UNIVERSITY OF GOTHENBURG

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

# HEAT TOLERANCE, ACCLIMATION AND STRESS IN TREE SPECIES COMMONLY USED IN SWEDISH CITIES

**Gustav Ståhl**

---

Degree project for **Master of Science** (120 hec) with a major in Environmental Science ES2500, Examination Course Environmental Science, 30 hec

Second cycle

**Semester/year**: Spring 2024

**Supervisor**: Johan Uddling Fredin & Olivier Jean Leonce Manzi, Department of Biology and Environmental Sciences

**Examiner**: Göran Wallin, Department of Biology and Environmental Sciences

# Abstract

The investigation of heat tolerance in Nordic urban trees represents an underexplored domain, yet it is a critical area of study due to escalating global temperatures, particularly in urban settings. This study examined heat tolerance, heat acclimation and the connection between tree traits and leaf temperature. The primary methodology employed in this study revolved around chlorophyll fluorescence for plant stress assessment, but measurements of leaf size, stomatal conductance, and leaf angles were also conducted. The four tree species included were *Acer platanoides, Betula pendula, Prunus avium*, and *Tilia cordata*, all native to southern Sweden.

The findings indicated a few differences in heat tolerance levels among the examined species, with *Tilia cordata* being slightly better than *Betula* and *Prunus*. The study further revealed a lack of significant heat acclimation among the experimental tree species since there were no treatment differences after heatwave 1 and heatwave 2, and also since the stress was the same in the control and heatwave groups after heatwave 3. Several significant regressions between temperature and tree traits (stomatal conductance, characteristic leaf dimension, and leaf angles) were identified, though the significance of these regressions varied across different heatwaves. Substantial disparities in thermal safety margins were also observed among the species, with *Tilia* having the largest thermal safety margin and Pav having the lowest one after the third heatwave.

This study offers valuable insights into select Swedish urban tree species' heat tolerance dynamics, bridging knowledge gaps about heat tolerance and acclimation mechanisms where data has remained scarce. The findings have significant implications for urban planners and foresters, providing guidance for managing urban heat challenges and enhancing the resilience of urban greenery. Future research should focus on long-term monitoring and interdisciplinary efforts to further understand the mechanisms regulating urban tree responses to temperature stress and to develop strategies for enhancing urban tree resilience in the face of climate change.

**Keywords:** Heat Tolerance, Chlorophyll Fluorescence, Heat Acclimation, Heat Stress

## Popular Science Summary

**The Earth is getting hotter (who knew, right?), so understanding how urban trees handle heat becomes more and more important.** There's not much data about heat and Nordic trees, so I tried to help fix that problem. I researched heat tolerance and heat adaptation of four tree species in Sweden: Norway maple, silver birch, wild cherry, and little-leaf linden. I measured how much heat stress the trees were under and I also measured leaf traits such as leaf size and leaf angles.

I tested the trees with three heatwaves, but they didn't really get used to the heat. There wasn't much difference in how well each type of tree handled it, either. If the heatwaves had been longer, I might have seen some heat adaptation. Their leaf size and angles seemed to relate to how hot their leaves got, but this changed with each heatwave. Also, what's interesting is that each type of tree had a different safe temperature interval.

My discoveries can help scientists understand how trees in Swedish cities handle hot weather, giving more knowledge about how they tolerate and adapt to heat. This information is really helpful for city planners and tree experts because it gives them ideas on how to care for city trees as temperatures rise. Knowing which tree types can handle the heat better helps cities get ready for climate change.

Looking ahead, it's important that we keep studying city trees, especially in long-term studies and work together with experts in different fields to learn more about trees and heat. This will be important for making city trees and cities stronger against climate change.

Ståhl, G. (2024). Heat Tolerance, Acclimation and Stress in Tree Species Commonly Used in Swedish Cities. Department of Biological and Environmental Sciences, University of Gothenburg.

## Table of Contents

## Abbreviations

AHW = After heatwave
AHW1 = After heatwave number 1
AHW2 = After heatwave number 2
Apl = *Acer platanoides* (Norway Maple)
Bpe = *Betula pendula* (Silver Birch)
C = Control group
$d$ = Characteristic (leaf) dimension
$F_m$ = Maximum chlorophyll fluorescence
$F_v$ = Variable chlorophyll fluorescence
$F_v/F_m$ = A measure of how effectively Photosystem II can utilize light energy for photosynthesis
$g_{sw}$ = Stomatal conductance to water vapor
H = Heatwave-exposed group
HW = Heatwave
HW1 = Heatwave number 1 (max. temperature was 33 °C)
HW2 = Heatwave number 2 (max. temperature was 33 °C)
HW3 = Heatwave number 3 (max. temperature was 38 °C)
IR = Infrared
Pav = *Prunus avium* (Sweet Cherry)
PPFD = Photosynthetic Photon Flux Density
PSII = Photosystem II
RH = Relative humidity
Sin = *Sorbus intermedia* (Swedish Whitebeam)
$T_5$ = The leaf temperature at which the $F_v/F_m$ ratio is decreased by 5 % from its unstressed value
$T_{50}$ = The leaf temperature at which the $F_v/F_m$ ratio is decreased by 50 % from its unstressed value
$T_{95}$ = The leaf temperature at which the $F_v/F_m$ ratio is decreased by 95 % from its unstressed value
$T_{air}$ = Air temperature
Tco = *Tilia cordata* (Small-leaved Lime/Little-leaf Linden)
$T_{leaf}$ = Leaf temperature
TSM = Thermal safety margin

# 1. Introduction and Theoretical Background

## Global Warming and Urban Trees

Heatwaves are projected to become more intense, last longer, and occur with greater frequency worldwide in the near future (De Boeck et al., 2016; Yao et al., 2013). The frequency of heatwaves is anticipated to increase by up to tenfold in the next four decades (De Boeck et al., 2016). Europe's five most severe heatwaves on record occurred during the 21st century (Yao et al., 2013). Extended high heat and drought periods between the 1990s and the 2000s led to extensive forest death and reductions in areas around the Mediterranean Sea (Allen et al., 2010). According to all climate forecasts, high-temperature events will become more common and extreme due to global warming (Coumou and Robinson, 2013).

More frequent and longer heatwaves pose a greater risk for urban vegetation, particularly for trees which face intensified warming due to the urban heat island effect (when cities are hotter than rural areas because of human activities like buildings and roads trapping heat), as well as major difficulties from heat, lack of water, and flooding (Sharma et al., 2022; Teskey et al., 2015). Heat stress events are intensified in urban areas because of the urban heat island effect, which occurs when the air temperature in urban areas is elevated compared to the surrounding rural regions (Leal Filho et al., 2018). Furthermore, heatwaves are now recognized as a significant public health issue since a higher human death rate has been detected in some instances (Sharma et al., 2022). Many factors contribute to the urban heat island effect, such as low urban evaporation and transpiration rates, buildings absorbing sunlight, heat-trapping between buildings through reflections, and radiation emission by cooling systems, which elevates temperatures in urban environments (Bhargava et al., 2017). Additional factors contributing to the urban heat island effect effect include the heating of buildings, traffic, and industrial processes. Moreover, some building materials absorb more heat than others, while fast urban runoff reduces evaporation and increases heat (Percival, 2023; Tan et al., 2010).

As heatwaves become more common, it is important to know how they can and will affect city trees and greenery. Urban trees play a crucial role in decreasing the intensity of the urban heat by providing shade and cooling through evapotranspiration. Planting urban trees is also considered the most efficient and economical method for mitigating city heat (Livesley et al., 2016). Much of the research on how heat stress affects trees has focused on analyzing the effects of heatwaves in forests. Some research has also been done to study the short-term effects on tree seedlings (Teskey et al., 2015).

Using trees and plants strategically helps cool cities by reducing the urban heat island effect (US EPA, 2014). They reduce temperatures by shading and releasing moisture into the air, lowering surface and air temperatures. Urban trees and vegetation offer several advantages. Firstly, they help reduce the need for air conditioning by shading buildings, which lowers energy use (US EPA, 2014). Reducing energy demand helps decrease air pollution and greenhouse gas emissions. Planting trees also helps filter air pollutants and store $CO_2$. Additionally, urban trees and the soil around these are crucial in managing stormwater by absorbing rainwater, reducing runoff, and improving water quality. Besides these practical benefits, trees and vegetation enhance the quality of life by providing visual appeal, creating habitats for various species, and reducing noise pollution (US EPA, 2014). Knowing how plants react to heat stress is vital for managing urban green spaces well and selecting the right tree species for cities. As urban populations grow worldwide, more people will live in cities in the future (Brune, 2016). This makes it even more crucial to ensure that future urban trees can handle higher temperatures and other tough conditions to help cool down city areas.

## Heat Tolerance

Continuous exposure to high temperatures beyond a plant's heat tolerance can harm its physiology, biochemistry, and gene regulation pathways, ultimately reducing its capacity to absorb carbon (Bilger et al., 1984; Bita and Gerats, 2013). Plants can use different methods to cope with heat. They might grow in shady areas or change their leaf angles to absorb less sunlight and cool down by water evaporation that requires energy in the form of heat. This heat is drawn from the leaf and the surrounding environment, thus cooling the plant and surrounding air (Fauset et al., 2018). Plants react differently to stress at various growth stages and in different plant parts, using various ways to adapt. Adjustments of lipid composition, ion transporters, osmoprotectants, and complex signaling pathways are essential for helping plants deal with stress. These methods, both for avoiding and tolerating stress, show how complicated it is for plants to handle heat (Wahid et al., 2007). The aforementioned temperature control methods are necessary because they help regulate leaf temperatures for photosynthesis, especially when the environment changes greatly, while also shielding leaves from harm during harsh conditions. Furthermore, the temperature can vary extensively among leaves and different types of trees because of variations in the microclimate inside the canopy (Fauset et al., 2018). Plants have different ways to become more tolerant to heat, from long-term adaptations over many generations to quick changes like adjusting their lipid makeup or stomatal conductance. In forestry, it has been seen that even trees of the same type can handle high temperatures differently (Percival, 2023). Knowing about these differences could be helpful in choosing urban trees that can handle hotter weather. Two effective strategies for plants to handle heat are to gather osmolytes (small organic compounds) or change the fatty acid composition in membrane lipids (Percival, 2023; Tarvainen et al., 2022). These osmolytes are vital since they help keep the plant's balance of water and other substances at a suitable level, especially during intense heat.

## Chlorophyll Fluorescence

Chlorophyll fluorescence is a nondestructive and noninvasive method for estimating damage to the photosynthetic system in leaves under environmental stress while also serving as a method for analyzing general plant health (Percival and Henderson, 2003). It often uses easy-to-operate, portable, and commercially available equipment (Tinus et al., 2010). Chlorophyll fluorescence detects shifts in leaf chlorophyll fluorescence resulting from changes in photosystem II (PSII) functioning generated by various stresses such as heat, droughts, and floods (Percival, 2023).

Studies have demonstrated that changes in the variable to maximum fluorescence ratio ($F_\mathrm{v}/F_\mathrm{m}$) can indicate plants' heat tolerance (Allakhverdiev et al., 2008). The temperature where the $F_\mathrm{v}/F_\mathrm{m}$ ratio decreases by 50% ($T_{50}$) is commonly used to measure heat tolerance (Knight and Ackerly, 2003). Tree species from warmer climates typically have higher $T_{50}$ values than those from colder climates (Zhu et al., 2018). When comparing species' heat tolerance, using the $T_{50}$ values instead of $T_5$ or $T_{95}$ is the most common. $T_5$ and $T_{95}$ represent the leaf temperatures at which the $F_v/F_m$ ratio decreases by 5% and 95%, respectively, from its unstressed value. $T_{50}$ values are favored over $T_5$ or $T_{95}$ values for a few reasons: they provide a well-rounded and statistically reliable measure of population sensitivity to temperature stress, are more practically meaningful and straightforward to interpret, and enable comparisons across studies since they are commonly used in scientific literature.

The damage from heat stress on trees depends on factors such as temperature, the duration of increased heat, and heat acclimation potential. Trees show various reactions to heat stress, including changes in morphology, growth, and function. Heat stress affects both tree physiology and biochemistry. Trees have mechanisms to cope with heatwaves at morphological, physiological, biochemical, and molecular levels, but further research is needed in these areas (Percival, 2023).

To understand how stress affects plants, studying how PSII works is important (Luo et al., 2016). Chlorophyll fluorescence measurements are useful for this purpose, especially for evaluating how well urban trees photosynthesize under heat stress. These measurements help understand how trees can handle and bounce back from heat stress by looking at PSII's performance. When temperatures rise, PSII gets disrupted, directly affecting how well plants can photosynthesize, impacting their overall health (Luo et al., 2016). In this study, the variation of the $F_v/F_m$ ratio among different urban tree species under and after heat stress is observed.

## Heat Stress

Heat stress occurs at temperatures exceeding a certain threshold (Hu et al., 2020). Long periods of heat stress negatively affect tree biology at all levels, from the cellular level to the whole tree (Wahid et al., 2007). Thermal stress has also been shown to change the duration of the growing season by impacting important developmental stages (Percival, 2023). Electron transport, PSII function, and thylakoid membrane fluidity can all be damaged by heat (Teskey et al., 2015). Extreme heat exposure also increases the production of reactive oxygen species and respiration rates (Devireddy et al., 2021). High concentrations of reactive oxygen species harm plants by oxidizing certain cellular membranes (Percival, 2017). This leads to reduced stomatal conductance, photosynthesis, and growth, all of which can result in premature leaf shedding, yellowing, and necrosis. The effects of extreme heat on trees, including scorching leaves, branches, and stems, as well as growth inhibition, also encompass leaf aging, shedding, and, in severe cases, tree death (O’Sullivan et al., 2017; Teskey et al., 2015). Tree mortality usually depends on the duration, frequency, and severity of the heat stress episodes (Wahid et al., 2007). It is common for trees to die not only due to direct heat and drought events but also to insect attacks and various diseases, which are triggered by many years of heat/drought stress limiting the trees' defensive capacities (Allen et al., 2010).

One paper found that short heatwaves can significantly affect the canopy of some temperate forests (Filewod and Thomas, 2014). Another study found that brief, very intense heatwaves during the growing season have a greater impact on the two studied tree species (*Pinus taeda* and *Quercus rubra*) than a slower, gradual accumulation of the same amount of heat (Bauweraerts et al., 2014). Interestingly, there is limited research on the long-term effects of heat stress on trees spanning more than five years; specifically, there is little data on whether heat stress in one year influences growth in the following year (Percival, 2023). Another unexplored domain regards fertilizers based on specific nutrients such as potassium, calcium, or magnesium that have been shown to enhance heat stress tolerance in several crops, indicating that this method could possibly apply to urban trees (Percival, 2023).

The lethal temperature limit for most trees is often considered to be 60°C for one minute (Kelsey and Westlind, 2017). Temperatures below this value do not directly kill the trees but generate heat stress. The threshold for heat damage (=physiological or biochemical disruptions caused by high temperatures) lies around 45-50°C (O’sullivan et al., 2017).

Furthermore, the timing is very crucial during heat stress; when leaves are fully developed for photosynthesis, a temperature increase of 10–15°C above surrounding levels is typically needed to produce any heat damage (Teskey et al., 2015; Yang et al., 2023). However, if the heatwave occurs during the growing season, even lower temperatures can lead to significant plant damage (Kitao et al., 1998; Wahid et al., 2007). Young and full-grown trees showed comparable levels of leaf damage from heat stress, but the young (i.e., not fully developed) trees produced significantly fewer new leaves afterward. This suggests that there could be differences in their ability to recover from heat stress, which may be affected by the amount of stored sugars (Filewod and Thomas, 2013; Teskey et al., 2015).

### Thermal Safety Margin (TSM)

Plant heat tolerance measurements are valuable tools for assessing vulnerability to rising environmental temperatures amidst climate change (Cook et al., 2021). A commonly used metric for evaluating plant thermal safety is the TSM. Thermal safety refers to the ability of an organism to cope with high temperatures without experiencing adverse effects. TSMs, on the other hand, represent the disparity between the plant's physiological heat tolerance and the environmental temperature (Cook et al., 2021). TSM can thus be calculated with the following equation:

$$TSM = T_{50} - T_{leaf}$$

($T_{leaf}$, leaf temperature.)

A large positive TSM indicates a substantial difference between the leaf and critical threshold temperatures, suggesting the tree possesses a significant buffer against heat stress and is more resilient to high temperatures (Tarvainen et al., 2022). Conversely, a small or negative TSM suggests that the leaf temperature is close to or exceeds the critical threshold temperature, indicating reduced tolerance to heat stress and an elevated risk of thermal damage. Investigations of TSM in urban trees are important for assessing their resilience to heat stress, particularly in urban areas where the urban heat island effect worsens temperature extremes. Additionally, leaf temperatures are often not the same as air temperatures, so TSMs utilizing $T_{leaf}$ instead of $T_{air}$ give more reliable predictions of plant vulnerability to heat stress (Tarvainen et al., 2022). TSM has been used extensively when studying animals but more rarely when it comes to plants (Kitudom et al., 2022), indicating the need to explore its potential applications in plant research further.

