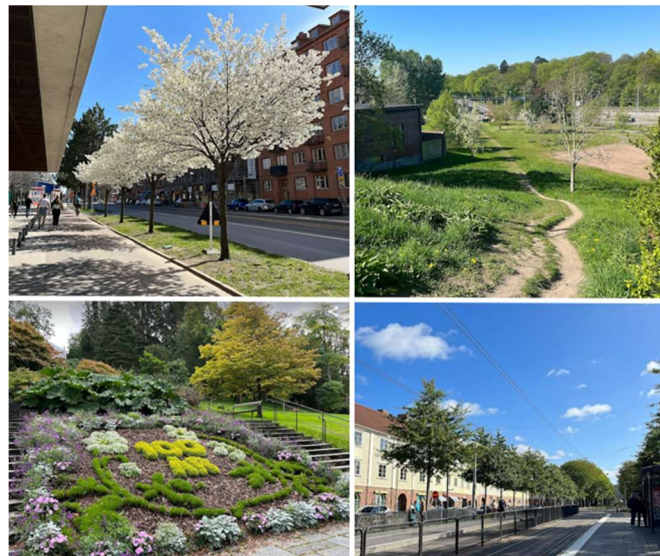




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## Department of Biological and Environmental Sciences

# Tolerance and acclimation capacity of four Swedish urban tree species to heatwaves



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## Abstract

With progression of global warming and concerning projections of increasing heatwave intensity and frequency, its' full effects on urban trees are still unclear. Harsh urban conditions like the heat-island effect call for robust tree species that can deal with several stressors. To gain knowledge about which tree species should be used in urban planning for viable future urban greenery, four urban tree species commonly grown in Swedish cities were assessed for heat-tolerance and thermal acclimation abilities to recurrent heatwaves. Namely, *Acer platanoides*, *Betula pendula*, *Prunus avium* and *Tilia cordata*. It was investigated (1) how heat tolerance varied among the tree species, (2) if they were able to thermally acclimate to heatwaves and (3) if plants repeatedly exposed to heatwaves displayed higher heat tolerance than those that have not. To address this, the plants were either subjected to a heat-treatment of heatwaves, two of those at 33 °C, or a control-treatment with common summer-temperatures. In a third heatwave of 38 °C, the previously control treated plants were exposed to heat as well. Physiological parameters like net photosynthesis, stomatal conductance and water use efficiency were assessed during the heatwaves. Meanwhile, photosynthetic capacity through  $V_{cmax}$  and  $J_{max}$  as well as dark respiration were assessed post-heatwave. The data shows that the heatwaves of 33 °C had small effects on almost all species, with strongest effects on plant physiology occurring in the last heatwave of 38 °C. *Acer platanoides* and *Tilia cordata* showed potential to tolerate heatwaves more than *Betula pendula* and *Prunus avium*. However, no signs of thermal acclimation to the heatwaves were found in any species and consequently repeated heatwave exposure did not improve heat tolerance in the stronger last heatwave. This overall suggests that moderate and short heatwaves might be tolerated by the four urban tree species.

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**Keywords:** Heat tolerance, thermal acclimation, urban trees, heatwave,  $V_{cmax}$  and  $J_{max}$

# 1. Introduction

## 1.1. Global warming and Heatwaves

According to the IPCC (2021) the global warming level of 1.5 °C is threatened to be crossed in the next decades unless vast reductions in greenhouse gas emissions occur. With global warming advancing, effects on climate extremes become larger. For example, heatwaves are clearly becoming more frequent and intense with increasing global temperature, along with other extreme weather events like heavy precipitation and droughts. Dosio et al. (2018) showed that under a 2 °C increase in global warming, most of the world would be affected by a higher frequency of extreme heatwaves. Looking at Sweden, an increase in the annual mean temperature has been observed (SMHI 2015) and it is projected that Scandinavia will experience a rise in temperature above the global mean, with summers becoming drier and warmer especially in southern Sweden (Holgerson 2007). In the recent extremely hot summer in 2018, a temperature of nearly 35 °C was measured in August in south-west Sweden, making it that year's maximum temperature (SMHI 2018). For Västra Götaland county, in the south-west of Sweden, the number of consecutive days with an average daily temperature above 20 °C was about 2,5 days in the period of 1961 to 1990. In the past 20 years this has increased a bit, with the number of days ranging from 1-3 days. Regarding future projections, longer periods of an average daily temperature above 20 °C can be expected (Berglöv et al. 2015).

