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Ramla Khan

ramla.khan@ndorms.ox.ac.uk

The Open University

Philip Wheeler

The Open University

David Gowing

The Open University

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Thermal tolerance of *Acer campestre* (field maple) under heat and drought stress derived from chlorophyll fluorescence

Ramla Khan[*1,2], Philip Wheeler[1], David Gowing[1]
[1] School of Earth, Environment and Ecosystem Sciences, The Open University, Milton Keynes, UK
[2] Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK

[*]ramla.khan@ndorms.ox.ac.uk

Abstract

Climate change is intensifying extreme heat events, making the thermal tolerance of street trees increasingly crucial for sustainable urban forestry. In this study, we quantified the physiological responses of *Acer campestre* (field maple) to heat stress under varying hydration conditions by measuring chlorophyll fluorescence parameters. We assessed critical temperature thresholds (T_{crit}) and temperature at 50% reduction in photosystem II efficiency (T_{50}) through four experimental trials, with the final trial incorporating drought stress. Our results demonstrated significant hydration-dependent plasticity in thermal tolerance thresholds. Well-watered specimens exhibited T_{crit} values of 37.0–44.0 °C, which declined sharply to 29.0 °C under drought conditions. Following 24-hour rehydration, T_{crit} significantly increased across all trials ($t = 7.63$, $df = 31$, $p < 0.01$), with the most pronounced recovery in drought-stressed specimens from 29.0 °C to 42.0 °C with a mean difference of 8.81 °C (95% CI: 6.46–11.17 °C). In contrast, T_{50} values remained relatively stable (47.0–50.0°C) with only modest post-hydration of mean difference 1.19 °C ($t = 2.83$, $df = 31$, $p < 0.01$, 95% CI: 0.33–2.04 °C). Principal component analysis revealed that hydration status explained 80.41% of variation in thermal responses, with K-means clustering (silhouette score = 0.45) separating samples into distinct hydrated and non-hydrated physiological groups. PERMANOVA confirmed significant treatment effects on multivariate thermal profiles ($F = 5.47$, $R^2 = 0.41$, $p = 0.001$). The significant correlation between T_{crit} and T_{50} ($r = 0.61$, $p < 0.01$, 95% CI: 0.43–0.74 °C)

suggested coordinated protection across different levels of thermal stress. These findings demonstrate that immediate hydration status, rather than prior drought conditioning, predominantly determines thermal resilience in *Acer campestre*, with critical implications for urban tree management under increasingly frequent heat extremes.

Introduction

Urban environments increasingly experience excessive temperatures due to climate change and the urban heat island effect, placing urban vegetation under unprecedented thermal stress. (IPCC, 2021, 2023; Kendon et al., 2022, 2022). As extreme heat events become more frequent and intense, the physiological limits of urban trees are tested, threatening their survival and capacity to provide ecosystem services (Coutts et al., 2015; Grilo et al., 2025). While urban trees can significantly mitigate local temperatures through shading and evapotranspiration (Meier & Scherer, 2012), their ability depends critically on maintaining their physiological function during heat stress.

Chlorophyll fluorescence offers a sensitive, non-invasive approach for quantifying plant responses to thermal stress by measuring photosystem II efficiency, which typically experiences damage before other physiological systems (Murchie & Lawson, 2013). This approach yields two particularly valuable thermal tolerance metrics: critical temperature (T_{crit}), indicating the onset of photosynthetic decline (Tiwari et al., 2021), and T_{50}, the temperature at which photosynthetic function is reduced by half (Perez & Feeley, 2020).

Previous research has documented considerable variation in thermal tolerance among tree species, with T_{50} generally between 45-54°C and T_{crit} between 41-51°C (Leon-Garcia & Lasso, 2019; O'sullivan et al., 2017). These studies have identified interspecific differences in thermal tolerance, often linked to species' native growing conditions and physiological adaptations. Studies suggest that thermal tolerance is not fixed but dynamic, responding to both genetic

factors and environmental conditions. For instance, studies in agricultural and forest systems have documented significant variation in thermal tolerance within species (Posch et al., 2022) and in response to environmental gradients such as elevation (Feeley et al., 2020). Researchers have also begun to explore potential relationships between drought tolerance and heat tolerance, suggesting complex interactions between water status and thermal resilience (Kunert et al., 2022).

Many existing studies assess thermal tolerance under standardized, optimal conditions, often using well-watered plants and fixed environments that do not fully capture the dynamic and co-occurring stressors typical of urban settings. This can lead to an overestimation of thermal resilience, particularly where drought and heat stress interact. Our study addresses this gap by explicitly manipulating hydration status under controlled conditions to reflect more realistic urban tree scenarios.