$T_{leaf}$, mentioned in the equation above, is the plant’s "body temperature," regulating leaf metabolic processes and impacting leaf carbon utilization (Michaletz et al., 2016). It can surpass air temperature by up to 15°C and exhibits variability across species and environmental conditions (Cook et al., 2021; Kitudom et al., 2022). Even in the same surroundings, $T_{leaf}$ can vary considerably due to differences in the leaves' physical and physiological traits (Fauset et al., 2018; Lin et al., 2017). $T_{leaf}$ directly influences leaf function at the micro-environmental level, suggesting that thermotolerance should acclimate to leaf temperature. Additionally, leaf traits may influence thermotolerance by affecting $T_{leaf}$.

Consistent trends regarding heat tolerance in plants have been identified. For instance, species originating from warmer habitats tend to exhibit inherently higher levels of thermotolerance (O’sullivan et al., 2017; Zhu et al., 2018). Moreover, thermotolerance has been observed to acclimate in response to growth temperature (Tarvainen et al., 2022; Zhu et al., 2018). Plants from dry habitats generally demonstrate greater thermotolerance than those from wetter environments (Curtis et al., 2016; Knight and Ackerly, 2003). TSMs also play a crucial role in forecasting the thermal safety of organisms in the context of global warming (Sunday et al., 2014). One report’s findings indicate that $T_{leaf}$ is more important than thermotolerance for assessing thermal safety in plants (Kitudom, 2022).

### Heat Acclimation

In recent decades, extensive research has explored how plants adapt to environmental pressures at the molecular level, particularly through acclimation (Charng et al., 2023). Different defense mechanisms are activated based on the pattern of stress stimuli, whether continuous or irregular. Acclimating to intermittent stress includes a priming mechanism triggered by prior exposure to milder stressors, giving tolerance to severe stressors. This phenomenon is widely observed across plant species in response to various stressors (Mauch-Mani et al., 2017).

When plants experience a gradual increase in heat stress, they adapt their leaves to withstand higher temperatures before their photosynthetic system starts malfunctioning. However, plants do not have enough time to adjust during sudden heat stress events, so their photosynthetic system breaks down at lower temperatures in these cases (Daas et al., 2008; Ghouil et al., 2003). The light intensity level can also impact the temperature at which damage occurs to the PSII, with higher intensities linked to more severe damage (Berry and Bjorkman, 1980). This finding could be significant in urban environments where reflective surfaces such as windows, traffic, and buildings may lead to remarkably high light levels, which can be a considerable issue for plant health (Berry and Bjorkman, 1980). PSII damage from temperatures below 40°C can usually be undone and reversed for almost all tree species. However, the damage usually becomes permanent once temperatures stay at or exceed 40-45°C for a certain period of time. The severity of PSII impairment depends on factors such as species, how long the heat stress lasts, and the temperature before the heat stress (Mathur et al., 2014). Based on a study involving various tree species, a temperature of at least 42°C seems to be a threshold value for city trees (Krause et al., 2013; Percival, 2023). One study found significant acclimation of leaf respiration to warming in many different tree species over several years (Reich et al., 2016). Another report found that exposing trees to moderate heat before planting has been shown to increase their heat stress tolerance, thus offering protection in the first growing season (Teskey et al., 2015).

### Stomatal Conductance and Heat Tolerance

Sustaining constant water levels is crucial during episodes of heat stress (Hassan et al., 2021), and plants usually keep their water levels stable at all temperatures when there is enough water in the surrounding soil (Mazorra et al., 2002). Predictably, heat stress can therefore significantly disrupt plant water balance when water is lacking (Machado and Paulsen, 2001). In times of water scarcity, stomata closure occurs to reduce water loss, consequently reducing photosynthesis. Generally, if the conditions are dry, transpiration rates will be low, resulting in higher $T_{leaf}$. Conversely, if conditions are moist, transpiration rates will be higher, leading to lower $T_{leaf}$. Because of this, an effective response to heat stress involves keeping stomata open during long heatwaves to cool down the leaf and maintain normal leaf function (Lu et al., 2000; Schulze et al., 1973). Nonetheless, prolonged heat stress can still negatively impact photosynthetic capacity, even if stomata remain open. Due to the fact that little research has been done in this area, it is not known exactly which urban trees can cool themselves using transpiration during high heat (Esperon-Rodriguez et al., 2021).

### Urban Trees with Favorable Qualities

Urban landscape managers face the complex task of selecting trees capable of handling hotter weather in the future. With more scorching heatwaves and higher temperatures expected during future growing seasons, choosing trees that can thrive in these conditions is vital (Diem et al., 2017). Since heat and drought often occur simultaneously (Percival, 2023), it is commonly assumed that selecting trees resistant to drought also means they can endure extreme temperatures. This presumption might need to be further studied; almost no research demonstrates a direct correlation between how trees handle high heat and how they handle drought stress (Coumou and Robinson, 2013; Diem et al., 2017; Percival, 2023). Regarding ornamental urban tree species, urban landscapers have many trees to choose from. Nevertheless, most scientific studies on heat tolerance have only focused on a small selection of genera like *Acer*, *Picea*, and *Quercus*. Additionally, only a handful of species within these genera have been extensively studied, which leaves big data and information gaps in this area (Percival, 2023). Creating more heat-tolerant urban trees through breeding is possible. However, relying only on traditional methods might not be the most efficient approach in this case, as it could be time-consuming and yield unpredictable results (Harfouche et al., 2011).

## Study Aim and Research Questions

This study aims to acquire more information about heat tolerance and acclimation in a few common urban trees naturally occurring in southern Sweden. This larger aim has been divided into the four following questions:

**Question 1:** What is the heat tolerance of the selected urban tree species, and does it vary between the different species?

**Question 2:** Do the selected urban tree species show heat acclimation after heatwave exposure, and if so, to what degree?

**Question 3:** Is there a correlation between urban tree traits (leaf size, leaf angles, and stomatal conductance) and temperature?

**Question 4:** Do the different species have different thermal safety margins?

## 2. Materials and Methods

### Experimental Approach and Design

Urban tree species were subjected to controlled heat stress within laboratory growth chambers. Chlorophyll fluorescence measurements were performed which give insights into the impact of heat stress on photosynthesis and leaf functioning. In *Table 1* below, one can see a timeline and a brief overview of the experiment and what measurements were done at what time.

**Table 1.** Timeline of heatwaves (HWs) and the after-heatwave (AHW) phases. In the AHW phases, the *elevated* temperature $F_v/F_m$ measurements were carried out, and *ambient* temperature $F_v/F_m$ measurements were also

made the day after the heatwaves ended to see the general stress levels of the plants post-heatwave. Data about leaf angles, leaf temperature ($T_{leaf}$), $g_{sw}$ (stomatal conductance to water vapor; this data was procured from another student), PAR (photosynthetically active radiation) measurements, and length and width of selected leaves (for characteristic leaf dimension determination - $d$) were collected during the heatwaves.

| Period | Date |
|---|---|
| **Heatwave 1** (HW1): Measurements of leaf traits (PAR, leaf angles, $T_{leaf}$, $g_{sw}$, and $d$) | 5-7 February 2024 |
| **After-Heatwave 1** (AHW1): Measurements of *elevated* temperature $F_v/F_m$ measurements and measurements of *ambient* temperature $F_v/F_m$ | 12-16 + 19-20 February 2024 |
| **Heatwave 2** (HW2): Measurements of leaf traits (PAR and $T_{leaf}$ only) | 21-23 February 2024 |
| **After-Heatwave 2** (AHW2): Measurements of *elevated* temperature $F_v/F_m$ measurements and measurements of *ambient* temperature $F_v/F_m$ | February 28-March 1 + 4-6 March 2024 |
| **Heatwave 3** (HW3): Measurements of leaf traits (PAR, leaf angles, $T_{leaf}$, $g_{sw}$, and $d$) | 11-13 March 2024 |

Below, in *Table 2*, is an overview of how many measurement replicates were made. For every individual tree, two suitable leaves were selected and marked, and the chosen ones were healthy and average-looking. This means that in total, there were 86 measured leaves in HW1 and 84 ones in HW2. In HW3, 129 leaf measurements could be done since some students from the course *Plant Ecophysiology in a Global Change Perspective* (BIO506, University of Gothenburg) were helping with these measurements. A few of the trees in HW3 had 4 leaves analyzed, but this had to be decreased to 3 or 2 leaves given the limited time available.

**Table 2.** The number of replicates that were done for each tree group during the different measurement phases.

The four measured tree species were *Acer platanoides* (Apl), *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco). H stands for "heatwave-exposed group," while C stands for "control group." HW means heatwave, and AHW signifies after heatwave. $F_v/F_m$ measures how effectively PSII can utilize light energy for photosynthesis and how much stress a tree is under.

| Tree Group | Nr. of measured trees in **HW1** | Nr. of measured trees in **HW2** | Nr. of measured trees in **HW3** | Nr. of measured trees during *elevated* temperature $F_v/F_m$ measurements **AHW1** | Nr. of measured trees during *elevated* temperature $F_v/F_m$ measurements **AHW2** | Nr. of measured trees during *ambient* temperature $F_v/F_m$ measurements **AHW1** | Nr. of measured trees during *ambient* temperature $F_v/F_m$ measurements **AHW3** |
|---|---|---|---|---|---|---|---|
| Apl C | 6 | 6 | 6 | - | - | 6 | 6 |
| Apl H | 6 | 6 | 6 | - | - | 6 | 6 |
| Bpe C | 6 | 6 | 6 | 4 | 4 | 6 | 6 |
| Bpe H | 6 | 6 | 6 | 4 | 4 | 6 | 6 |
| Pav C | 5 | 4 | 4 | 4 | 4 | 5 | 5 |
| Pav H | 5 | 5 | 5 | 4 | 4 | 5 | 5 |
| Tco C | 4 | 4 | 5 | 4 | 4 | 4 | 5 |
| Tco H | 5 | 5 | 6 | 4 | 4 | 5 | 6 |
| Sum (all trees together) | 43 | 42 | 43 | 24 | 24 | 43 | 45 |

### Plant Material and Growth Conditions

46 urban trees were used in the experiment belonging to four different Swedish urban tree species: *Acer platanoides* (Apl) - Norway maple, *Betula pendula* (Bpe) - Silver birch, *Prunus avium* (Pav) - Sweet cherry, and *Tilia cordata* (Tco) - Little-leaf linden. One other urban tree species was originally included, but it was excluded from the experiment due to low development. Additionally, a few extra experiment trees were available for use if anything would have happened with any of the main experiment trees. Additionally, a few other trees had to be excluded from the measurements due to one Pav tree being wilted and a few Tco trees not having leaves close enough to the growing lamps.

The four tree species are naturally occurring in southern Sweden. The trees used for this study ranged from 50 to 80 cm in height, were 2 to 3 years old, and were brought up by a plant nursery (Splendor Plant AB, Jonstorp, Sweden). All the trees originate from Denmark, ensuring their comparability and indicating that they are not genetically adapted to very warm conditions. The trees were already potted when received from the plant nursery, and the soil compositions were adjusted to each species for favorable growing conditions. The trees were watered to avoid water shortages, meaning larger trees with higher transpiration received more water than smaller trees. Maintaining well-watered plants was crucial for isolating and studying the effects of temperature without the confounding factor of dehydration stress. The primary determinant of a tree's water intake was the dryness of its soil. Every week, the trees were given the same amount of nutrients adapted for growing trees of their size and age. They received 200 ml of 1:100 nutrient-to-water mix every week (Stroller Blå Trädgårdsnäring, SBM Life Science, Lund, Sweden).

Growth chambers were used to simulate controlled heatwaves by regulating the temperature, relative humidity (RH), and $CO_2$ levels, maintaining temperatures within a specified range. In *Figure 1,* one of the growth chambers used for control and heatwave conditions can be observed. Light intensity (PPFD - photosynthetic photon flux density) was also controlled and maintained at 1200 µmol $m^{-2}$ $s^{-1}$ 15 cm from the lamps in HW1, while after the first heatwave, the PPFD was increased to 1500 µmol $m^{-2}$ $s^{-1}$ at the same distance from the growing lamps. These carefully controlled chamber settings allowed for examining realistic heatwaves and control conditions. The complete data on PPFD and RH can be found in *Appendix A*.

**Figure 1**. One of the two growing chambers, used as control and heatwave chambers, that was utilized in the experiment.

The selected time period for the three different heatwaves in this experiment was three days. In HW1 and HW2, 33°C was the peak day temperature set for the heat chambers from 10:00 to 17:00. During the nights, the lowest temperature was set to 19°C. The peak day temperature in HW3 was 38°C from 10:00 to 17:00, while the lowest night temperature was 21°C. The trees in the control chamber reached a peak day temperature of 23°C from 10:00 to 10:00 to 17:00, while the lowest night temperature was 17°C. A peak temperature of 33°C was chosen because this is the heat record in Gothenburg between 1990 and 2020. The other peak temperature, 38°C, was chosen since it is the current Swedish heat record. The control temperatures were chosen to represent a relatively warm day in Gothenburg between 1990 and 2020, being in the 75th temperature percentile for July. A full overview of the temperatures, RH, and light levels for all three heatwaves and the control chambers can be found in *Appendix A.*

In the chambers, the positions of the trees were regularly switched to avoid any positioning-related errors. Furthermore, two chambers were used—one for control plants and one for the heatwaves. To avoid any bias caused by unknown or unwanted chamber differences, the control and heatwave chambers and trees were switched once per week. A few times, algal growth had occurred on the top soil layers of some of the pots, likely due to high moisture levels. In these cases, the top soil layer was stirred with unaffected soil from the same pot to prevent further algal growth.

**Heat Tolerance Measurements**

The *elevated* temperature $F_v/F_m$ measurements in this experiment were performed with the help of an infrared heating lamp to calculate tree species' $T_{50}$ values. The *ambient* temperature $F_v/F_m$ measurements, on the other hand, were performed one day after HW1 and HW3 at a room temperature of 23°C to see how much the heat episodes had stressed the trees (because of scheduling problems, *ambient* temperature $F_v/F_m$ measurements could not be conducted after HW2). The measurements of the traits were performed during the heat waves.

The evaluation of tree heat tolerance in this thesis involves comparing tree performance in regular conditions to that during stress, with the aim of understanding the impact of heat stress on trees and their adaptive capacity. *Figure 2* illustrates the experimental setup for one part of the experiment – the *elevated* temperature $F_v/F_m$ measurements. The experiment was performed in the phytotron facility at the University of Gothenburg. Two infrared heating lamps are shown, which were controlled with dimmers (not shown in the figure) to change the temperature (*Figure 2*). On the bench to the left, a *Testo thermometer* is shown with a thermocouple attached to a leaf clip, which in turn is attached to a Bpe leaf (*Figure 2*). This setup allowed for the recording of $T_{leaf}$, and the data was transferred to a smartphone via Bluetooth. A pocket PEA fluorimeter (Hansatech Instruments Ltd, King's Lynn, Norfolk, UK) is shown to the right on the bench (*Figure 2*). The black tripod was used to position the leaf close to the heating lamps (*Figure 2*).

**Figure 2**. Experimental setup when performing the *elevated* temperature $F_v/F_m$ measurements in the phytotron laboratory at the University of Gothenburg. The most important equipment is shown.

In order to start with the *elevated* temperature $F_v/F_m$ measurements, selected leaves were detached from the experimental trees. Leaf clips were put on the detached leaves and allowed to dark-adapt for 20 minutes. During dark adaptation, a metal shutter plate shadowed a small circle area on the leaf. After dark-adaptation, a pocket PEA fluorimeter was activated and positioned on the leaf, with its head inserted into the clip's shutter plate. After this step, the shutter plate was pulled back, and measurements could be performed. Values obtained from the pocket PEA fluorimeter were manually noted and saved electronically.

The leaves were detached from the trees to facilitate leaf heating control and to avoid heating plant parts other than those of the two chosen experiment leaves. One leaf clip was attached per examined leaf, and the shutter plate was closed to shadow the desired leaf area. Two leaves were selected per tree to reduce the time each leaf was examined and thereby avoid excess leaf dehydration, which would have been more probable if only one leaf had been used. For the first leaf, the target temperatures for the measurements were 23, 34, 42, 46, and 50°C. The target temperatures for the second leaf from the same plant were 23, 38, 44, and 48°C. Care was taken to avoid more prominent leaf veins in the leaf clip opening. A layer of nail polish was added to the cut end of the petiole immediately after the cut to prevent water loss.

During the *elevated* temperature $F_v/F_m$ measurements, the leaves (with clips attached) were placed in plastic bags with a wet paper towel to ensure they would not lose excessive moisture and turgor when they were not being used. Temperature sensors (*Testo thermometers*) were connected to the leaf via the aforementioned leaf clips, and the leaves were placed horizontally under infrared heating lamps. A dimmer was used to control the leaf's temperature. The target leaf temperatures were 23 (ambient temperature in this experiment), 34, 38, 42, 44, 46, 48, and 50°C.