## 1.2. Urban environment

Urban environments face several added challenges in face of global warming to increased and more severe heatwaves. There, warming is locally intensified, and further urbanization in addition to increased heat extremes will likely increase the overall severity of heatwaves in the more or less near future (IPCC 2021). Urban settings are subject to the so-called heat-island effect, meaning the temperature is usually a bit higher in cities compared to the more rural surroundings. This is mostly due to materials like asphalt, concrete and sheet metal having a high heat capacity and thus storing more heat (Czaja et al. 2020). There have been findings of up to a 4°C difference between urban and rural areas, and findings of significant correlations of air temperature and urban forest-cover have been made (Wong and Yu 2005; Hamada and Ohta 2010). The cooling by trees of their surroundings is an important ecosystem service and works through providing shade as well as through evapotranspiration (Konarska et al. 2016). This evapotranspiration describes the process where a plant loses water, mainly through the stomata, in form of water vapour. In this process solar radiation energy is transformed into

latent heat, thereby cooling the leaf and air temperature surrounding it (Bowler et al. 2010; Konarska et al. 2016). Aside from cooling benefits, urban trees provide other ecosystem services. There have been findings on improved wellbeing and mental health through exposure to forest settings (Morita et al. 2007, Park et al. 2011, Kühn et al. 2017, as cited in Wolf 2020). But also, physical health is improved by urban trees, as they help reduce noise pollution (Farina 2014, Van Renterghem 2019, as cited in Czaja et al. 2020), as well as immobilizing and absorbing a range of air pollutants (Nowak et al. 2006).

### 1.3. Temperature effects on plants

Temperature plays an important role in many plant processes such as nutrition, gas exchange and reproductive growth and it is a limiting factor in these processes. There are three base point temperatures regarding plant functioning, commonly known as the minimum, optimum and maximum temperature. Basic processes to sustain life will continue in plants at minimum and maximum temperature but growth will stop (Wang et al. 2024). If temperatures are exceeded below the minimum or above the optimum, plant growth and development will be harmed (Allakhverdiev et al. 2008; Mittler et al. 2012 as cited in Wang et al. 2024).

The photosynthetic rate at light-saturation is usually low at extremely cold and hot temperatures and has its optimum at a temperature between 20 °C and 30 °C in most species. Temperatures above that could lead to decreased productivity in trees (Hikosaka et al. 2006; Yamori et al. 2014). That is because important enzymes as well as other photosynthetic components are susceptible to heat damage. For example, the active state of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) decreases in high temperatures, affecting photosynthetic productivity negatively (Kobza and Edwards 1987; Craft and Brandner 2000; Hikosaka et al. 2006; Allakhverdiev et al. 2008). Also, the chloroplast thylakoid membrane and the embedded Photosystem II can be compromised by high temperature (Gounaris et al. 1984; Bukhov et al. 1999; Allakhverdiev et al. 2008). This underlines the importance of understanding which tree species and to what extent, can tolerate heat and thermally acclimate in their photosynthetic capacity. Heat tolerance is defined as the ability of an organism, to withstand the effect of higher temperatures without permanent damage (Spektrum n.d.). By acclimation, a plant's capacity to adapt on a physiological level to changes in their environment within the plant's lifetime is meant (Kleine et al. 2021). In response to higher temperatures, upregulating photosynthesis or downregulating respiration can facilitate this thermal acclimation so a positive carbon balance is maintained (Dusenge et al. 2019, Lombardozzi et al. 2015, Slot and Kitajima 2015, as cited in Hara et al. 2021).

There has been previous research on thermal acclimation in plants, exposing them to moderately or highly elevated temperatures and assessing acclimation to growth temperature or along latitudinal or altitudinal gradients. Many found evidence of thermal acclimation (Gunderson et al. 2000; Campbell et al. 2007; Kattge and Knorr 2007; Wittemann et al. 2022). However, Dillaway and Kruger (2010) found that photosynthetic thermal acclimation along a latitudinal gradient did not occur in almost any of the tested species. In recurring heatwave experiments on boreal trees, also no thermal acclimation was found (Gagne et al. 2020). In Miami, subtropical tree species did not acclimate photosynthetically in a thermal urban gradient (Kullberg et al., 2023). Hara et al. (2021) as well as Okubo et al. (2023) have found varying results on thermal acclimation in tree species growing in Japanese cities, underlining that there is species specificity in heat tolerance and acclimation. The different findings emphasize the importance of studying thermal acclimation and heat tolerance in trees. Especially heatwaves and recurrent heatwaves need to be analysed due to their expected increasing frequency and intensity. Research on urban trees to those is crucial to help equip cities with the necessary knowledge to plan urban greenery sustainably. The benefits of urban trees are indispensable now and will be of greater importance in a hotter future climate. To provide cities and their inhabitants with healthy urban trees, their response to elevated temperatures, and in particular to heatwaves is researched in this thesis.

## 2. Aim

This thesis aims to examine the heat tolerance and thermal acclimation capacities of four tree species that currently grow in south-western Swedish cities. The purpose is to identify tree species that can withstand a future hotter urban climate.

The following research questions are addressed:

- 1) How do the selected tree species differ in their heat tolerance?
- 2) Are the selected species able to thermally acclimate to heatwaves?
- 3) Does repeated exposure to heatwaves result in a higher heat tolerance, compared to unexposed plants?