The relationship between hydration status and thermal tolerance represents a critical yet underexplored dimension of plant heat resilience. Unlike controlled experimental conditions, urban trees frequently experience varying degrees of water stress that may significantly alter their effective thermal thresholds. Recent investigations suggest that immediate hydration status may be a primary determinant of thermal tolerance, potentially even surpassing species-specific adaptations or drought acclimation in importance (Münchinger et al., 2023). This hypothesis remains inadequately tested, particularly for common urban tree species under field-relevant conditions.

This knowledge gap has significant implications for urban forestry practice. Current approaches to species selection and management often rely on thermal tolerance values derived under optimal conditions, potentially misrepresenting performance during real-world heat events where water stress frequently co-occurs. Understanding how hydration status modulates

thermal thresholds would enable more accurate vulnerability assessments and targeted irrigation interventions during extreme heat.

Our study addresses this critical gap by quantifying the relationship between hydration status and thermal tolerance in *Acer campestre* (field maple), a widely planted urban tree in the UK and Europe. Through controlled experimental manipulations of both water availability and temperature exposure, we examine how chlorophyll fluorescence parameters respond to heat stress under varying hydration conditions. Unlike previous studies that typically assess thermal tolerance at a single point in time or hydration state, our experimental design specifically isolates the effects of immediate hydration status versus prior drought conditioning, allowing us to disaggregate their relative contributions to thermal resilience.

Using multivariate statistical techniques, including principal component analysis and clustering, we identify the primary drivers of variation in thermal response patterns. This approach reveals whether thermal tolerance in Acer campestre is primarily determined by fixed, species-level characteristics or is dynamically regulated by hydration status, a distinction with important implications for urban tree management under climate change.

Specifically, our research aims to quantify the range and plasticity of thermal tolerance thresholds in *Acer campestre* under varying hydration conditions; determine the relative importance of immediate hydration status versus prior drought exposure in modulating these thresholds; characterize the relationship between different metrics of thermal tolerance (T_{crit} and T_{50}); and develop practical implications for urban tree management during heat extremes.

Methods

Four independent experimental trials/tests were conducted on eight field maple trees growing in containers/pots (Figure 1a) on the Open University grounds in Milton Keynes, UK, using the OSp5+ chlorophyll modulated fluorometer (ADC, Hoddesdon, UK) (Opti-Sciences, 2013).

These trees were growing in a mesocosm system to keep hydrated automatically (Figure 1b). The first three trials were conducted under normal weather conditions in July of 2023, with a ten-day gap between them, but in the final trial, drought stress was imposed by withholding water to simulate extreme drought conditions, and the measurements were taken in late August.

Figure 1: a) Trees growing inside pots/containers, b) Mesocosm system inside the pots/containers

Tree species of study

Acer campestre is a common urban tree species in the United Kingdom (Treezilla, 2024). It is a deciduous tree native to the British Isles and widespread across Europe. Typically reaching 12–15 m in height (up to 25 m in some cases), it is common in hedgerows, woodland edges, and parklands. The species has light grey, flaking bark and a rounded, low-branching crown. Its foliage emerges bright green in spring, turning golden to red in autumn. Small yellow-green flowers appear in late April, followed by twin-winged samaras that disperse by wind in October. Germination occurs after 18 months of dormancy, with peak growth between ages 5 and 25 (Zecchin et al., 2016).

Controlled experiments for thermal tolerance

Small branches were collected from the trees and brought to the lab. Part of the stem was cut under water to trap the transition bubble. Leaf discs (2 cm in diameter) from these branches were extracted using a cork borer to ensure uniform sampling. To maintain aerobic conditions

and prevent anaerobiosis during heating, the leaf discs were enclosed within a tea cloth, with one layer placed on the adaxial side and another on the abaxial side. The wrapped discs were then placed in a sealed plastic bag and fully submerged in a water bath, secured with weights to ensure complete immersion while preventing moisture contamination.

The maximum quantum yield of photosystem II (F_v/F_m) was initially measured at 20 °C to establish baseline values and verify the physiological integrity of the sampled leaves. The leaf discs were then subjected to controlled heat treatments in a darkened water bath for fifteen minutes. Treatment temperatures ranged from 37 °C to 60 °C in 4 °C increments.

Following heat treatments, the leaf discs were retrieved using protective gloves to prevent physical damage and transferred onto petri dishes lined with moistened paper towels soaked in distilled water. The samples were subsequently acclimated in a light-free environment under a dark cloth for fifteen minutes before measuring F_v/F_m using an OS5p+ fluorometer (Opti-Sciences, 2013). After the initial fluorescence measurements, the leaf discs were stored in petri dishes with closed lids under laboratory conditions (Figure 2) to undergo a 24-hour recovery phase. Post-recovery F_v/F_m measurements were recorded to evaluate the extent of recovery or degradation in photosynthetic efficiency.