$T_{leaf}$ was monitored using a *Testo thermometer*, and readings were recorded every two seconds using the *Testo Smart Probes* app. The pocket PEA measurement was made after two minutes with temperatures stable around the desired temperature. The measurements were made within ±1°C of the target (so when the target was 42°C, measurement at temperatures of 41-43°C was considered acceptable). The maximum temperature variation during the two-minute period was within 1°C between the highest and lowest values. Care was taken to avoid overshooting the maximum value prior to the measurement with the pocket PEA (i.e., temperatures higher than those allowed during the two-minute measurement period). Heating the leaves took between 4 and 20 minutes, with an average of 8 minutes per leaf, to reach the target temperature level. The obtained data were later transferred from the pocket PEA to a computer via Bluetooth.

### Traits Measurements and Leaf Temperature

The leaf tree traits examined in this report were stomatal conductance to water vapor ($g_{sw}$), characteristic leaf dimension ($d$), and leaf angles. These specific traits were chosen due to the fact that they have properties that affect the energy balance of the leaves and, thus, $T_{leaf}$ and TSM. Another master's student provided the stomatal conductance values and conducted a related experiment using the same plants and heat chambers. This collaboration helped broaden the research.

The leaf trait, *d*, refers to the specific measurements of a leaf's length and width (i.e., the average distance air travels over a leaf), which can vary significantly among plant species. These dimensions are essential, e.g., for identifying and classifying plants. The characteristic leaf dimension was calculated from measurements of leaf length and width as follows:

$$d = \frac{(L+W)}{2} \times 0.7$$

(L, leaf length; W, leaf width).

This characteristic leaf dimension is not fully comprehensive for all species due to variations in leaf morphology, such as differences in serration, lobing, or other structural features, which may not be adequately captured by length and width measurements alone. Nonetheless, it is still a viable measurement that easily considers both length and width. For the trees used in this experiment, the equation works well for Bpe, Pav, and Tco, but it may not work optimally for Apl. It would still be possible to notice if there are any relationships between temperature and leaves' characteristic dimensions within each species.

The final plant trait measured in this study, leaf angles, was assessed using a protractor during HW1 and HW3. In this experiment, 0° is classified as the left end of a circle at the horizontal plane, which means that 90° is at the top of the circle. Initially, the angles were classified between 0° and 360°, but they were later converted to the scale 0° to 90° for simplicity and comparability purposes. This means the angles were measured based on how many degrees they differed from the horizontal plane. This was done because it does not matter if the light hits the leaf's top or bottom.

Measurements of the PAR were made to see how much sunlight every measured leaf got since PAR drives plant growth. The PAR levels were measured for each selected leaf. Both the horizontal and the leaf angle PAR were measured, and generally, these were quite similar except when the leaves were partially shaded or had specific angles. The $T_{leaf}$ values measured during the three heatwaves had to be corrected for leaf emissivity since the instrument used assumed black body emissivity (i.e., an emissivity equal to 1). First, the actual measure of $T_{leaf}$ values had to be corrected with the help of *Boltzmann law* as stated below (Jones and Rotenberg, 2001):

$$\Phi_L = \varepsilon \times \sigma \times T^4$$

($\Phi_L$, thermal radiation; $\varepsilon$, emissivity (a property of the surface emissivity); $\sigma$, the Stefan–Boltzmann constant ($5.67 \times 10^{-8}$ W $m^{-2}$ $K^{-4}$); T, the temperature in degrees Kelvin.)

The emissivity of typical vegetation generally falls within the range of 0.95 to 0.995 (Jones and Rotenberg, 2001). In this experiment, the value of 0.98 was used. The emissivity effect on $T_{leaf}$ was relatively small but not negligible. After all the temperatures had been transformed using the *Boltzmann law*, the measured horizontal PAR values were used to make regression graphs in *Excel*. Each leaf's $T_{leaf}$ was normalized to a value at PAR = 900 µmol/m²/s based on the ratio between $T_{leaf}$ and horizontal PAR for each species and treatment. When this transformation had been performed, the $T_{leaf}$ at a standardized PAR could be used to see whether there were any significant regressions between temperature and the chosen leaf traits in this study. The standardization process was implemented to ensure consistency across measurements and accurately assess the influence of PAR on the tree responses to heat stress.

### Data Handling and Statistical Analyses

*Microsoft Excel* was used to employ linear regression analysis to investigate the relationship between plant traits and temperature. This aimed to quantitatively assess how temperature variations influence some key traits of urban tree species, thereby gaining valuable information about heat tolerance and their response to heat stress.

The $F_v/F_m$ graphs were made using the statistics program *RStudio*, which facilitates the making of appropriate graphs displaying $T_5$, $T_{50}$, and $T_{95}$ values and associated statistical data such as standard errors. The equation was fitted for each species, treatment, and heatwave.

*Welch's t-test* was used in this report to compare two groups to see if they differ significantly. If the average values (x) and standard errors (SE) fulfilled the equation above (larger than zero), the two groups (species, treatments, or heatwaves) were considered to differ significantly. The equation is presented as follows (Vårhammar et al., 2015):

$$(x_1 - x_2) - 2.58 \times \sqrt{SE_1^2 + SE_2^2} > 0$$

*Welch's t-test* assesses the means of two groups. Unlike the traditional *Student's t-test*, Welch's method does not assume equal variances between the compared groups. Because of this, it proves particularly valuable in scenarios where equal variance and sample size assumptions are not fulfilled.

When calculating the TSMs, the $T_{leaf}$ values came from the heatwaves, while the $T_{50}$ values were calculated from the AHW phases using the *elevated* temperature $F_v/F_m$ measurements. The $T_{50}$ values used to create the HW3 TSMs came from the AHW2 values since no AHW3 phase was included in the experiment. The TSM was calculated for each registered temperature value using the thermal safety margin equation mentioned earlier (TSM = $T_{50}$ - $T_{leaf}$). Each registered $T_{leaf}$ value was utilized to get many individual TSM values. Ultimately, these numbers were averaged group-wise to get TSMs for Bpe, Pav, and Tco.

## 3. Results

### $T_{leaf}$ and its Relationships with Leaf Traits

Several significant differences emerged when comparing C with C and H with H, which can be seen in *Figures 3, 4,* and *5*. The letters assigned to each group denote statistical significance levels. If groups share the same letter, they are not statistically different. Lowercase letters can only be compared with other lowercase letters, while uppercase letters can only be compared with other uppercase letters. These several differences indicate that the different species generally experience different $T_{leaf}$ (at PAR = 900 µmol/m²/s for each species and treatment in order to get a fair comparison) and that this difference could be linked to the three leaf traits measured in this study.

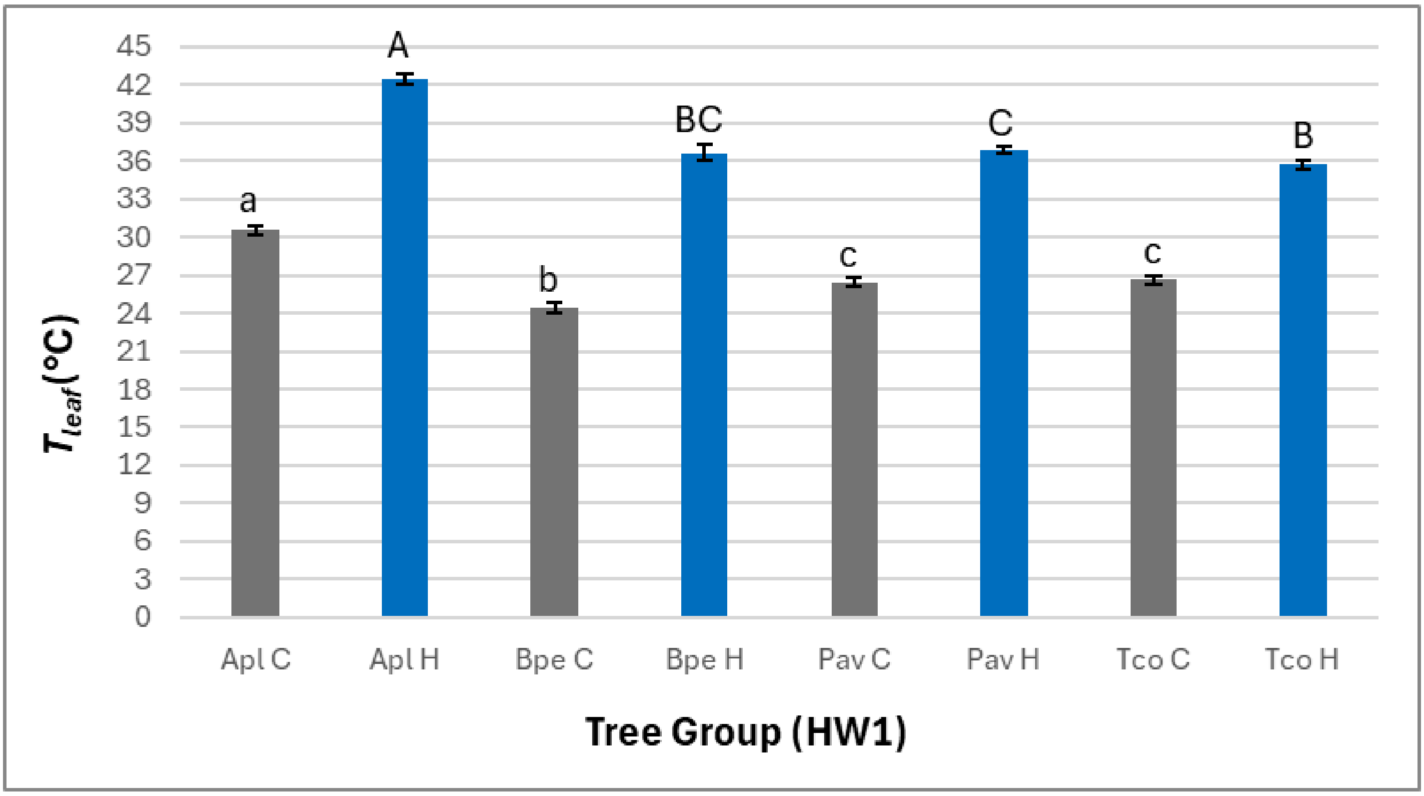


**Figure 3**. The average leaf temperature ($T_{leaf}$) during HW1. The air temperature ($T_{air}$) was 33°C at the time of measurement. The four measured tree species were *Acer platanoides* (Apl), *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco). H stands for “heatwave-exposed group,” while C stands for “control group.” The letters assigned to each group denote statistical significance levels. If groups share the same letter, they are not statistically different. It is important to note that lowercase letters are used only for comparisons within C groups, while uppercase letters are used for comparisons within H groups.

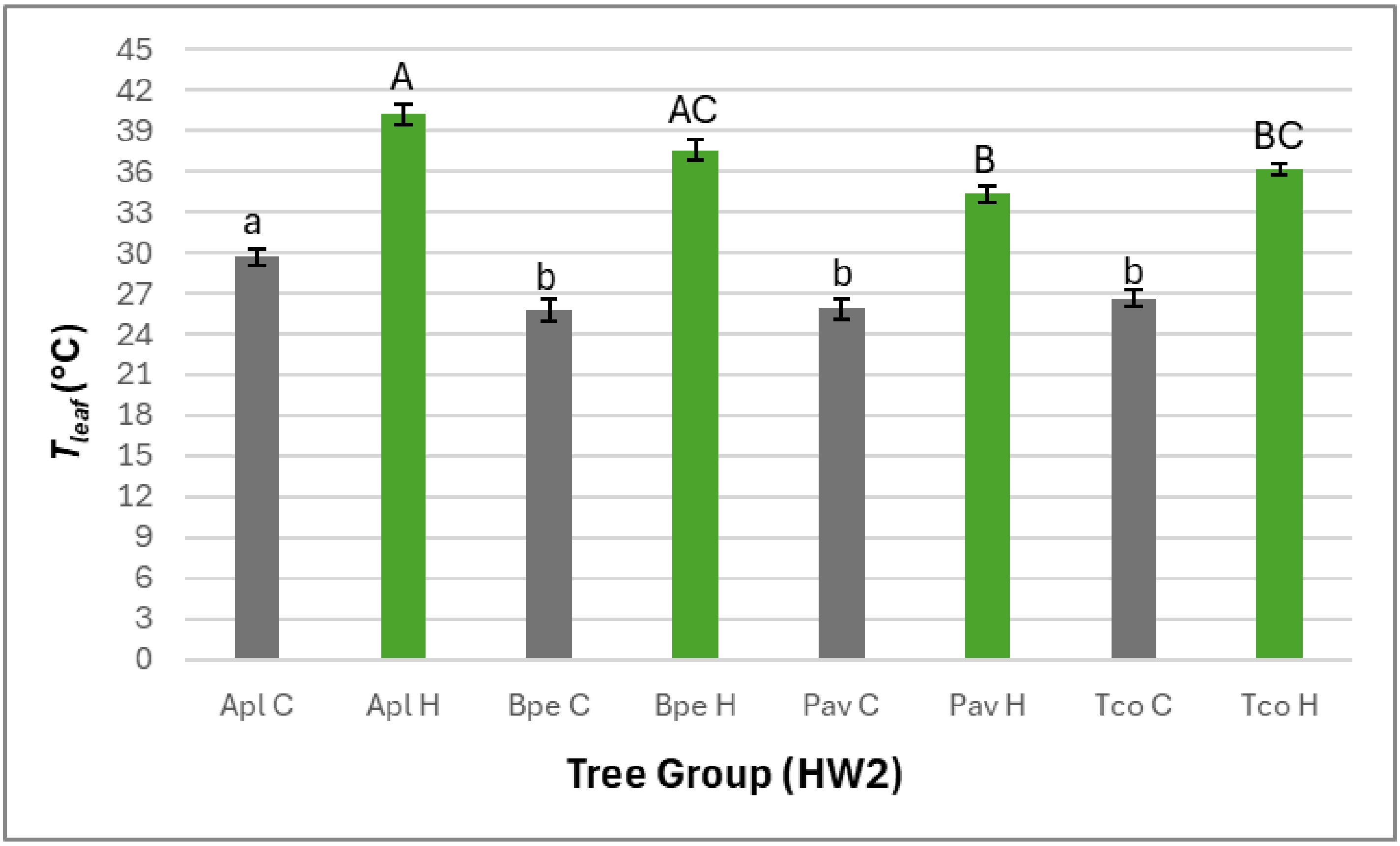


**Figure 4**. The average leaf temperature ($T_{leaf}$) during HW1. The air temperature ($T_{air}$) was 33°C at the time of measurement. The four measured tree species were *Acer platanoides* (Apl), *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco). H stands for “heatwave-exposed group,” while C stands for “control group.” The letters assigned to each group denote statistical significance levels. If groups share the same letter, they are not statistically different. It is important to note that lowercase letters are used only for comparisons within C groups, while uppercase letters are used for comparisons within H groups.

