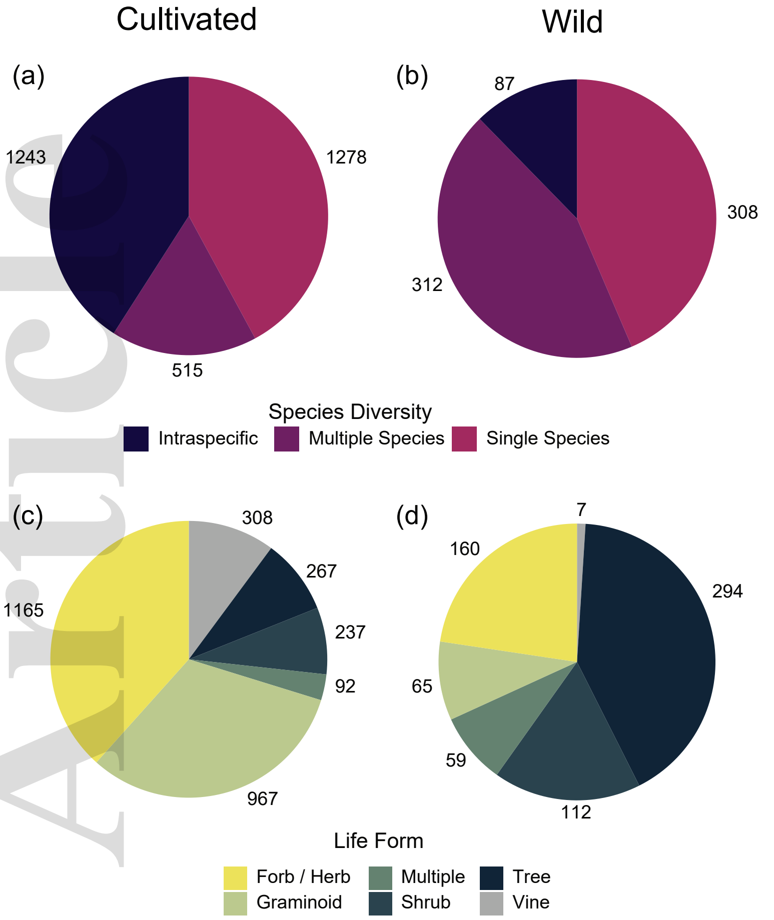
(g) Cultivated: heat

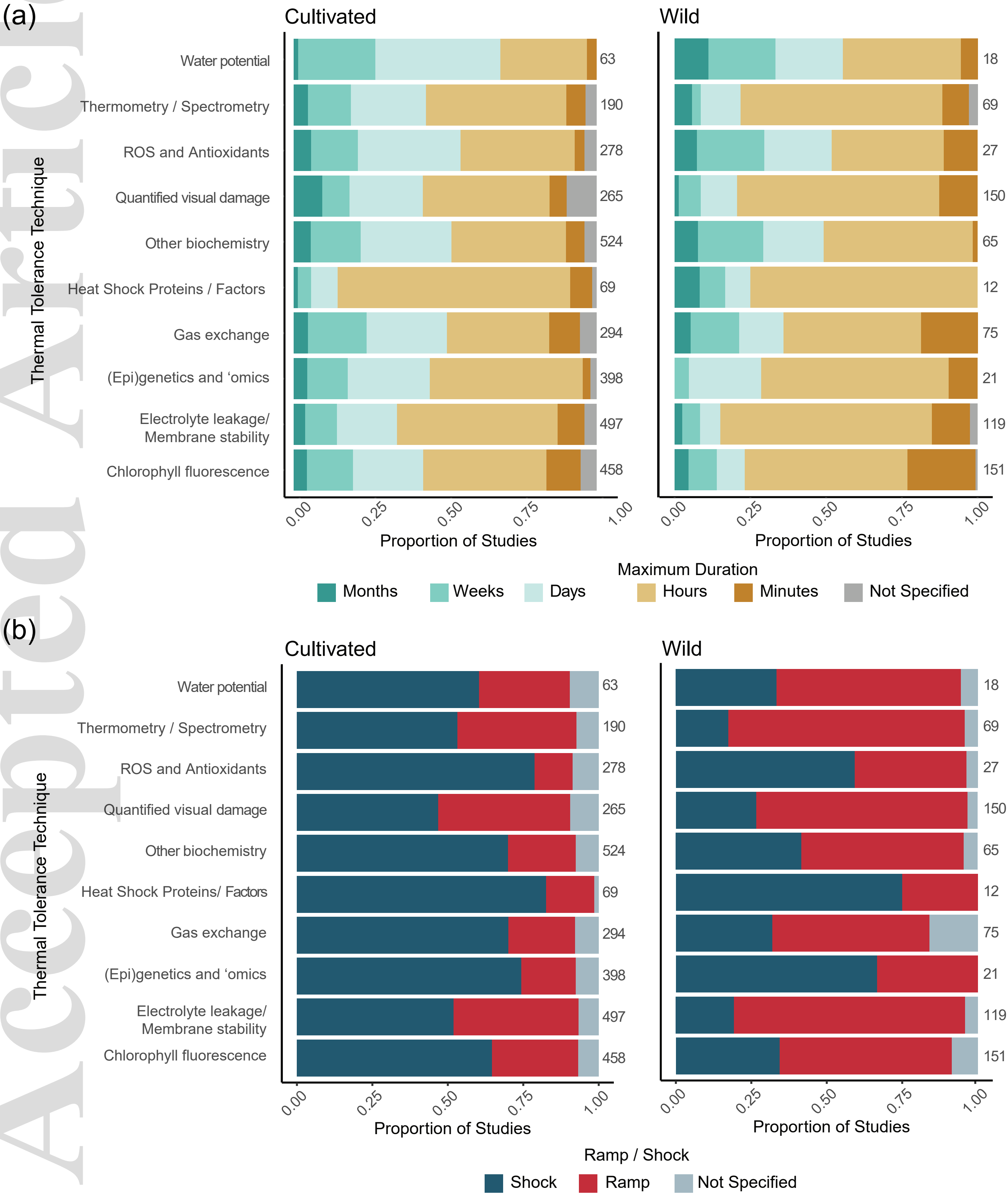


(h) Wild: heat




(a) Cultivated: Type of cultivation
Not specified
Multiple
Fibre
Viticulture
Plantation forestry
Pasture grasses
Other crop
Legumes
Arabidopsis
Cereals
Horticulture / Vegetables
0
250
500
750
1000
Number of Studies
(c)
Number of Articles
800
400
0
1960
1980
2000
2020
Year of Publication
(b) Wild: Biome
Not specified
Boreal forest
Mediterranean
Arid / Semi-arid / Savannah
Tropical / Subtropical
Alpine / Arctic Tundra / Subalpine
Multiple
Temperate
0
100
200
300
Number of Studies
(d)
Number of Articles
800
400
0
1960
1980
2000
2020
Year of Publication
Heat
Heat and Cold
Cold


Cultivated
Wild
(a)
1243
1278
515
(b)
87
308
312
Species Diversity
Intraspecific
Multiple Species
Single Species
(c)
308
267
1165
237
92
967
(d)
7
160
294
65
59
112
Life Form
Forb / Herb
Graminoid
Multiple
Shrub
Tree
Vine


(a)
Cultivated
Wild
Thermal Tolerance Technique
Water potential
Thermometry / Spectrometry
ROS and Antioxidants
Quantified visual damage
Other biochemistry
Heat Shock Proteins / Factors
Gas exchange
(Epi)genetics and ‘omics
Electrolyte leakage/ Membrane stability
Chlorophyll fluorescence
63
190
278
265
524
69
294
398
497
458
18
69
27
150
65
12
75
21
119
151
0.00
0.25
0.50
0.75
1.00
Proportion of Studies
Maximum Duration
Months
Weeks
Days
Hours
Minutes
Not Specified
(b)
Cultivated
Wild
Thermal Tolerance Technique
Water potential
Thermometry / Spectrometry
ROS and Antioxidants
Quantified visual damage
Other biochemistry
Heat Shock Proteins/ Factors
Gas exchange
(Epi)genetics and ‘omics
Electrolyte leakage/ Membrane stability
Chlorophyll fluorescence
63
190
278
265
524
69
294
398
497
458
18
69
27
150
65
12
75
21
119
151
0.00
0.25
0.50
0.75
1.00
Proportion of Studies
Ramp / Shock
Shock
Ramp
Not Specified