Figure 2: Samples left overnight in the lab, with the numbers divided into one horizontal and one vertical, representing the eight trees and the value of temperature treatment at the top

Estimating the thermal thresholds

To estimate T_{crit} and T_{50}, a logistic nonlinear least squares model was used to characterise the relationship between F_v/F_m and treatment temperature for each sample (Kunert et al., 2022; Münchinger et al., 2023; Tiwari et al., 2021). The modelling was implemented using the 'nls' function within the R 'stats' package (Bolar, 2022). T_{crit} was defined as the temperature at which a noticeable decline in Fv/Fm began, identified by detecting the point where the slope of the Fv/Fm–temperature curve reached 15% of its steepest (most negative) value (Tiwari et al., 2021). T_{50} was computed was calculated using Equation 1 as the temperature at which Fv/Fm dropped to 50% of the average value observed in the control (non-stressed) treatment (Perez & Feeley, 2020).

$$\frac{F_v}{F_m} = c + \frac{d - c}{1 + Exp[b \log(\frac{T}{T_{50}})]} \quad \text{(eq.1)}$$

In eq.1, T denotes temperature, while *c* represents the F_v/F_m value at the lower plateau, and d corresponds to the higher plateau. T_{50} refers to the temperature at which F_v/F_m declines to 50% of the total reduction, and b signifies the slope of the curve at $T = T_{50}$.

Principal component and cluster analysis

To explore multivariate patterns in physiological responses across treatments and time points, a Principal Component Analysis (PCA) was conducted on scaled data using the 'prcomp()' function in R from the base package 'stats' (Bolar, 2022). This dimensionality reduction technique was used to identify the primary axes of variation.

Unsupervised k-means clustering was applied to identify natural groupings in the data using the 'stats' package (Bolar, 2022). The optimal number of clusters (K) was determined using the silhouette method from the 'cluster' package (Mächler et al., 2012). To statistically test

158 treatment and cluster differences in multivariate space, Permutational Multivariate Analysis of
159 Variance (PERMANOVA) was conducted using the 'adonis2()' function in the 'Vegan'
160 package (Oksanen et al., 2025). The distance matrix was calculated using Euclidean distances
161 on scaled variables.

162 Results

163 Thermal threshold values

164 In our study, the T_{crit} and T_{50} values varied significantly across trials and hydration treatments,
165 reflecting dynamic changes in thermal tolerance (Table 1; Figures 3 and 4).

166 *Table 1: T_{crit} and T_{50} values across four experimental trials, with measurements taken initially and after a 24-hour hydration*
167 *period. Values are shown with 95% confidence intervals.*

Trial Date	**Treatment**	T_{crit} **(°C) [95% CI]**	T_{50} **(°C) [95% CI]**
03/07/2023	Initial	37 [37.9–44.0]	50 [50–52]
	After 24 hours	42 [41–45]	50 [48–49]
13/07/2023	Initial	37 [34–39]	48 [47–49]
	After 24 hours	43 [41–45]	48 [48–49]
23/07/2023	Initial	36 [29–41]	48 [45–49]
	After 24 hours	43 [41–45]	48 [48–49]
28/08/2023	Initial (drought)	29 [24–33]	48 [46–49]
	After 24 hours	42 [38–45]	50 [49–52]

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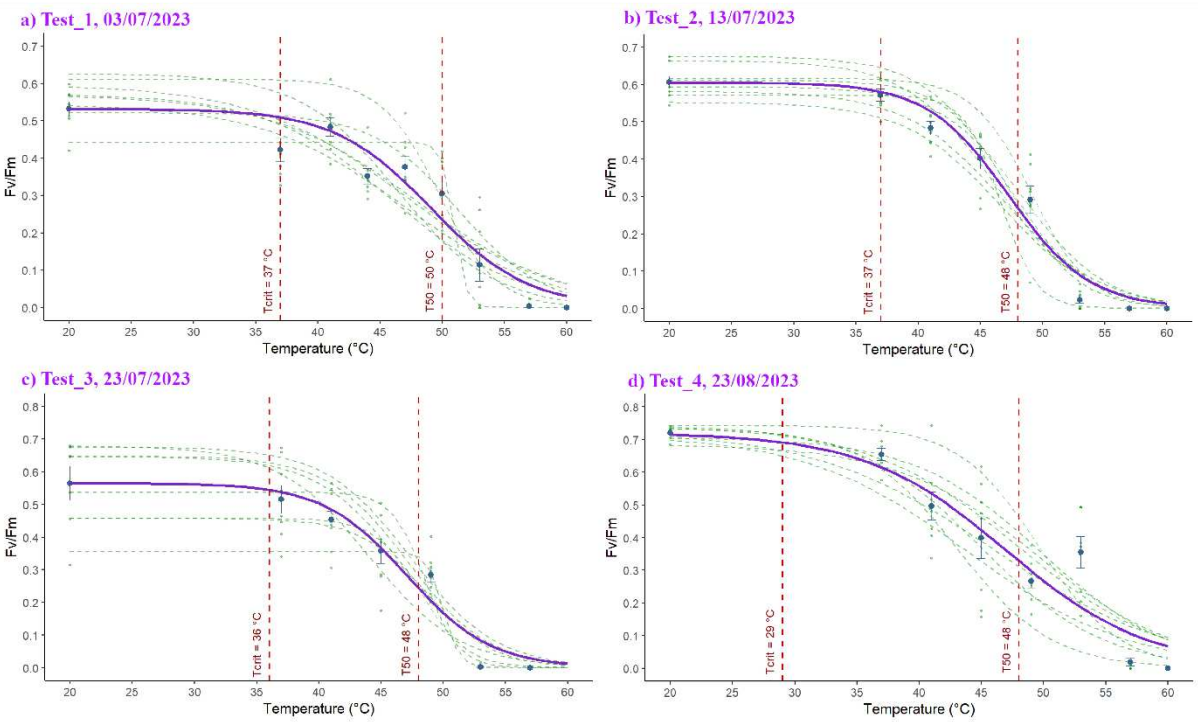