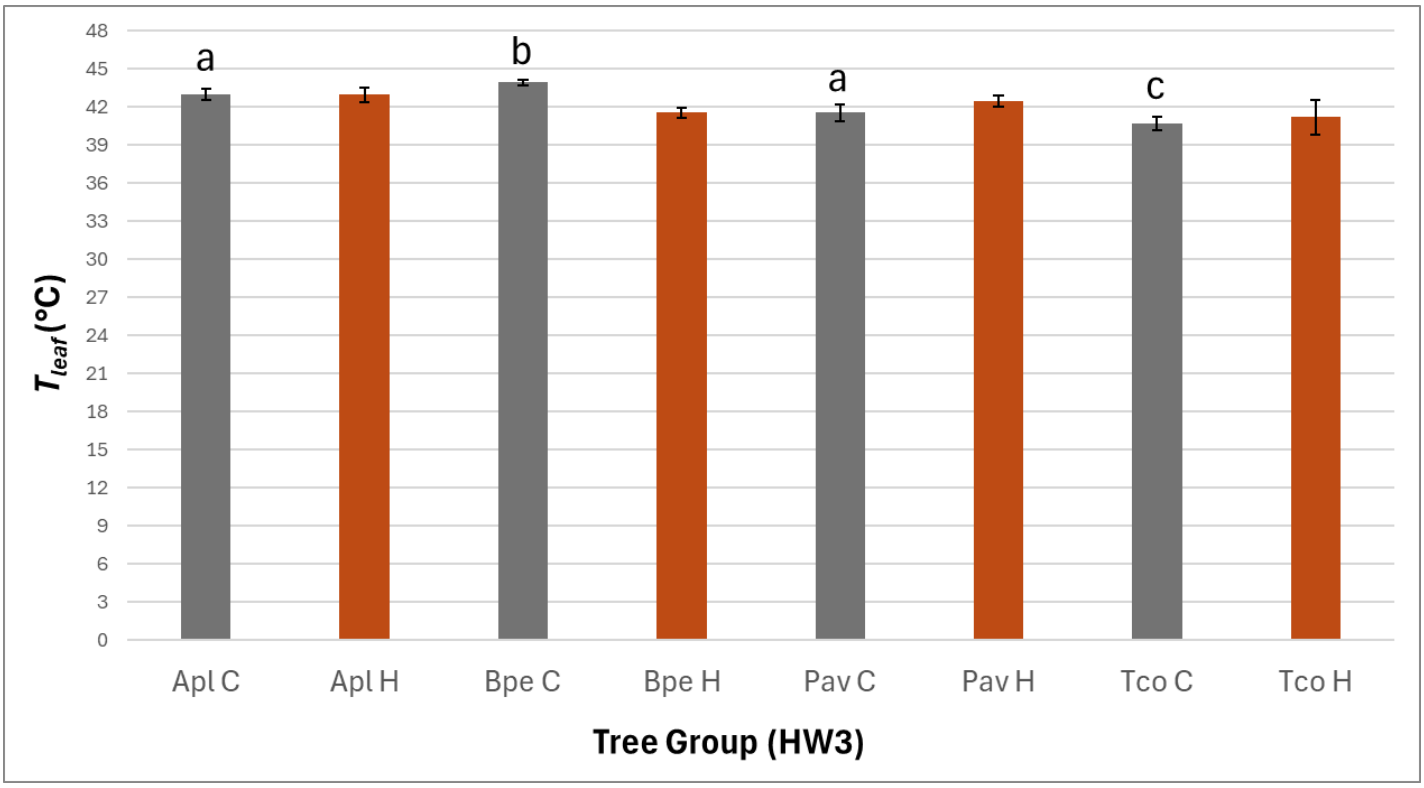
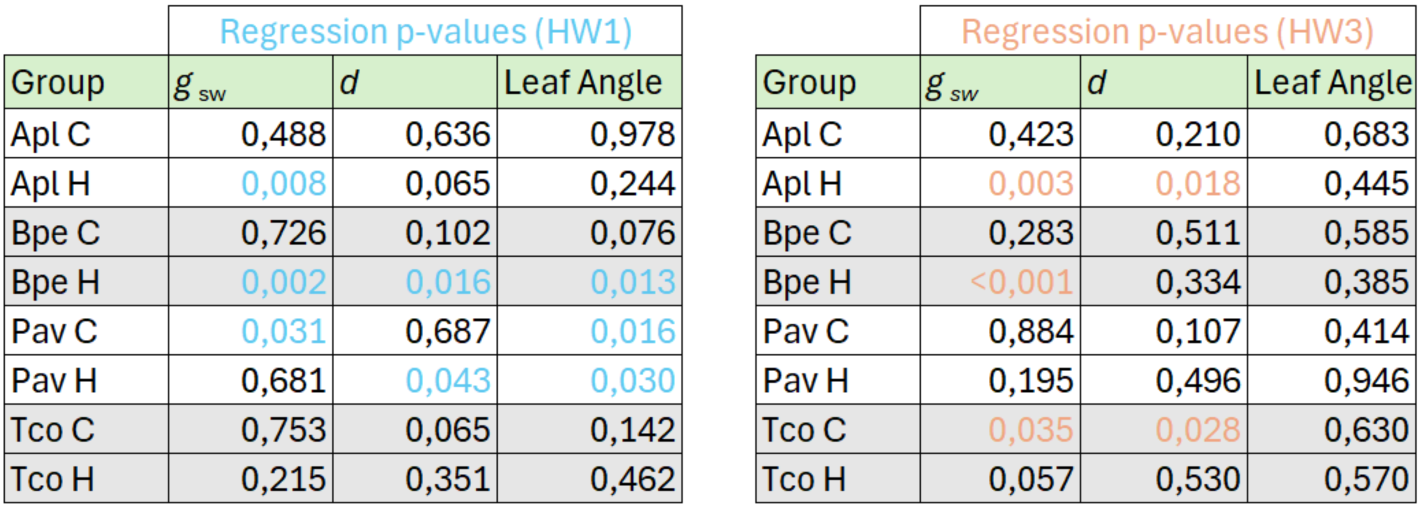


**Figure 5**. The average leaf temperature ($T_{leaf}$) during heatwave 3 (HW3). Note that the third heatwave had an air temperature ($T_{air}$) of 38°C, and the control groups were also subjected to 38°C instead of the earlier control temperature of 23°C as in HW1 and HW2. The four measured tree species were *Acer platanoides* (Apl), *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco). H stands for “heatwave-exposed group,” while C stands for “control group.” The letters assigned to each group denote statistical significance levels. If groups share the same letter, they are not statistically different. Lowercase letters are used for comparisons within C groups. There were no significant differences between the H groups.

In order to see if there were any significant regression/correlations between traits and temperature, data of the three traits above ($g_{sw}$, $d$, and leaf angles) were plotted against $T_{leaf}$ measured during these heatwaves. After this, regressions and regression statistical tests were made in *Excel*. The acquired p-values showed if the regressions were significant between the adjusted $T_{leaf}$ and these three specific leaf traits. A regression is deemed significant if the p-value is less than 0.05. This suggests substantial evidence to reject the null hypothesis, indicating that the independent variable significantly influences the dependent variable. The significant p-values are marked in blue for HW1 and orange for HW3 in *Table 3* below. The three traits analyzed here were $g_{sw}$, $d$, and leaf angle. All significant $g_{sw}$ regressions had a negative slope (both HW1 and HW3), white the $d$ regressions had positive slope values in HW1 and negative ones in HW3. For the leaf angle regressions, two regressions had a positive slope, while one had a negative slope. As can be seen in *Table 3*, there were not many clear patterns in significance. The only two values that were significant in both HW1 and HW3 were the $g_{sw}$ for Apl H and Bpe H. Eight regression values were significant during HW1. At the same time, only five were significant in HW3, and as stated before – not necessarily the same ones.

**Table 3**. P-values from all regressions. The regressions were conducted to analyze the relationship between $T_{leaf}$ and three specific traits. The three traits considered here were stomatal conductance to water ($g_{sw}$), characteristic leaf dimension (d), and leaf angle. The significant p-values are marked in blue for HW1 and orange for HW3. The scale for leaf angles ranged between 0 and 90 degrees, meaning the angles were measured based on their deviation in degrees from the horizontal plane.



| | Regression p-values (HW1) | | |
|---|---|---|---|
| Group | $g_{sw}$ | $d$ | Leaf Angle |
| Apl C | 0,488 | 0,636 | 0,978 |
| Apl H | 0,008 | 0,065 | 0,244 |
| Bpe C | 0,726 | 0,102 | 0,076 |
| Bpe H | 0,002 | 0,016 | 0,013 |
| Pav C | 0,031 | 0,687 | 0,016 |
| Pav H | 0,681 | 0,043 | 0,030 |
| Tco C | 0,753 | 0,065 | 0,142 |
| Tco H | 0,215 | 0,351 | 0,462 |

| | Regression p-values (HW3) | | |
|---|---|---|---|
| Group | $g_{sw}$ | $d$ | Leaf Angle |
| Apl C | 0,423 | 0,210 | 0,683 |
| Apl H | 0,003 | 0,018 | 0,445 |
| Bpe C | 0,283 | 0,511 | 0,585 |
| Bpe H | <0,001 | 0,334 | 0,385 |
| Pav C | 0,884 | 0,107 | 0,414 |
| Pav H | 0,195 | 0,496 | 0,946 |
| Tco C | 0,035 | 0,028 | 0,630 |
| Tco H | 0,057 | 0,530 | 0,570 |

*Figures 6, 7,* and *8* are all merely three visual examples of the 13 significant regressions in *Table 3* above. *Figure 6* shows a clear and significant regression between $g_{sw}$ and $T_{leaf}$. Significant regressions are also shown in *Figures 7* and *8* (showing *d* and leaf angles instead of $g_{sw}$), indicating notable trends in the respective datasets. *Figure 9* shows the three traits (average leaf angles, *d*, and $g_{sw}$) during HW1. Notably, it can be seen that Apl has very low $g_{sw}$ and that Apl and Pav have more horizontal leaves (closer to 0°) than Bpe and Tco. Moreover, Tco had the largest leaves (*d*) while Bpe had the smallest ones.

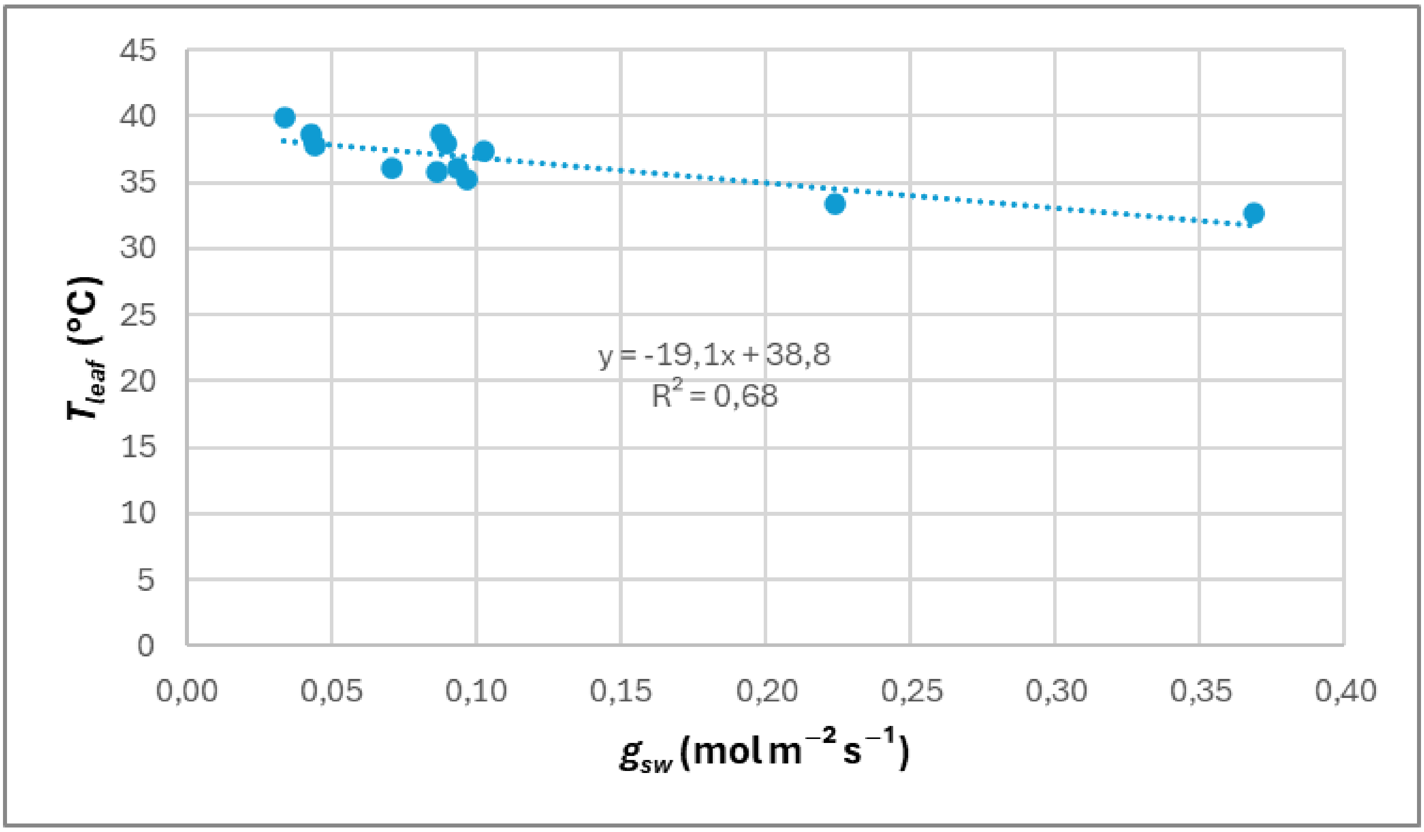


**Figure 6**. Leaf temperature ($T_{leaf}$) in relation to stomatal conductance to water vapor ($g_{sw}$) in Bpe H during HW1. The p-value was 0.00197.

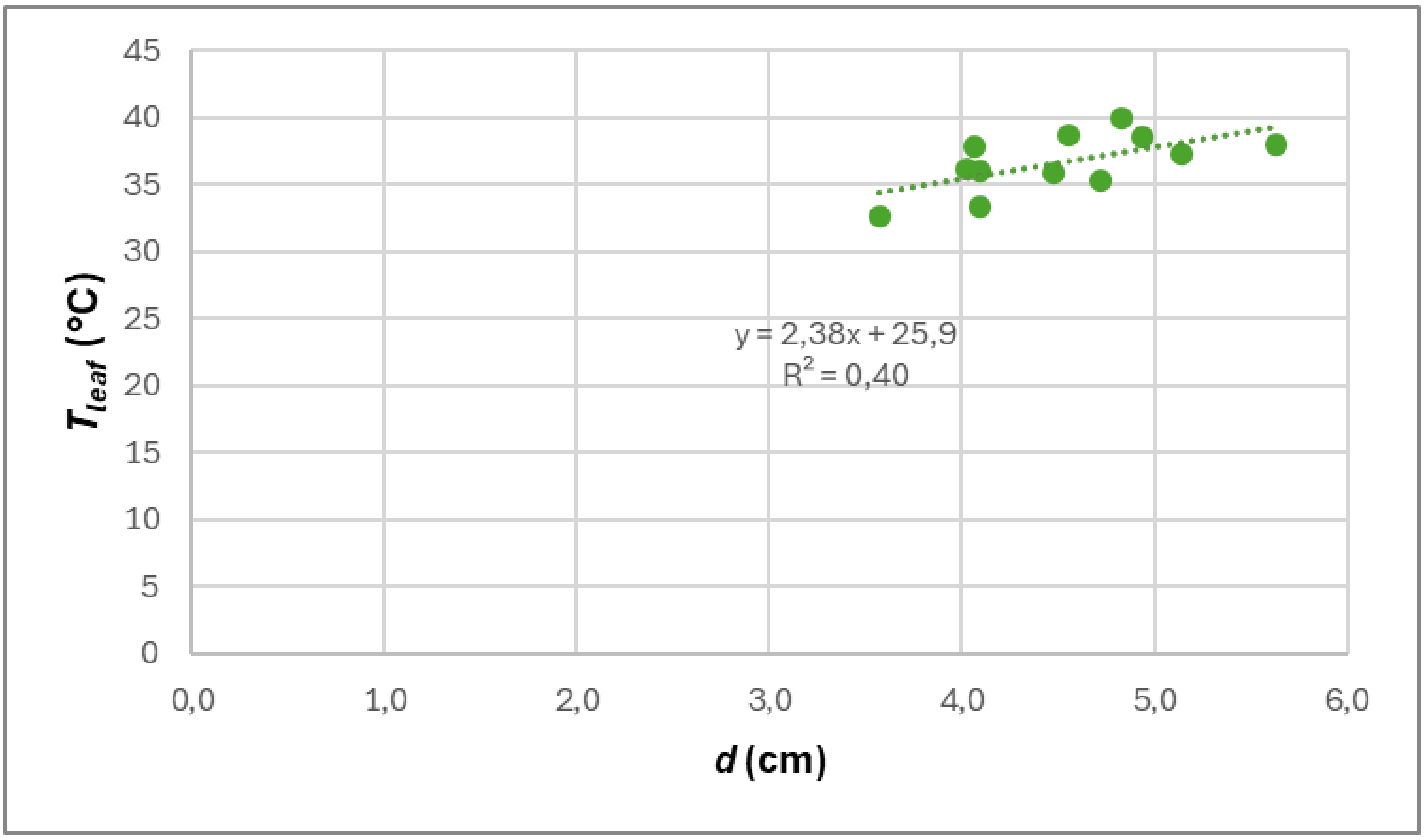


**Figure 7**. Leaf temperature ($T_{leaf}$) in relation to characteristic leaf dimension (*d*) in Bpe H during HW1. The p-value was 0,0161.