Figure 3:Temperature response curves of maximum quantum yield (Fv/Fm) for Acer campestre across four experimental trials. The solid purple line represents the mean response curve across all replicates, while the green dotted lines show individual tree replicates (n=8). Vertical red dashed lines indicate the critical temperature thresholds.

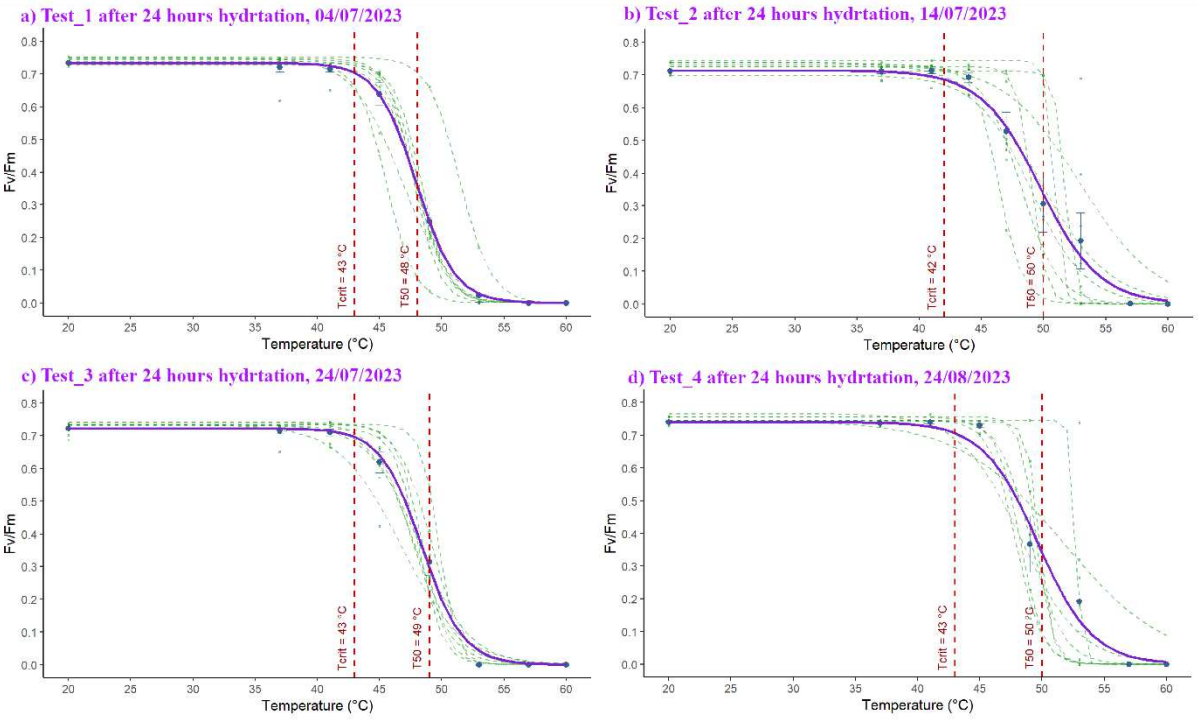


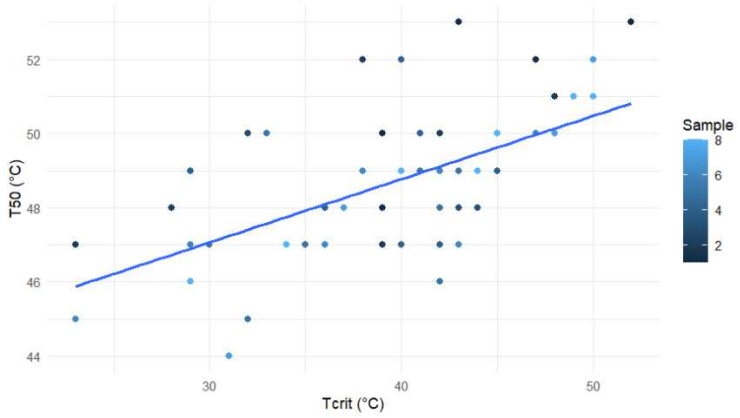
Figure 4: Temperature response curves of maximum quantum yield (Fv/Fm) for Acer campestre after 24 hours of hydration across four experimental trials. The solid purple line represents the mean response curve across all replicates, while the green dotted lines show individual tree replicates (n=8). Vertical red dashed lines indicate the critical temperature thresholds.

T_{crit} declined throughout the experiment, ranging from 37.0–44.0 °C in the well-watered Trial 1 to as low as 29 °C under extreme drought conditions in Trial 4. This sharp decrease highlighted the sensitivity of initial damage thresholds to sustained water stress. In contrast, T_{50} values that represent the temperature at which 50% of maximum photochemical efficiency is lost remained more stable, typically ranging between 47.0–49.0 °C, with the highest values (50.0–52.0 °C) observed in Trials 1 and 4.

The 24-hour hydration treatment significantly improved thermal tolerance across all trials. In every case, T_{crit} increased following the rehydration, with the most striking change observed in Trial 4, where T_{crit} rose from 29.0 °C to 42.0 °C. This recovery was statistically significant ($t = 7.63$, $df = 31$, $p < 0.01$), with a mean difference of 8.81 °C (95% CI: 6.46–11.17 °C). T_{50} also increased modestly post-hydration, with a mean difference of 1.19 °C ($t = 2.83$, $df = 31$, $p < 0.01$; 95% CI: 0.33–2.04 °C). These findings indicate that while both thermal traits responded to water availability, T_{crit} was more sensitive to short-term hydration changes, making it a valuable early-warning indicator of thermal vulnerability.

Correlation between T_{crit} and T_{50}

A Pearson correlation analysis revealed a significant positive association between T_{crit} and T_{50} ($r = 0.61$, $t = 6.03$, $df = 62$, $p < 0.01$; 95% CI: 0.43–0.74 °C; Figure 5). This relationship suggested that individuals with higher thresholds for the onset of thermal damage (T_{crit}) also tend to maintain function at higher peak stress levels (T_{50}). The distinct response magnitudes underscore that these metrics capture complementary aspects of thermal stress physiology.



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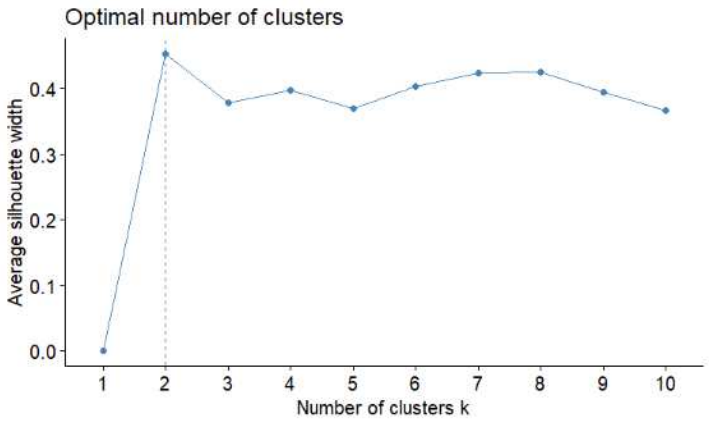
199 *Figure 5: Pearson correlation between T_{50} and T_{crit} values. The samples in the legend represent the eight replicates across*
200 *each temperature treatment.*

201 ## Principal component and cluster analysis

202 To explore underlying patterns in thermal tolerance, a Principal Component Analysis (PCA)
203 was performed on T_{crit} and T_{50} values. The first principal component (PC1) explained 80.41%
204 of the total variance, primarily capturing differences in T_{crit}, while PC2 accounted for 19.59%
205 (Figure 6a). K-means clustering, validated by silhouette analysis (optimal K = 2, score = 0.45),
206 separated samples into two distinct physiological groups (Figure 6).



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208 *Figure 6: Number of clusters derived through the silhouette method*

209 Cluster 1 consisted predominantly of post-hydration samples and exhibited consistently high
210 thermal tolerance (mean T_{crit} ~43.0 °C, T_{50} ~49.0 °C). Cluster 2 included mainly pre-hydration
211 measurements and was characterized by lower values (T_{crit} ~31.0 °C, T_{50} ~47.0 °C; Figure 7).

The clear separation between these clusters reflects the dominant role of water availability in driving physiological plasticity in thermal thresholds.