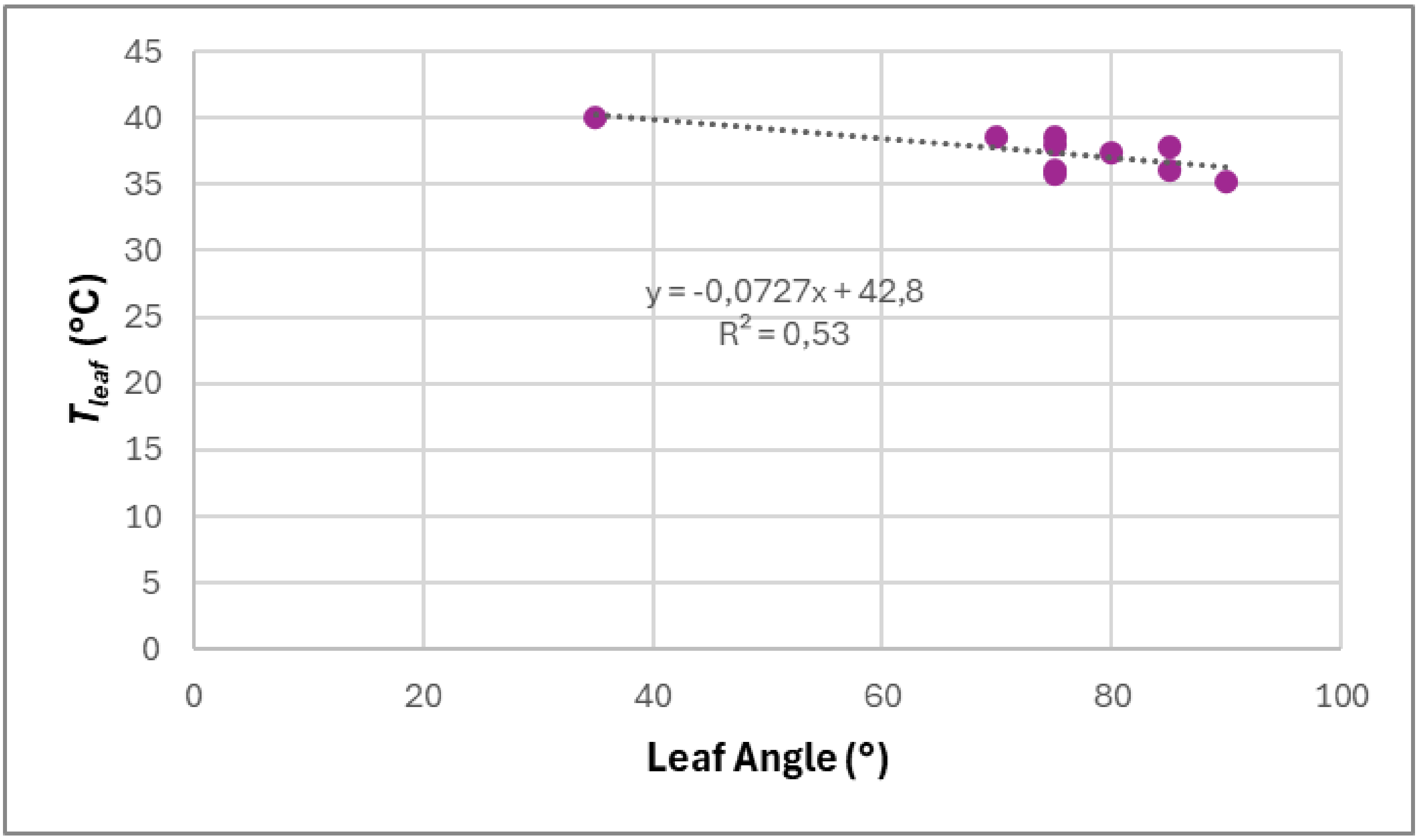


**Figure 8**. Leaf temperature ($T_{leaf}$) in relation to leaf angles in Bpe H during HW1. The scale for leaf angles ranged between 0 and 90 degrees, meaning the angles were measured based on their deviation in degrees from the horizontal plane. The p-value was 0,0129.

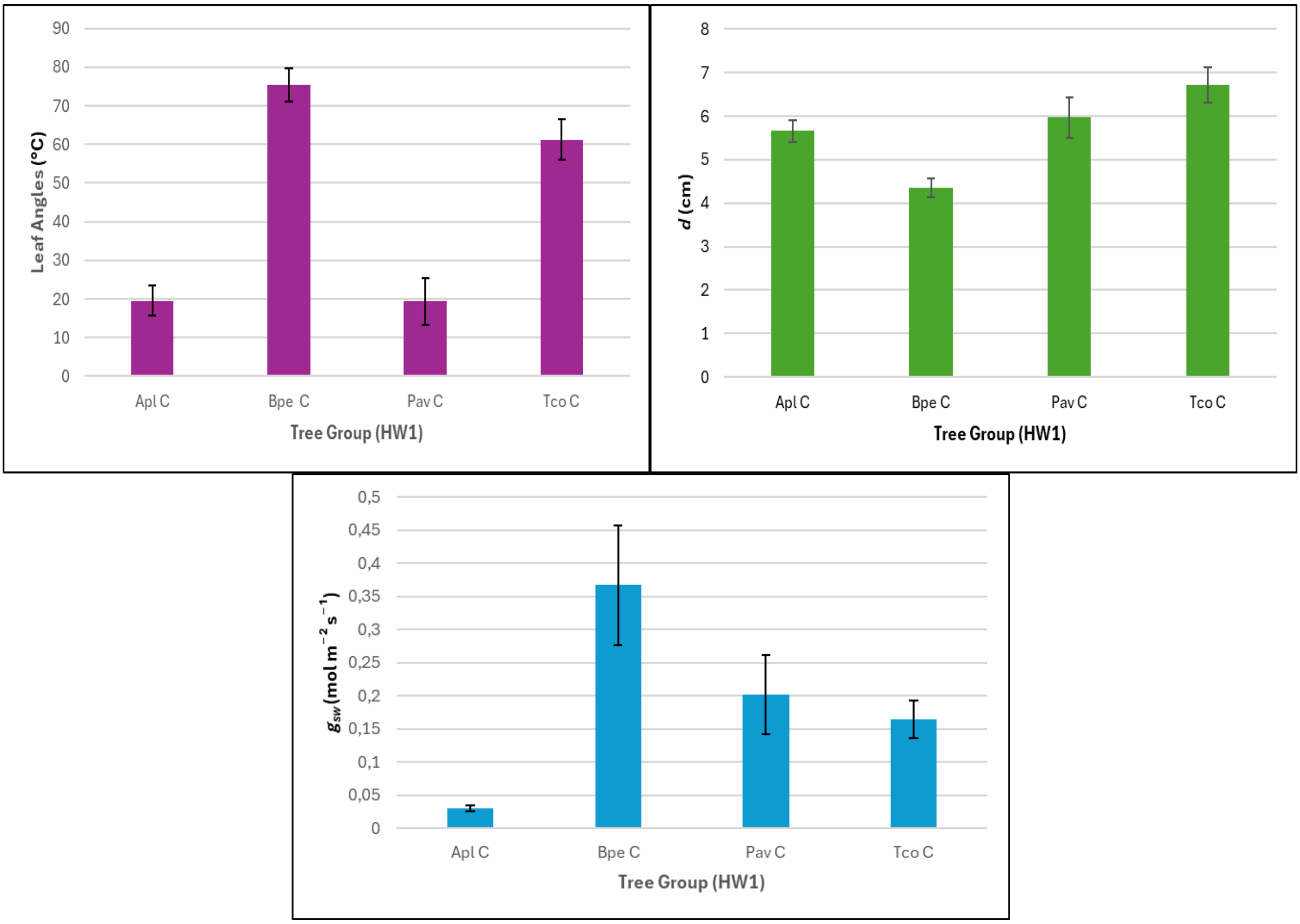


**Figure 9.** Showing the average leaf angles, characteristic leaf dimensions (*d*), and stomatal conductance to water vapor ($g_{sw}$) for control (C) trees during heatwave 1 (HW1) with added standard error bars. The scale for leaf angles ranged between 0 and 90 degrees, meaning the angles were measured based on their deviation in degrees from the horizontal plane. The four measured tree species were *Acer platanoides* (Apl), *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco).

### Results Acquired After the Heatwaves

*Figures 10* and *11* show the elevated temperature responses of $F_v/F_m$ during AHW1 and AHW2. From these values, $T_5$, $T_{50}$, and $T_{95}$ could be determined.

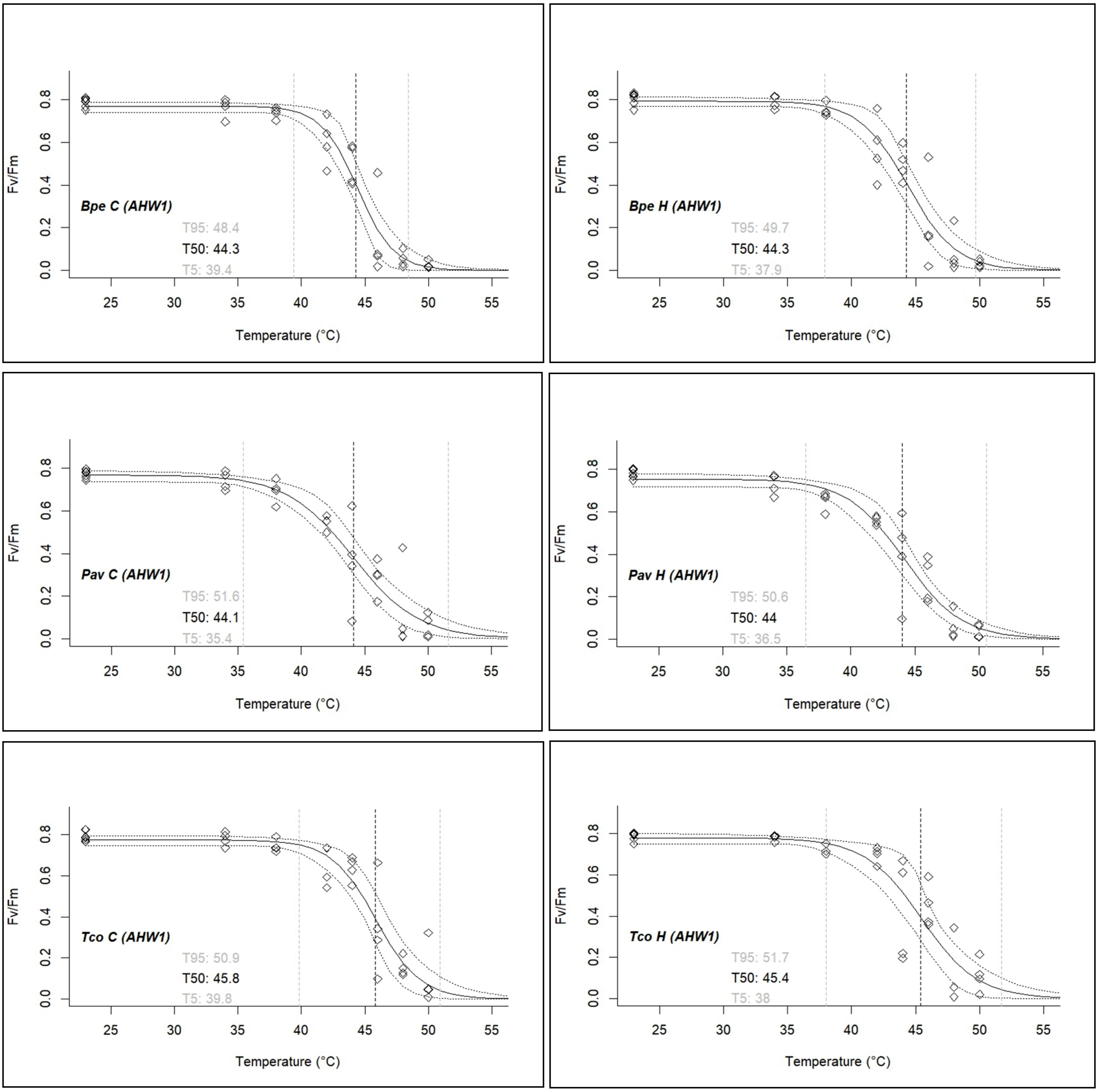


**Figure 10**. The temperature responses of $F_v/F_m$ after heatwave 1 (AHW1). From these $F_v/F_m$ values, $T_5$, $T_{50}$, and $T_{95}$ could be determined. $F_v/F_m$ is the maximal quantum efficiency of Photosystem II (PSII) and its decline can be seen as a general measurement of plant stress (in this case, heat stress). $T_5$, $T_{50}$, and $T_{95}$ represent the leaf temperatures where the $F_v/F_m$ ratio decreases by 5%, 50%, and 95%, respectively, from its unstressed value. The three measured tree species were *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco). H stands for "heatwave-exposed group," while C stands for "control group."

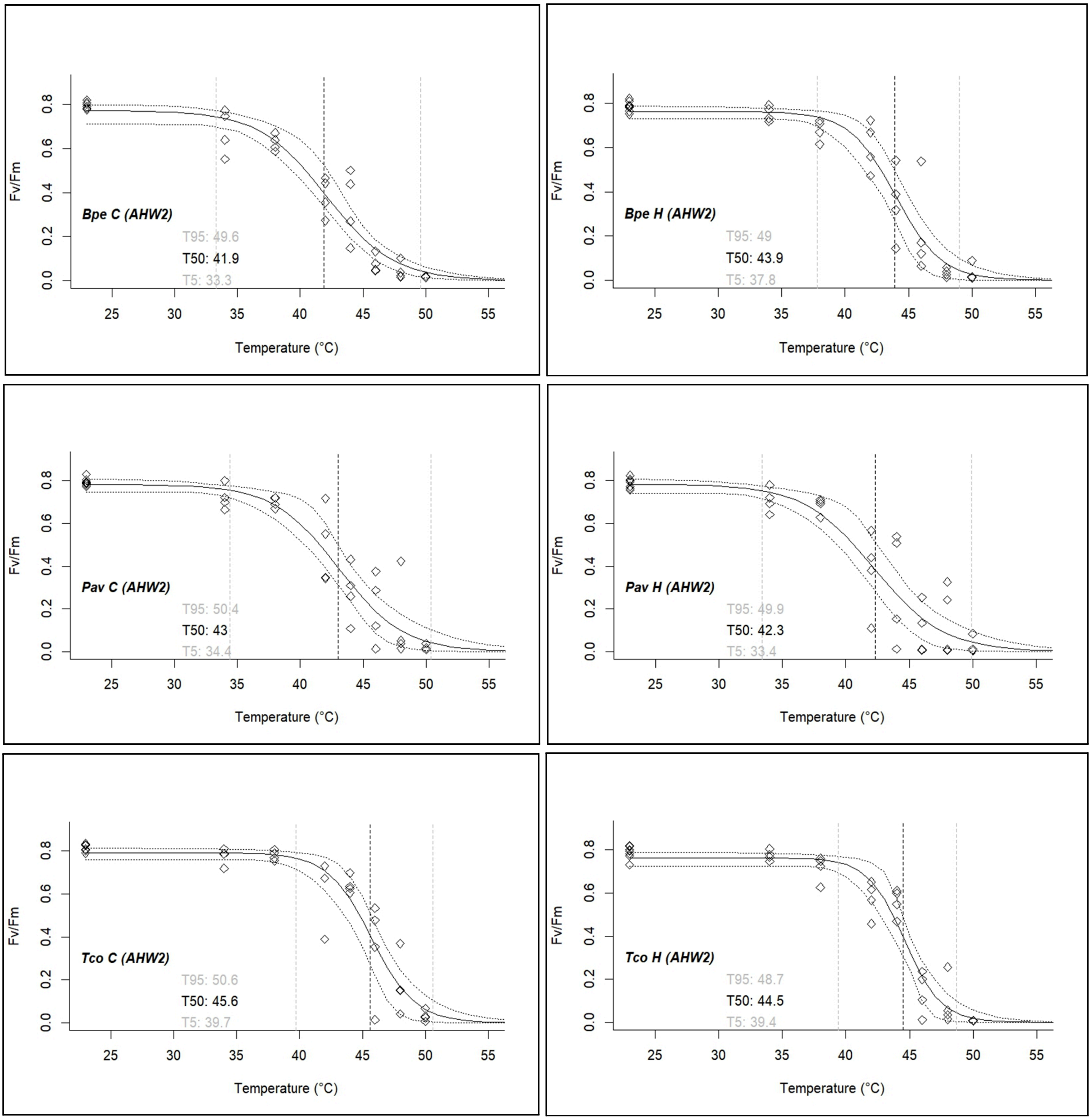


**Figure 11**. The temperature responses of $F_v/F_m$ after heatwave 2 (AHW2). From these $F_v/F_m$ values, $T_5$, $T_{50}$, and $T_{95}$ could be determined. $F_v/F_m$ is the maximal quantum efficiency of Photosystem II (PSII) and its decline can be seen as a general measurement of plant stress (in this case, heat stress). $T_5$, $T_{50}$, and $T_{95}$ represent the leaf temperatures where the $F_v/F_m$ ratio decreases by 5%, 50%, and 95%, respectively, from its unstressed value. The three measured tree species were *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco). H stands for "heatwave-exposed group," while C stands for "control group."

Below, the $T_5$, $T_{50}$, and $T_{95}$ values for different species and treatments are displayed in AHW1 and AHW2 (*Figures 12-14*). *Figures 12-14* provide a more detailed representation of the data presented in *Figures 9* and *10*, incorporating error bars to illustrate variability and enhance the precision of the results. *Welch's t-test* was used to see if there were any significant differences between the tested groups. The following comparisons were performed: a *species comparison* (control groups vs. control groups for AHW1 and AHW2), a *control and heatwave comparison* (C vs. H groups in AHW1 and AHW2) as well as a *heatwave group comparison* (H group in AHW1 vs. H group in AHW2). There were only two significant differences out of all of these comparisons: for $T_{50}$ between Tco C and Bpe C and between Tco C and Pav C in AHW2 (*Figure 13*). No significant differences existed between treatments or between the heatwaves. A substantial variation in T50 between the C and H groups would be considered evidence of heat acclimation within a particular species (Okubo et al., 2023), but this was not found (*Figure 13*).

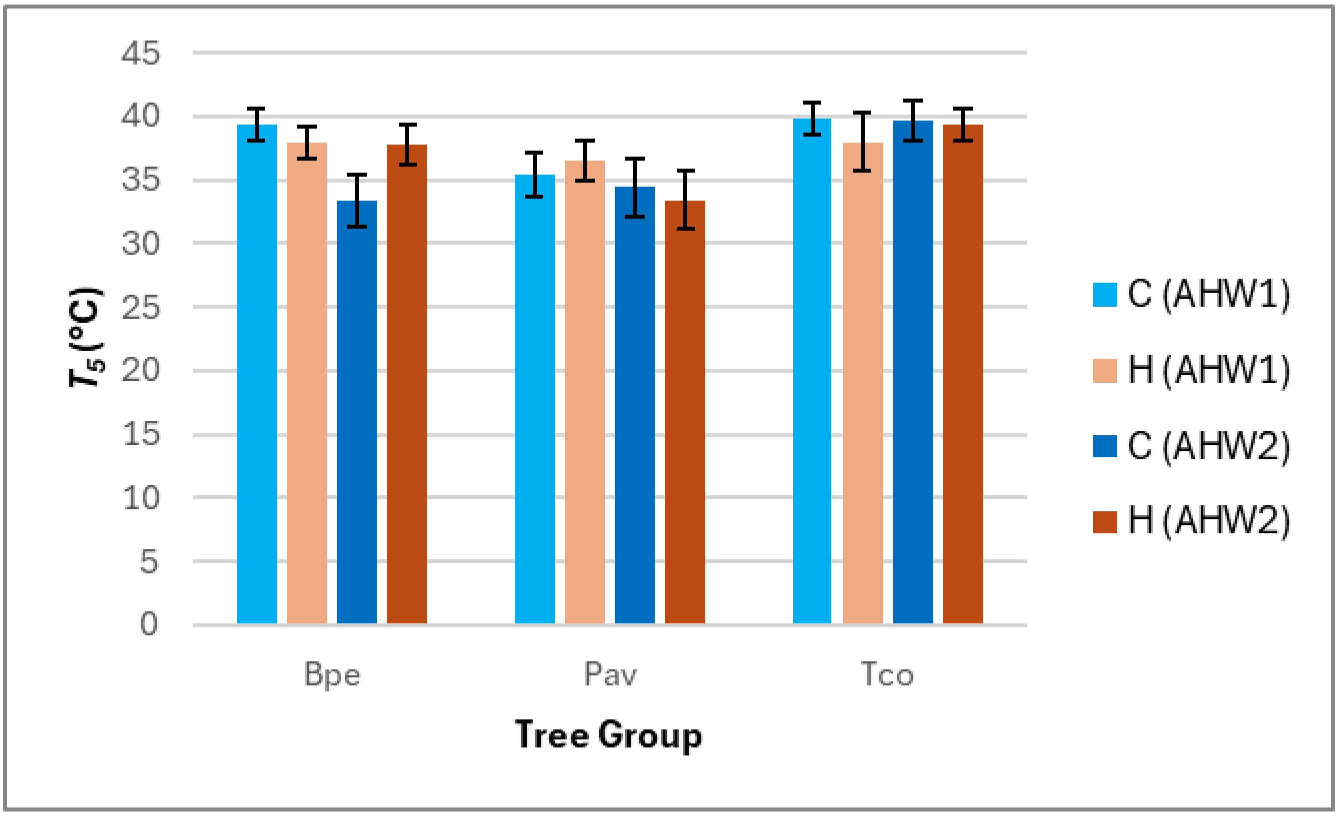


**Figure 12.** $T_5$ values after HW1 (AHW1) and after HW2 (AHW2). $T_5$ is the leaf temperature at which the $F_v/F_m$ ratio is decreased by 5 % from its unstressed value. The three measured tree species were *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco). H stands for “heatwave-exposed group,” while C stands for “control group.”

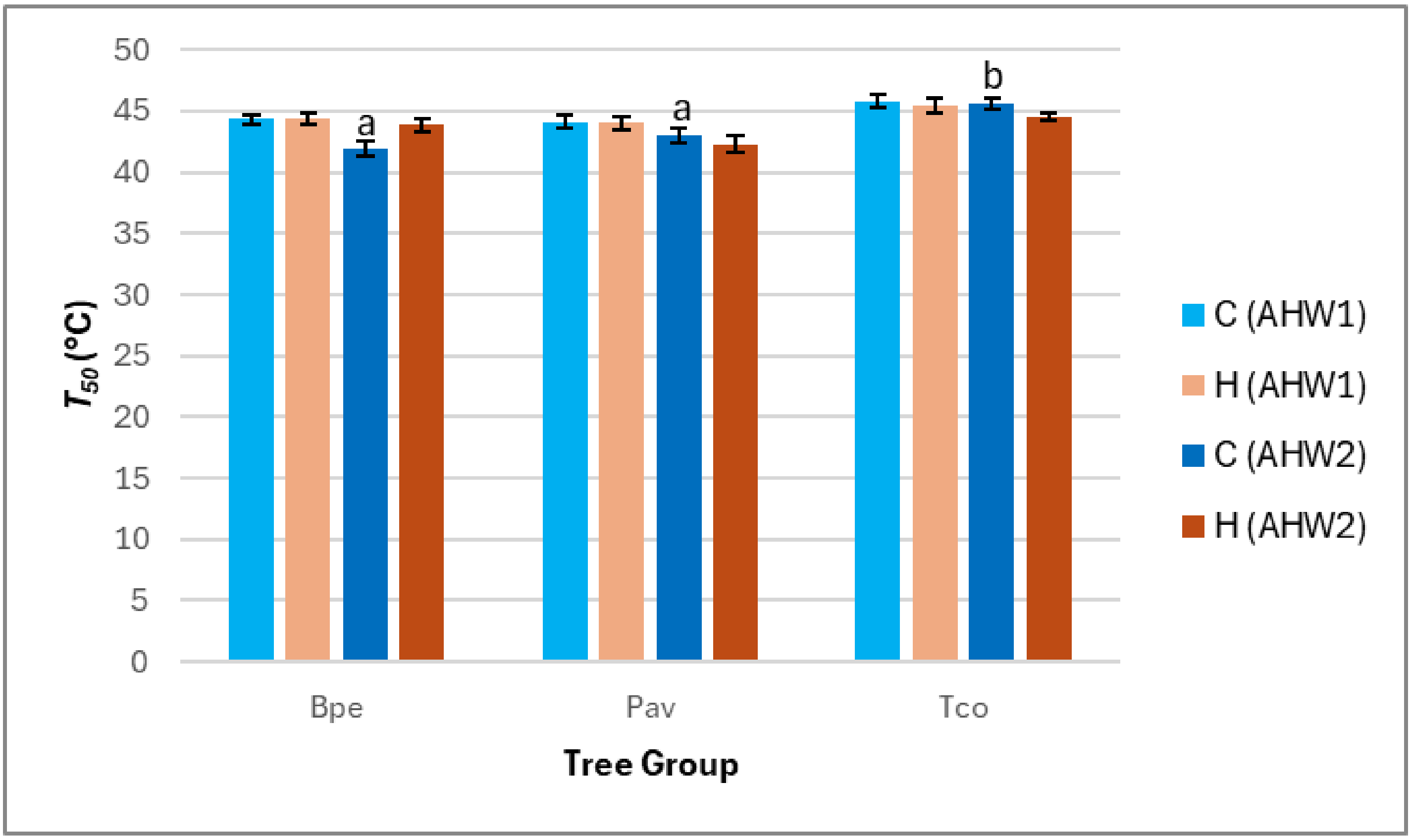


**Figure 13.** $T_{50}$ values after HW1 (AHW1) and after HW2 (AHW2). $T_{50}$ is the leaf temperature at which the $F_v/F_m$ ratio is decreased by 50 % from its unstressed value. The three measured tree species were *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco). H stands for "heatwave-exposed group," while C stands for "control group." The only occurring significant differences are labeled with significance letters. Bars sharing the same letter are not significantly different from each other (p > 0.05).

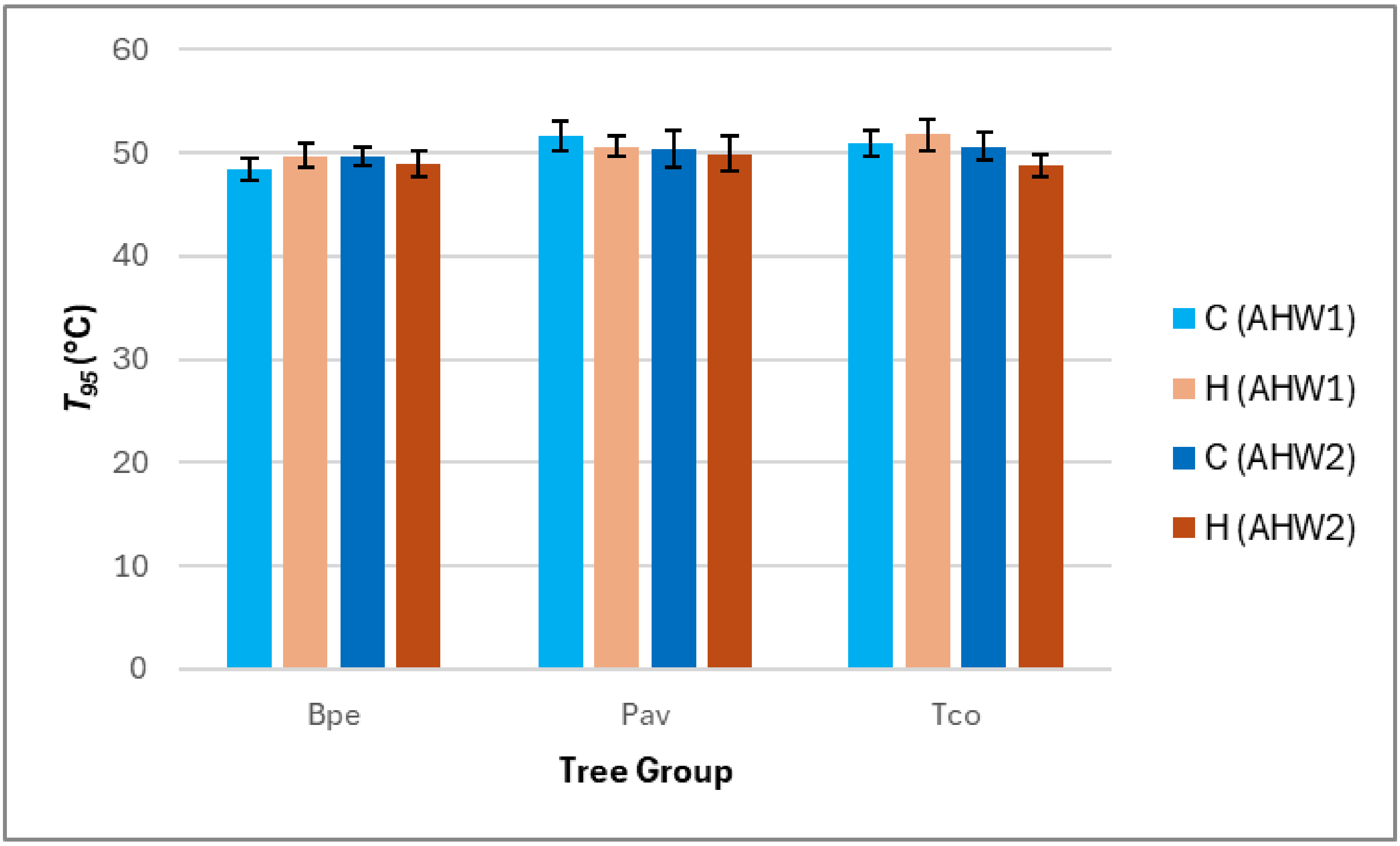


**Figure 14.** $T_{95}$ values after HW1 (AHW1) and after HW2 (AHW2). $T_{95}$ is the leaf temperature at which the $F_v/F_m$ ratio is decreased by 95 % from its unstressed value. No significant differences were found. The three

measured tree species were *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco). H stands for "heatwave-exposed group," while C stands for "control group."

*Figure 15* illustrates the individual tree groups in AHW1 and AHW3. The extreme heatwave (HW3 with a daytime peak temperature of 38°C) damaged the leaves. Still, the trees that previously experienced the milder heatwaves (HW1 and HW2 with a daytime peak temperature of 33°C) had no benefits from experiencing the milder heatwaves before being subjected to the more extreme heatwave, which is consistent with the results that no acclimation of $T_{50}$ occurred. In AHW3, however, all groups showed a decrease in their $F_v/F_m$ ratio except Pav C (*Figure 15*). Bpe H showed a higher $F_v/F_m$ than Bpe C AHW3, but this difference was not statistically significant.

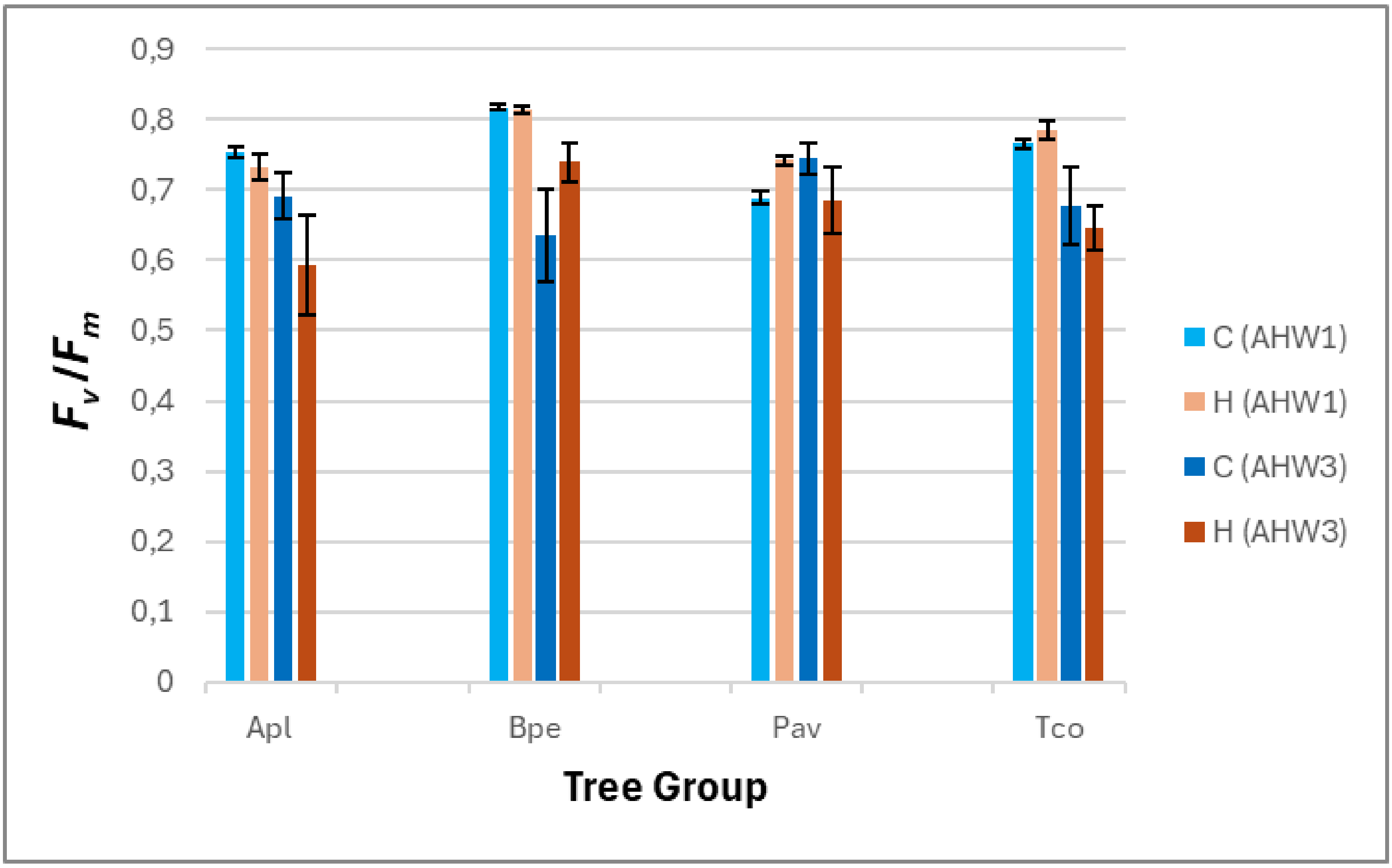


**Figure 15**. Different tree group $F_v/F_m$ average values after heatwave 1 (AHW1) and after heatwave 3 (AHW3) are shown with standard error bars. H stands for "heatwave-exposed group," while C stands for "control group." $F_v/F_m$ is the maximal quantum efficiency of PSII and its decline can be seen as a general measurement of plant stress (in this case, heat stress). The four measured tree species were *Acer platanoides* (Apl), *Betula pendula* (Bpe), *Prunus avium* (Pav), and *Tilia cordata* (Tco).

### 4.3 Thermal Safety Margin (TSM)

*Figure 16* was produced using data from both the HW and AHW phases and displays the average TSM values for the three heatwaves. Tco H consistently had the highest TSM throughout all three heatwaves. In contrast, Pav H had a negative TSM value during HW3, which indicates a potential high vulnerability to heat stress in this species, particularly under extreme heat events such as HW3. Finally, Bpe H had a small positive TSM during HW3, indicating that it experienced mild stress conditions during that heatwave period.