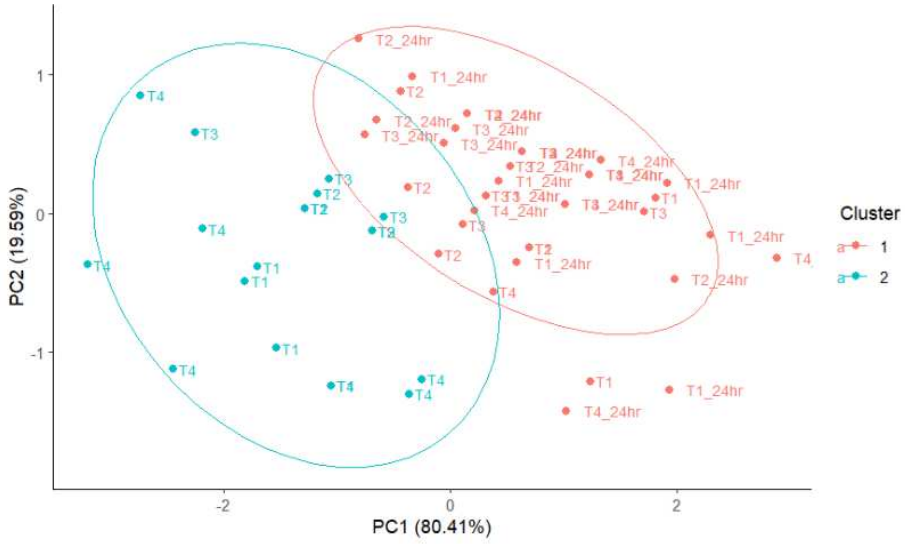

Figure 7: Principal Component Analysis (PCA) plot showing sample distribution along PC1 (80.41%) and PC2 (19.59%). Cluster 1 is colour coded in red and Cluster 2 in blue, with the Ellipses representing the 95% confidence intervals for each cluster. Labels "T1", "T2", "T3", and "T4" present the trials/tests, and their 24-hour post-hydration versions.

Multivariate differences between treatments

To statistically evaluate the combined influence of hydration and experimental treatment, a PERMANOVA was conducted on scaled T_{crit} and T_{50} values using Euclidean distances. The analysis confirmed significant treatment effects ($F = 5.47$, $R^2 = 0.41$, $p = 0.001$), indicating that variation in thermal responses was strongly explained by hydration status and trial conditions. This supports the interpretation that short-term water availability is a key determinant of both individual thermal performance and population-level response structure.

Impact of drought treatments

Drought-stressed samples (T4 treatment) showed greater dispersion in thermal thresholds than controls (Figure 8). However, PCA revealed substantial overlap between drought and control

treatments along PC1, indicating that hydration status had a more immediate and pronounced effect on thermal threshold values than drought stress alone.

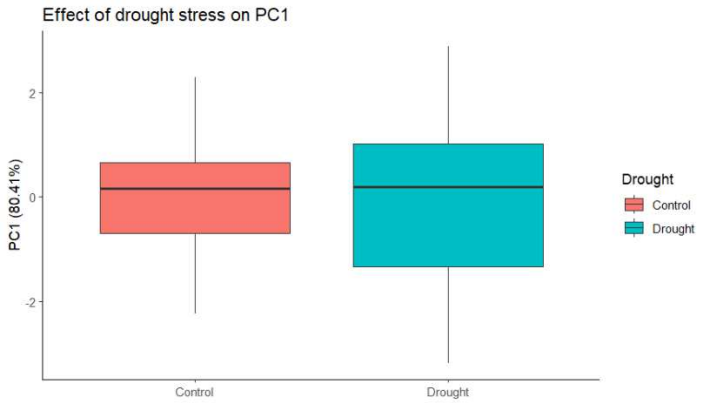

Figure 8: Boxplot illustrating the effect of drought stress on PC1 (80.41%) in blue, while the other tests (control) are in red

Discussion

This study investigated the thermal tolerance of *Acer campestre* under heat and drought stress through a series of controlled laboratory experiments, incorporating chlorophyll fluorescence-derived metrics (T_{crit} and T_{50}). Our findings revealed significant plasticity in thermal tolerance thresholds primarily driven by hydration status, with important implications for urban tree management under climate change.

Findings from our research show the dominant role of hydration status in modulating thermal tolerance in *Acer campestre*. Across all experimental trials, short-term water availability emerged as the principal factor influencing thermal response patterns, particularly for T_{crit} values. The consistent and significant improvement in T_{crit} following 24 hours of rehydration with mean increases of up to 13.31 °C in drought-stressed trees demonstrates remarkable physiological plasticity. This plasticity was confirmed through PCA, where hydration status explained over 80% of variation in thermal responses, and through cluster analysis, which separated pre- and post-hydration samples.

246 The intense recovery of T_{crit} from 29.0 °C to 42.0 °C after rehydration in drought-stressed trees
247 suggests that water availability acts as an immediate and powerful modulator of photosystem
248 II stability. This aligns with established physiological mechanisms where adequate hydration
249 maintains cellular turgor, stabilizes thylakoid membranes, supports enzyme function, and
250 facilitates repair processes within the photosynthetic apparatus (Kim & Portis, 2005). The rapid
251 recovery observed here likely reflects the re-establishment of these protective mechanisms
252 rather than acclimation through new protein synthesis, which would require longer timeframes.

253 Our results revealed contrasting patterns between T_{crit} and T_{50} responses to hydration and
254 drought stress. While both metrics showed significant correlations ($r = 0.61$, $p < 0.01$), their
255 markedly different sensitivities to water availability suggest they capture distinct aspects of
256 thermal tolerance. T_{crit}, representing the onset of thermal damage, was highly responsive to
257 short-term hydration changes, varying by as much as 13.31 °C between treatments. In contrast,
258 T_{50} remained relatively stable (47.0-50.0°C) across experimental conditions, showing only
259 modest changes with hydration status.

260 This differential response pattern suggests a physiological decoupling between the mechanisms
261 governing initial stress detection (T_{crit}) and those protecting against the onset of permanent
262 damage (T_{50}). Initial thermal damage likely involves thermolabile proteins and disruption of
263 electron transport processes that are highly sensitive to the immediate cellular environment,
264 including water status (Sharkey, 2005). In contrast, resistance to severe heat stress (T_{50}) may
265 depend more on inherent structural properties of the photosynthetic apparatus and heat shock
266 protein expression, which are less rapidly modified by hydration alone (Bourgine & Guihur,
267 2021).

268 These distinct response patterns have important implications for interpreting thermal tolerance
269 studies. Our findings suggest that T_{crit} is a more sensitive indicator of current physiological
270 condition, while T_{50} may better reflect intrinsic species-level thermal limits. This distinction

may explain some of the contradictory results in the literature regarding species rankings for heat tolerance, particularly when hydration status is not controlled or reported.

The thermal tolerance thresholds observed in *Acer campestre* provide valuable context for understanding its potential resilience relative to other urban tree species. Under optimal conditions, the T_{crit} values of 42-44 °C observed in hydrated samples align with those reported for moderately heat-tolerant deciduous species (Slot et al., 2019). However, the substantial decline in T_{crit} to 29 °C under drought stress places water-limited *Acer campestre* in a highly vulnerable category, especially considering that urban surface temperatures can exceed 40 °C during extreme heat events.

Compared to other common urban trees such as *Quercus* and *Platanus* that typically exhibit T_{50} values up to 52-54 °C (Gillner et al., 2015; Teskey et al., 2015), the T_{50} values we observed (47-50°C) position *Acer campestre* in the mid-range of thermal tolerance. This suggests that *Acer campestre* may withstand short-duration heat events when adequately hydrated but could be vulnerable to prolonged high temperatures, particularly when combined with drought stress.

The substantial recovery capacity demonstrated by *Acer campestre* following rehydration is noteworthy in the context of urban tree resilience. This ability to rapidly restore thermal tolerance with improved water availability could be an important adaptive feature for surviving the fluctuating moisture conditions typical of urban environments. However, the incomplete recovery observed in some metrics after severe drought suggests there may be limits to this plasticity, particularly under repeated or prolonged stress events.

Our findings have significant implications for urban forestry management as cities face increasing heat stress. The strong dependence of thermal tolerance on hydration status suggests that irrigation strategies may be as important as species selection for maintaining resilient urban

forests. Given that T_{crit} declined to potentially dangerous levels (29°C) under drought conditions, ensuring adequate soil moisture should be a priority for tree management.

The significant correlation between T_{crit} and T_{50} indicates that individuals with higher thresholds for initial damage also tend to withstand higher peak stress levels. This relationship could potentially be leveraged in screening programs to identify especially resilient individuals within species for propagation and urban planting. However, the substantial intra-species variation observed in our study, particularly under variable hydration conditions, cautions against overgeneralizing from small sample sizes or single-condition measurements.

The patterns of thermal decline and recovery observed in our study reflect the complex interplay between water status and photosynthetic function under heat stress. The rapid improvement in T_{crit} following rehydration suggests involvement of mechanisms such as membrane stabilization, osmotic adjustment, and restoration of electron transport efficiency. These immediate physiological responses likely precede longer-term acclimation processes such as heat shock protein synthesis or changes in membrane composition.

The relatively stable T_{50} values across treatments suggest that protection against severe heat damage may involve more constitutive mechanisms less dependent on immediate water status. This could include structural adaptations in the photosynthetic apparatus, baseline levels of heat shock proteins, or other genetically determined protective features (Sharkey, 2005). The partial maintenance of these protections even under drought stress may represent an important survival strategy, preserving core photosynthetic function while allowing peripheral processes to fluctuate with environmental conditions.

The incomplete recovery of thermal tolerance after severe drought, particularly in the final trial, raises questions about potential cumulative effects of repeated stress exposure. While short-term recovery was substantial, the persistence of some stress effects suggests that consecutive

heat and drought events could progressively erode resilience, potentially leading to lasting damage or mortality. This aligns with observations from field studies showing increased tree mortality following multiple extreme events (Allen et al., 2015; Teskey et al., 2015).