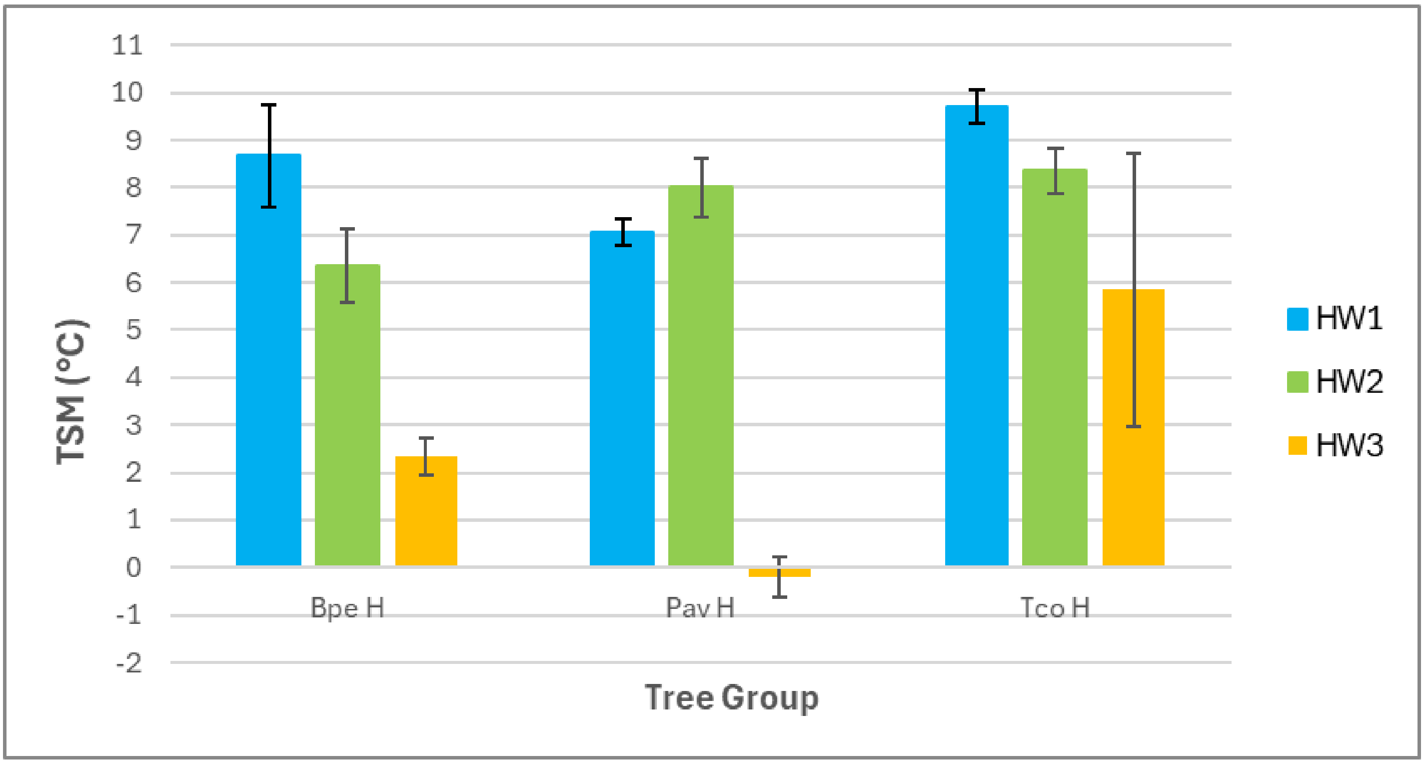


**Figure 16.** Average thermal safety margins (TSMs) during the three experiment heatwaves. TSM was calculated as the difference between the measured $T_{leaf}$ and $T_{50}$. HW1 and HW2 (heatwave 1 and 2) had a peak air temperature of 33°C, while heatwave 3 (HW3) had a peak air temperature of 38°C.

# 4. Discussion

The findings of this study shed light on heat tolerance in a few Swedish urban tree species, offering valuable insights into heat tolerance and acclimation in species where this data is largely missing. Thus, the information acquired through the making of this report can provide implications and some further guidance for urban city planners and urban foresters.

## Research Question 1

The first question of this report was to investigate the heat tolerance of different species and whether there are differences among selected urban trees. This question was answered by collecting the $F_v/F_m$ temperature response curves data and calculating the $T_{50}$ values. The only statistically significant differences were those between Tco C and Bpe C and between Tco C and Pav C after HW2 (*Figure 13*). Interestingly, there was a significant difference between control trees during HW2 but not during HW1. Further research is needed to elucidate the specific mechanisms underlying these differences and their impact on heat tolerance in urban tree species. According to the results received in this report, the findings indicate that the selected Swedish urban tree species with the highest heat tolerance would be *Tilia cordata* (small-leaved lime/little-leaf linden). A high $T_{50}$ contributed to generally quite high TSMs for this species (more about this under the subheading “Research Question 4” below). If this study were to be improved, more species would have been included, and more replicates would have been made to get a large sample size. How the experiment was set up, including how long and intense the heatwave exposures were, when measurements were taken, and how well confounding variables were controlled, could have impacted how the results were interpreted.

Zhu et al. (2018) highlight methodological challenges associated with comparing $T_{50}$ values across different studies. Variations in measurement approaches and environmental conditions must be acknowledged to ensure the reliability and comparability of $T_{50}$ values. Moreover, Teskey et al. (2015) discuss how environmental factors such as light intensity, humidity, and $CO_2$ levels impact heat stress and $T_{50}$ values. In another study by Münchinger et al. (2023), the $T_{50}$ ranged between 46.1 ± 0.4 and 53.6 ± 0.7 °C, which is generally noticeably higher than the ones received in this study. That experiment included e.g. *Acer platanoides, Acer pseudoplatanus, Prunus avium,* and *Tilia platophyllus* (and thus, there was a significant species and genera overlap), which likely means that differing environmental factors led to differing $T_{50}$ between the studies. Additionally, the lack of comparable $T_{50}$ data for Nordic urban trees underscores the importance of performing more studies like this.

### Research Question 2

The report's second question, whether the trees show heat acclimation after heatwave exposures or not, can be answered with the help of *Figures 11-15*. The $F_v/F_m$ quotient provides valuable insights into the photosynthetic efficiency of the trees after heatwave exposures. C and H groups showed no significant differences after HW1 or HW2, indicating no heat acclimation (*Figures 12-14*). Observing $F_v/F_m$ values trends across successive heatwave periods can offer valuable insights into the trees' photosynthetic efficiency and ability to acclimate to heat stress. If $F_v/F_m$ values stabilize or show improvement over time, it may suggest that the trees have undergone acclimation. The extreme heatwave, HW3, damaged the leaves. Nevertheless, the trees that previously experienced the milder heatwaves (HW1 and HW2) had no benefits from experiencing the milder heatwaves before being subjected to the more extreme heatwave, which is consistent with the results that no acclimation of $T_{50}$ occurred.

Since both C and H tree groups were subjected to 38°C in HW3, it is possible to see if there would be any acclimation. Based on the findings depicted in *Figure 15*, it can be observed that there was no significant acclimation. Assessing $F_v/F_m$ values in even additional heatwave periods beyond AHW3 could have elucidated more explicitly whether the trees undergo acclimation processes to better cope with recurring heatwave events. Examining other physiological parameters alongside $F_v/F_m$ values, such as chlorophyll content and enzyme activity, could help better understand the trees' acclimation capabilities in the future.

It is important to note that rapid heat stress episodes do not allow sufficient time for trees to acclimate (Daas et al., 2008; Ghouil et al., 2003), which could mean that the three days long heatwaves in this experiment were too short for these trees to acclimate to. A study by Reich et al. (2016) showed that leaf respiration was acclimated to temperature over many months, which further implies that the heatwaves were too short for acclimation. Another study, however, found that trees show limited capacity for acclimation to elevated temperatures (Kullberg and Feeley, 2022), which is strengthened by the findings of this report.

### Research Question 3

The third question in this report is whether there is a correlation between the three urban tree traits studied in this report (leaf size, leaf angles, and stomatal conductance) and temperature. In *Table 3*, it can be observed that several of the regressions made are significant. All significant $g_{sw}$ regressions had a negative slope (both HW1 and HW3), white the $d$ regressions had positive slope values in HW1 and negative ones in HW3. All of the $g_{sw}$ regressions values were negative which suggests that there is a pattern, but depending on the variability in the data, this is sometimes significant and sometimes not. For the leaf angle regressions, two regressions had a positive slope while one had a negative slope. The comparison between the significant regression values observed during HW1 and HW3 sheds light on the dynamic nature of the relationship between urban tree traits and temperature. Interestingly, while eight regression values were significant during HW1, indicating a correlation between tree traits and temperature under initial heatwave conditions, only five were significant during HW3. Notably, the eight significant values in HW1 were not always the same as those in HW3, suggesting a dynamic relationship between tree traits and temperature responses across different heatwave periods. This discrepancy raises questions about long-term trends and resilience of urban tree responses to varying heatwave intensities. If there was a robust, irrefutable correlation between these traits and temperature, the same trait-temperature correlations would have been expected to be significant in both HW1 and HW3 and not only in one. The observed differences in the number of significant regressions between the two heatwave events could lead to further investigation into the underlying factors causing these variations. Differences in the heatwaves' intensity or timing may have influenced the correlations' strength.

Studies by Lu et al. (2000) and Schulze et al. (1973) discuss how $g_{sw}$ plays a crucial role in regulating $T_{leaf}$, highlighting that open stomata during heat stress can help cool leaves via transpiration. This finding aligns with the significant negative $g_{sw}$ regression values noted in this research. Machado and Paulsen (2001) also explain how water availability impacts $g_{sw}$ and $T_{leaf}$, suggesting that different water conditions (the trees were not watered uniformly each time) during heatwaves might explain the variations in regression significance between HW1 and HW3.

O'Sullivan et al. (2017) stress the importance of stomatal behavior and $T_{leaf}$ regulation in alleviating heat stress, which supports the significant differences in $T_{leaf}$ for Apl due to its low $g_{sw}$ and horizontal leaf orientation. Berry and Bjorkman (1980) and Fauset et al. (2018) examine the impact of leaf angles on heat stress, indicating that horizontal leaf orientations can exacerbate damage from high light intensity. These findings support the importance of leaf angles in mitigating heat stress.

Tarvainen et al. (2022) confirm the effectiveness of elevated transpiration rates in mitigating heat stress, highlighting the key role of $g_{sw}$ and leaf cooling mechanisms. Zhu et al. (2018) provide context on the heat tolerance of different tree species, which supports this study's findings on the variability of $T_{leaf}$ among different trees. A report by Kitudom et al. (2022), the maximum $T_{air}$ and $T_{leaf}$ explained 68% of the variance of thermotolerance, while other leaf traits only explained 6%. This suggests that traits such as the ones measured in this study might not alter thermotolerance very much. Faucet et al. (2018), on the other hand, emphasize the importance of leaf functional traits in leaf thermoregulation.

Two reasons why there were several significant differences between Apl's $T_{leaf}$ in *Figures 3-5,* and the other trees are that Apl was found to have a very low $g_{sw}$ and the most horizontal leaves (thus receiving much radiation). Consequently, research that found that elevated transpiration is effective in mitigating heat stress seems to be further implied by this report. The leaf angles also seem to be important when it comes to mitigating heat stress (*Figure 9).*

## Research Question 4

This paper's last and fourth question was whether the different tree species have different TSMs. *Figure 16* shows the TSMs of all three heatwaves for Bpe, Pav, and Tco. The results show that these three species have differing TSMs in all three heatwaves. Tco had the highest TSM in all heatwaves, while Pav had the lowest TSM in the third and last heatwave. The elevated TSM in Tco is attributable to their high $T_{50}$ in combination with moderate $T_{leaf}$. Conversely, the low TSM in Pav during HW3 is due to relatively low $T_{50}$ and relatively high $T_{leaf}$. (Additionally, it should be noted that TSM does not exist for Apl due to time constraints when measuring $F_v/F_m$ temperature responses.) Variability in TSMs influences species distribution and resilience in urban environments, which is crucial for informed urban forestry decisions and climate adaptation. Integrating TSM knowledge into urban forestry management optimizes species selection, which mitigates climate impacts and improves sustainability.

Kitudom et al. (2022) showed that leaves' thermotolerances exhibited opposite trends with TSMs. In this study, however, no such trend could be identified. Tarvainen et al. (2022), on the other hand, found that TSMs were smaller in species with high $T_{leaf}$. Once more, no such relationship could be found. The differences could likely be attributed to variations in methodology, environmental conditions, species studied, temporal aspects, and statistical analysis. More research would be needed to ensure reliable conclusions in this matter.

### Methodological Limitations

Chlorophyll fluorescence is a suitable method for assessing heat stress and understanding plant heat tolerance, particularly in challenging conditions (Baker, 2004; Haque et al., 2014). When performing chlorophyll fluorescence experiments, it is important to be aware of the method's drawbacks. Although several commercial systems are available for measuring chlorophyll fluorescence, most of them assess fluorescence at specific leaf points rather than capturing the overall variability across a canopy. Consequently, multiple measurements are often required for a comprehensive understanding, making the process time-consuming and laborious (Mishra et al., 2014). Additionally, the fluorometer instrument in this experiment required leaf clamps during measurement, which sometimes can damage the plant sample (Arief et al., 2023).

The chlorophyll fluorescence method was chosen because it can evaluate how urban trees respond to heat stress directly and easily. Despite efforts to simulate natural conditions in the laboratory, no single method can fully represent the complex responses of plants to heat stress. However, through careful experiment planning and a suitable tree selection, this approach provides valuable insights into urban tree thermal tolerance. By combining various measurements, this report gained some further understanding of a few less-studied urban tree species and enhanced knowledge of plant responses to heat stress.

### Future Research

The findings of this report highlight the importance of understanding how different species respond to heat stress, particularly in the context of climate change and urban forestry management. While this study sheds light on current heat tolerance dynamics, future research should explore ways to enhance urban tree resilience further. Climate change models offer a promising tool for identifying heat-tolerant tree species suitable for specific urban environments (Percival, 2023). Short-term management strategies, including suitable irrigation and nutrient management focusing on potassium, magnesium, and calcium, show some promise in increasing tree survival during heat spells (Percival, 2023). However, there is a need for further research to better understand and refine these strategies. One of the challenges in understanding heat tolerance in urban trees is the limited focus of scientific research on a small number of tree genera (Percival, 2023). Therefore, it is essential to explore differences in heat tolerance between additional species and assess genetic variation in high-temperature tolerance within species.

## 5. Conclusions

In conclusion, this study offers insights into Swedish urban tree species' heat tolerance and acclimation. *Tilia cordata* showed slightly better heat tolerance than *Betula pendula* and *Prunus avium*. The trees showed no significant signs of acclimation since there were no treatment differences after HW1 and HW2, and since the stress was the same in C and H after HW3. The relationships that generally resulted in high $T_{leaf}$ seemed to be horizontal leaves and low $g_{sw}$, with *Acer platanoides* having the highest $T_{leaf}$. Furthermore, the examined trees had differing TSMs with *Tilia cordata* having the largest one, and *Prunus avium* having the smallest one. The findings highlight the importance of considering the complex relationship between urban tree responses to temperature and environmental factors in informing effective urban forestry and climate improvement strategies. Moving forward, long-term monitoring and interdisciplinary research efforts will be decisive in further understanding the mechanisms regulating urban tree responses to temperature stress and facilitating the development of management strategies to enhance urban tree resilience in a world facing climate change.

## Acknowledgments

I want to express my thanks to my supervisors, Johan Uddling Fredin and Olivier Jean Leonce Manzi, for their guidance during this project. Special thanks go to Astrid Fridell and Lasse Tarvainen for their practical assistance in the lab and valuable advice. Lastly, I want to thank Antonia Schauer for working alongside me to care for the experiment trees and for providing me with stomatal conductance data. Each of you has played a critical role in the success of this project.

## References

Allakhverdiev, S.I., Kreslavski, V.D., Klimov, V.V., Los, D.A., Carpentier, R., Mohanty, P., 2008. Heat stress: an overview of molecular responses in photosynthesis. Photosynth. Res. 98, 541–550. https://doi.org/10.1007/s11120-008-9331-0

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H. (Ted), Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running, S.W., Semerci, A., Cobb, N., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manag., Adaptation of Forests and Forest Management to Changing Climate 259, 660–684. https://doi.org/10.1016/j.foreco.2009.09.001

Arief, M.A.A., Kim, H., Kurniawan, H., Nugroho, A.P., Kim, T., Cho, B.-K., 2023. Chlorophyll Fluorescence Imaging for Early Detection of Drought and Heat Stress in Strawberry Plants. Plants 12, 1387. https://doi.org/10.3390/plants12061387

Bauweraerts, I., Ameye, M., Wertin, T.M., McGuire, M.A., Teskey, R.O., Steppe, K., 2014. Water availability is the decisive factor for the growth of two tree species in the occurrence of consecutive heat waves. Agric. For. Meteorol. 189–190, 19–29. https://doi.org/10.1016/j.agrformet.2014.01.001

Berry, J., Bjorkman, O., 1980. Photosynthetic Response and Adaptation to Temperature in Higher Plants. Annu. Rev. Plant Biol. 31, 491–543. https://doi.org/10.1146/annurev.pp.31.060180.002423

Bhargava, A., Lakmini, S., Bhargava, S., 2017. Urban Heat Island Effect: It’s Relevance in Urban Planning. J. Biodivers. Endanger. Species 05. https://doi.org/10.4172/2332-2543.1000187

Bilger, H.-W., Schreiber, U., Lange, O.L., 1984. Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. Oecologia 63, 256–262. https://doi.org/10.1007/BF00379886

Bita, C.E., Gerats, T., 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front. Plant Sci. 4, 273. https://doi.org/10.3389/fpls.2013.00273

Brune, M., 2016. Urban trees under climate change.

Charng, Y., Mitra, S., Yu, S.-J., 2023. Maintenance of abiotic stress memory in plants: Lessons learned from heat acclimation. Plant Cell 35, 187–200. https://doi.org/10.1093/plcell/koac313

Cook, A.M., Berry, N., Milner, K.V., Leigh, A., 2021. Water availability influences thermal safety margins for leaves. Funct. Ecol. 35, 2179–2189. https://doi.org/10.1111/1365-2435.13868

Coumou, D., Robinson, A., 2013. Historic and future increase in the global land area affected by monthly heat extremes. Environ. Res. Lett. 8, 034018. https://doi.org/10.1088/1748-9326/8/3/034018

Curtis, E.M., Gollan, J., Murray, B.R., Leigh, A., 2016. Native microhabitats better predict tolerance to warming than latitudinal macro-climatic variables in arid-zone plants. J. Biogeogr. 43, 1156–1165.

https://doi.org/10.1111/jbi.12713

Daas, C., Montpied, P., Hanchi, B., Dreyer, E., 2008. Responses of photosynthesis to high temperatures in oak saplings assessed by chlorophyll-a fluorescence: inter-specific diversity and temperature-induced plasticity. Ann. For. Sci. 65, 305–305. https://doi.org/10.1051/forest:2008002

De Boeck, H.J., Van De Velde, H., De Groote, T., Nijs, I., 2016. Ideas and perspectives: Heat stress: more than hot air. Biogeosciences 13, 5821–5825. https://doi.org/10.5194/bg-13-5821-2016

Devireddy, A.R., Tschaplinski, T.J., Tuskan, G.A., Muchero, W., Chen, J.-G., 2021. Role of Reactive Oxygen Species and Hormones in Plant Responses to Temperature Changes. Int. J. Mol. Sci. 22, 8843. https://doi.org/10.3390/ijms22168843

Diem, J.E., Stauber, C.E., Rothenberg, R., 2017. Heat in the southeastern United States: Characteristics, trends, and potential health impact. PLOS ONE 12, e0177937. https://doi.org/10.1371/journal.pone.0177937

Esperon-Rodriguez, M., Power, S.A., Tjoelker, M.G., Marchin, R.M., Rymer, P.D., 2021. Contrasting heat tolerance of urban trees to extreme temperatures during heatwaves. Urban For. Urban Green. 66, 127387. https://doi.org/10.1016/j.ufug.2021.127387

Fauset, S., Freitas, H.C., Galbraith, D.R., Sullivan, M.J.P., Aidar, M.P.M., Joly, C.A., Phillips, O.L., Vieira, S.A., Gloor, M.U., 2018. Differences in leaf thermoregulation and water use strategies between three co-occurring Atlantic forest tree species. Plant Cell Environ. 41, 1618. https://doi.org/10.1111/pce.13208

Filewod, B., Thomas, S.C., 2014. Impacts of a spring heat wave on canopy processes in a northern hardwood forest. Glob. Change Biol. 20, 360–371. https://doi.org/10.1111/gcb.12354

Ghouil, H., Montpied, P., Epron, D., Ksontini, M., Hanchi, B., Dreyer, E., 2003. Thermal optima of photosynthetic functions and thermostability of photochemistry in cork oak seedlings. Tree Physiol. 23, 1031–1039. https://doi.org/10.1093/treephys/23.15.1031

Harfouche, A., Meilan, R., Altman, A., 2011. Tree genetic engineering and applications to sustainable forestry and biomass production. Trends Biotechnol. 29, 9–17. https://doi.org/10.1016/j.tibtech.2010.09.003

Hassan, M.U., Chattha, M.U., Khan, I., Chattha, M.B., Barbanti, L., Aamer, M., Iqbal, M.M., Nawaz, M., Mahmood, A., Ali, A., Aslam, M.T., 2021. Heat stress in cultivated plants: nature, impact, mechanisms, and mitigation strategies—a review. Plant Biosyst. - Int. J. Deal. Asp. Plant Biol. 155, 211–234. https://doi.org/10.1080/11263504.2020.1727987

Hu, S., Ding, Y., Zhu, C., 2020. Sensitivity and Responses of Chloroplasts to Heat Stress in Plants. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00375

Jones, H., Rotenberg, E., 2001. Energy, Radiation and Temperature Regulation in Plants, in: eLS. https://doi.org/10.1038/npg.els.0003199

Kelsey, R.G., Westlind, D.J., 2017. Physiological Stress and Ethanol Accumulation in Tree Stems and Woody Tissues at Sublethal Temperatures from Fire. BioScience 67, 443–451. https://doi.org/10.1093/biosci/bix037

Kitao, M., Lei, T.T., Koike, T., 1998. Application of chlorophyll fluorescence to evaluate Mn tolerance of deciduous broad-leaved tree seedlings native to northern Japan. Tree Physiol. 18, 135–140. https://doi.org/10.1093/treephys/18.2.135

Kitudom, N., Fauset, S., Zhou, Y., Fan, Z., Li, M., He, M., Zhang, S., Xu, K., Lin, H., 2022. Thermal safety margins of plant leaves across biomes under a heatwave. Sci. Total Environ. 806, 150416. https://doi.org/10.1016/j.scitotenv.2021.150416

Knight, C.A., Ackerly, D.D., 2003. Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area and leaf size: congeneric species from desert and coastal environments. New Phytol. 160, 337–347. https://doi.org/10.1046/j.1469-8137.2003.00880.x

Krause, G.H., Cheesman, A.W., Winter, K., Krause, B., Virgo, A., 2013. Thermal tolerance, net CO2 exchange and growth of a tropical tree species, *Ficus insipida*, cultivated at elevated daytime and nighttime temperatures. J. Plant Physiol. 170, 822–827. https://doi.org/10.1016/j.jplph.2013.01.005

Kullberg, A.T., Feeley, K.J., 2022. Limited acclimation of leaf traits and leaf temperatures in a subtropical urban heat island. Tree Physiol. 42, 2266–2281. https://doi.org/10.1093/treephys/tpac066

Leal Filho, W., Echevarria Icaza, L., Neht, A., Klavins, M., Morgan, E.A., 2018. Coping with the impacts of urban heat islands. A literature based study on understanding urban heat vulnerability and the need for resilience in cities in a global climate change context. J. Clean. Prod. 171, 1140–1149. https://doi.org/10.1016/j.jclepro.2017.10.086

Lin, H., Chen, Y., Zhang, H., Fu, P., Fan, Z., 2017. Stronger cooling effects of transpiration and leaf physical traits of plants from a hot dry habitat than from a hot wet habitat. Funct. Ecol. 31, 2202–2211. https://doi.org/10.1111/1365-2435.12923

Livesley, S.J., McPherson, E.G., Calfapietra, C., 2016. The Urban Forest and Ecosystem Services: Impacts on Urban Water, Heat, and Pollution Cycles at the Tree, Street, and City Scale. J. Environ. Qual. 45, 119–124. https://doi.org/10.2134/jeq2015.11.0567

Lu, Z., Quiñones, M.A., Zeiger, E., 2000. Temperature dependence of guard cell respiration and stomatal conductance co-segregate in an F2 population of Pima cotton. Funct. Plant Biol. 27, 457–462. https://doi.org/10.1071/pp98128

Luo, H., Merope, T., Zhang, Y., Zhang, W., 2016. Combining gas exchange and chlorophyll a fluorescence measurements to analyze the photosynthetic activity of drip-irrigated cotton under different soil water deficits. J. Integr. Agric. 15, 1256–1266. https://doi.org/10.1016/S2095-3119(15)61270-9

Machado, S., Paulsen, G.M., 2001. Combined effects of drought and high temperature on water relations of wheat and sorghum. Plant Soil 233, 179–187. https://doi.org/10.1023/A:1010346601643

Mathur, S., Agrawal, D., Jajoo, A., 2014. Photosynthesis: Response to high temperature stress. J. Photochem. Photobiol. B, Stress and Photosynthesis 137, 116–126. https://doi.org/10.1016/j.jphotobiol.2014.01.010

Mazorra, L.M., Núñez, M., Hechavarria, M., Coll, F., Sánchez-Blanco, M.J., 2002. Influence of Brassinosteroids on Antioxidant Enzymes Activity in Tomato Under Different Temperatures. Biol. Plant. 45, 593–596. https://doi.org/10.1023/A:1022390917656

Michaletz, S.T., Weiser, M.D., McDowell, N.G., Zhou, J., Kaspari, M., Helliker, B.R., Enquist, B.J., 2016. The energetic and carbon economic origins of leaf thermoregulation. Nat. PLANTS 2, 16129. https://doi.org/10.1038/NPLANTS.2016.129

Mishra, A., Heyer, A.G., Mishra, K.B., 2014. Chlorophyll fluorescence emission can screen cold tolerance of cold acclimated Arabidopsis thaliana accessions. Plant Methods 10, 38. https://doi.org/10.1186/1746-4811-10-38

Münchinger, I.K., Hajek, P., Akdogan, B., Caicoya, A.T., Kunert, N., 2023. Leaf thermal tolerance and sensitivity of temperate tree species are correlated with leaf physiological and functional drought resistance traits. J. For. Res. 34, 63–76. https://doi.org/10.1007/s11676-022-01594-y

Okubo, N., Inoue, S., Ishii, H.R., 2023. Tolerance and Acclimation of the Leaves of Nine Urban Tree Species to High Temperatures. Forests 14, 1639. https://doi.org/10.3390/f14081639

O'sullivan, O.S., Heskel, M.A., Reich, P.B., Tjoelker, M.G., Weerasinghe, L.K., Penillard, A., Zhu, L., Egerton, J.J.G., Bloomfield, K.J., Creek, D., Bahar, N.H.A., Griffin, K.L., Hurry, V., Meir, P., Turnbull, M.H., Atkin, O.K., 2017. Thermal limits of leaf metabolism across biomes. Glob. Change Biol. 23, 209–223. https://doi.org/10.1111/gcb.13477

Percival, G.C., 2023. Heat tolerance of urban trees − A review. Urban For. Urban Green. 86, 128021. https://doi.org/10.1016/j.ufug.2023.128021

Percival, G.C., 2017. The influence of glyphosate on carotenoid pigments, reactive oxygen species scavenging enzymes and secondary stress metabolites within leaf tissue of three Acer species. Urban For. Urban Green. 24, 19–25. https://doi.org/10.1016/j.ufug.2017.03.018

Percival, G.C., Henderson, A., 2003. An assessment of the freezing tolerance of urban trees using chlorophyll fluorescence. J. Hortic. Sci. Biotechnol. 78, 254–260. https://doi.org/10.1080/14620316.2003.11511614

Reich, P.B., Sendall, K.M., Stefanski, A., Wei, X., Rich, R.L., Montgomery, R.A., 2016. Boreal and temperate trees show strong acclimation of respiration to warming. Nature 531, 633–636. https://doi.org/10.1038/nature17142

Schulze, E.-D., Lange, O.L., Kappen, L., Buschbom, U., Evenari, M., 1973. Stomatal responses to changes in temperature at increasing water stress. Planta 110, 29–42. https://doi.org/10.1007/BF00386920

Sharma, A., Andhikaputra, G., Wang, Y.-C., 2022. Heatwaves in South Asia: Characterization, Consequences on Human Health, and Adaptation Strategies. Atmosphere 13, 734. https://doi.org/10.3390/atmos13050734

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T., Huey, R.B., 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proc. Natl. Acad. Sci. 111, 5610–5615. https://doi.org/10.1073/pnas.1316145111

Tan, J., Zheng, Y., Tang, X., Guo, C., Li, L., Song, G., Zhen, X., Yuan, D., Kalkstein, A.J., Li, F., Chen, H., 2010. The urban heat island and its impact on heat waves and human health in Shanghai. Int. J. Biometeorol. 54, 75–84. https://doi.org/10.1007/s00484-009-0256-x

Tarvainen, L., Wittemann, M., Mujawamariya, M., Manishimwe, A., Zibera, E., Ntirugulirwa, B., Ract, C., Manzi, O.J.L., Andersson, M.X., Spetea, C., Nsabimana, D., Wallin, G., Uddling, J., 2022. Handling the heat – photosynthetic thermal stress in tropical trees. New Phytol. 233, 236–250. https://doi.org/10.1111/nph.17809

Teskey, R., Wertin, T., Bauweraerts, I., Ameye, M., McGuire, M.A., Steppe, K., 2015. Responses of tree species to heat waves and extreme heat events. Plant Cell Environ. 38, 1699–1712. https://doi.org/10.1111/pce.12417

US EPA, O., 2014. Using Trees and Vegetation to Reduce Heat Islands [WWW Document]. URL https://www.epa.gov/heatislands/using-trees-and-vegetation-reduce-heat-islands (accessed 12.17.23).

Vårhammar, A., Wallin, G., McLean, C.M., Dusenge, M.E., Medlyn, B.E., Hasper, T.B., Nsabimana, D., Uddling, J., 2015. Photosynthetic temperature responses of tree species in Rwanda: evidence of pronounced negative effects of high temperature in montane rainforest climax species. New Phytol. 206, 1000–1012. https://doi.org/10.1111/nph.13291

Wahid, A., Gelani, S., Ashraf, M., Foolad, M.R., 2007. Heat tolerance in plants: An overview. Environ. Exp. Bot. 61, 199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011

Yang, Z., Jiang, Y., Qiu, R., Gong, X., Agathokleous, E., Hu, W., Clothier, B., 2023. Heat stress decreased transpiration but increased evapotranspiration in gerbera. Front. Plant Sci. 14, 1119076. https://doi.org/10.3389/fpls.2023.1119076

Yao, Y., Luo, Y., Huang, J., Zhao, Z., 2013. Comparison of Monthly Temperature Extremes Simulated by CMIP3 and CMIP5 Models. J. Clim. 26, 7692–7707. https://doi.org/10.1175/JCLI-D-12-00560.1

Zhu, L., Bloomfield, K.J., Hocart, C.H., Egerton, J.J.G., O’Sullivan, O.S., Penillard, A., Weerasinghe, L.K., Atkin, O.K., 2018. Plasticity of photosynthetic heat tolerance in plants adapted to thermally contrasting biomes. Plant Cell Environ. 41, 1251–1262. https://doi.org/10.1111/pce.13133

# Appendix

## Appendix A

| | Control | | Heatwave 1 & 2 | | Heatwave 3 | | Light (15 cm from lamps) |
|---|---|---|---|---|---|---|---|
| | T (°C) | RH (%) | T (°C) | RH (%) | T (°C) | RH (%) | PPFD (μmol/m$^2$/s) |
| 00:00 | 17 | 80 | 19 | 80 | 21 | 80 | 0 |
| 01:00 | 17 | 80 | 19 | 80 | 21 | 80 | 0 |
| 02:00 | 17 | 80 | 19 | 80 | 21 | 80 | 0 |
| 03:00 | 17 | 80 | 19 | 80 | 21 | 80 | 0 |
| 04:00 | 17 | 80 | 19 | 80 | 21 | 80 | 300 |
| 05:00 | 18 | 75 | 21 | 75 | 22 | 75 | 600 |
| 06:00 | 19 | 70 | 23 | 65 | 23 | 65 | 900 |
| 07:00 | 20 | 65 | 26 | 55 | 24 | 55 | 1200 |
| 08:00 | 21 | 60 | 29 | 45 | 29 | 45 | 1200 |
| 09:00 | 22 | 55 | 31 | 40 | 34 | 40 | 1200 |
| 10:00 | 23 | 50 | 33 | 40 | 38 | 40 | 1200 |
| 11:00 | 23 | 50 | 33 | 40 | 38 | 40 | 1200 |
| 12:00 | 23 | 50 | 33 | 40 | 38 | 40 | 1200 |
| 13:00 | 23 | 50 | 33 | 40 | 38 | 40 | 1200 |
| 14:00 | 23 | 50 | 33 | 40 | 38 | 40 | 1200 |
| 15:00 | 23 | 50 | 33 | 40 | 38 | 40 | 1200 |
| 16:00 | 23 | 50 | 33 | 40 | 38 | 40 | 1200 |
| 17:00 | 23 | 50 | 33 | 40 | 38 | 40 | 1200 |
| 18:00 | 22 | 55 | 31 | 40 | 31 | 40 | 900 |
| 19:00 | 21 | 60 | 29 | 45 | 28 | 45 | 600 |
| 20:00 | 20 | 65 | 27 | 50 | 26 | 50 | 300 |
| 21:00 | 19 | 70 | 25 | 55 | 24 | 55 | 0 |
| 22:00 | 18 | 75 | 23 | 65 | 23 | 65 | 0 |
| 23:00 | 18 | 75 | 21 | 75 | 22 | 75 | 0 |

**(T, temperature; RH, relative humidity.)**