Several limitations should be considered when interpreting our results. First, our experiments used potted trees, which may experience different root conditions than those in urban settings. The controlled nature of the study, while allowing for precise manipulation of variables, may not fully capture the complexity of urban microclimates. Additionally, our focus on short-term responses does not address potential seasonal acclimation or long-term adaptation processes.

Future research should expand this approach to include a wider range of urban tree species, allowing for comparative assessment of hydration-dependent thermal plasticity. Longer-term studies that incorporate repeated stress events and recovery periods would help elucidate potential cumulative effects and acclimation capacity. Field-based assessments that measure thermal tolerance in situ across urban moisture gradients would complement our controlled findings and provide more direct management insights.

Investigation of the cellular and molecular mechanisms underlying the observed thermal responses would further advance our understanding of heat tolerance in woody plants. Particular attention should be paid to the physiological basis for the differential sensitivity of T_{crit} and T_{50} to hydration status, which may involve distinct protective pathways.

Conclusion

This study demonstrates that *Acer campestre* exhibits remarkable physiological plasticity in thermal tolerance, primarily driven by hydration status. Our results revealed distinct thermal response patterns across experimental treatments, with significant implications for urban forestry management under climate change.

The most substantive finding was the dramatic variability in T_{crit} values based on hydration condition. Well-watered specimens maintained T_{crit} values between 37.0-44.0 °C, which declined to as low as 29.0 °C under severe drought stress. Following 24-hour rehydration, T_{crit} significantly increased across all trials ($t = 7.63$, $df = 31$, $p < 0.01$), with the most striking recovery observed in drought-stressed specimens (from 29.0 °C to 42.0 °C). This recovery was statistically significant with a mean difference of 8.81 °C (95% CI: 6.46-11.17 °C), demonstrating the profound impact of short-term hydration on photosystem II resilience.

In contrast, T_{50} values showed more stability, typically ranging between 47.0-50.0 °C across treatments. While T_{50} also increased modestly post-hydration (mean difference 1.19°C, $t = 2.83$, $df = 31$, $p < 0.01$; 95% CI: 0.33-2.04 °C), the magnitude of change was substantially smaller than for T_{crit}. This differential response pattern suggests distinct physiological mechanisms govern initial thermal damage versus catastrophic photosystem failure.

Principal Component Analysis confirmed the dominant role of hydration in driving thermal tolerance, with the first principal component explaining 80.41% of total variance, primarily capturing differences in T_{crit}. K-means clustering (optimal $K = 2$, silhouette score = 0.45) separated samples into two distinct physiological groups: Cluster 1, consisting predominantly of post-hydration samples with high thermal tolerance (mean T_{crit} ~43.0 °C, T_{50} ~49.0 °C), and Cluster 2, comprised mainly of pre-hydration measurements with lower thresholds (T_{crit} ~31.0 °C, T_{50} ~47.0 °C). PERMANOVA confirmed significant treatment effects ($F = 5.47$, $R^2 = 0.41$, $p = 0.001$), with variation in thermal responses strongly explained by hydration status.

The significant positive correlation between T_{crit} and T_{50} ($r = 0.61$, $p < 0.01$; 95% CI: 0.43-0.74 °C) indicates that individuals with higher thresholds for initial damage also tend to maintain function at higher peak stress levels. This relationship suggests coordinated thermal protection mechanisms operating across different levels of stress intensity.

Our findings have important practical implications for urban tree management. Given that T_{crit} declined to potentially hazardous levels under drought conditions (29.0 °C), well below temperatures commonly experienced in urban areas during summer, maintaining adequate soil moisture during heat waves appears critical for preserving physiological function. The rapid recovery of thermal tolerance following rehydration suggests that timely irrigation interventions could substantially improve tree resilience during extreme heat events.

As climate change intensifies urban heat and drought stress, understanding the dynamic relationship between hydration and thermal tolerance becomes increasingly valuable for urban forestry. Future research should explore whether similar hydration-dependent thermal plasticity exists across other common urban tree species and investigate the long-term consequences of repeated stress exposure. Integrating these physiological insights into urban tree selection and management strategies could significantly enhance the resilience of urban forests under future climate scenarios.

Statements and Declarations

Funding: The research was funded by the Engineering and Physical Sciences Research Council of the UK.

Competing interests: The authors have no relevant financial or non-financial interests to disclose

Clinical Trial Registration: Not applicable.

Consent to Publish declaration: Not applicable.

Ethics and consent to Participate declaration: The plant material used in this study, *Acer campestre*, was cultivated on the Open University grounds and not collected from the wild. All

procedures complied with local and national guidelines for plant research. No permits or licenses were required.

Author Contributions: Ramla Khan wrote the draft, collected the data, and performed analysis as part of her Phd work. David Gowing and Philip Wheeler, in the position of supervisors, revised the document, gave suggestions for the analysis protocol and data collection.

Data: The data that support the findings of this study are openly available at Zenodo on the link: Ramla101/Thermal-Tolerance-of-Acer-Campestre: Thermal Tolerance of Acer Campestre

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