### 3. Material and Methods

#### 3.1. Plant growth conditions

Four tree species that commonly grow in south-western Swedish cities were chosen for this study, with 9 to 12 replicates each (Table 1). They were received bare-rooted and planted in planting soil (Emmaljunga Torvmull AB, Vittsjö, Sweden) and grown in phytochambers at the Department of biology and environmental science of the University of Gothenburg. The species *Betula pendula* (*Bpe*), *Prunus avium* (*Pav*) and *Tilia cordata* (*Tco*) were planted on the 17<sup>th</sup> of November 2023, while *Acer platanoides* (*Apl*) was planted on the 5<sup>th</sup> of December 2023. To simulate the conditions of a warm summer day in July the plants were grown in 23 °C during the day and 17 °C during the night in two different phytochambers. The exact temperature conditions the chambers were set to are presented in table A1 (Appendix). The trees were rotated between the chambers and placed in a random spot in each chamber on a weekly basis. This approach eliminates potential confounding effects from individual chambers, enabling a reliable statistical analysis even with just a single replicated chamber per treatment.

To exclude limitations due to other factors than heat exposure, the plants were watered biweekly until saturation. Additionally, 200 mL of 1:100 nutrient to water mix were given to the plants on a weekly basis (Stroller blå Trädgårdsnäring SBM Life Science AB, Lund, Sweden). Before the start of the heatwave experiment, the tree height and stem diameter were measured (Table 2).

Table 1: Plant species with number of replicates used and their basic characteristics.

Species	Short	Replicates	Family name	Mean annual air Temp (°C)	Strategy	Reference
<i>Acer platanoides</i>	<i>Apl</i>	12	Aceraceae	6-10.5	Pioneer	FVA, 2017a
<i>Betula pendula</i>	<i>Bpe</i>	12	Betulaceae	(-2)-13	Pioneer	FVA, 2017b
<i>Prunus avium</i>	<i>Pav</i>	10	Rosaceae	5-15	Pioneer	FVA, 2017c
<i>Tilia cordata</i>	<i>Tco</i>	9	Malvaceae	7-11.5	Climax	FVA, 2017d

Table 2.: Average height and stem diameter per species at the start of the experiment (Mean  $\pm$  Standard deviation (SD)).

Species	Average height (cm)	Stem diameter (mm)
<i>Apl</i>	60.5 $\pm$ 1.5	5.9 $\pm$ 0.8
<i>Bpe</i>	60.1 $\pm$ 18.4	10.0 $\pm$ 2.1
<i>Pav</i>	85.9 $\pm$ 2.9	10.6 $\pm$ 1.3
<i>Tco</i>	61.8 $\pm$ 1.7	13.4 $\pm$ 1.9

### 3.2. Experimental setup

The goal of this experiment was to periodically expose half of the plants to heatwave conditions, while the other half remained in control conditions. The heatwaves were simulated in the plant growth chambers. The heat-treated plants (H) were exposed to in total three heatwaves of three days each, referred to as HW1, HW2 and HW3 from here on. In the first two heatwaves the growth chamber was set to 33 °C/19 °C day/night temperature. In the third heatwave, both control (C) and heat-treated plants were exposed to a temperature of 38 °C/21 °C day/night (Table 3) to study if previous heatwave exposure resulted in greater heat tolerance. The humidity conditions were set to 40% relative humidity in the heated chamber and 50% in the control chamber. The light was set to a photosynthetic active radiation (PAR) of 1200 W/m<sup>2</sup> measured 15 cm below the lamps. Measurements were made during and after the heatwaves to capture direct heat responses, potential thermal acclimation and after-effects.

The first heatwave was started in early February (05.02.-07.02.2024) with four days in between heatwave and heat acclimation measurements (Post-heatwave 12.02.-16.02.2024). Four days after the measurements the second heatwave was started (21.02.-23.02.2024) with an acclimation period of again 4 days (Post-heatwave 28.02.-05.03.2024). Again after 4 days the third heatwave was started (11.03.-13.03.2024) and the post heatwave measurements were done after four days of acclimation time (18.03.-22.03.2024 and 25.03.2024).

Table 3: Temperature treatments for the first, second and third heatwave.

Treatment	Cycle 1 and 2		Cycle 3	
	Control	Heated	Control	Heated
Day Temperature	23 °C	33 °C	38 °C	38 °C
Night Temperature	17 °C	19 °C	21 °C	21 °C

### 3.3. Visible injury to tree and leaves

To keep track of visible effects, like leaf loss or leaf damage, the number of leaves was counted before the start of the experiment as well as after each heatwave. After the first heatwave, the leaf number was counted after 8 days, after the second it was counted after 14 days. On the day after the third heatwave ended, leaf damage was evaluated through visual evaluation in addition to the leaf count. On trees with many branches and leaves, specific branches were chosen for leaf counting and were revisited for each counting instance. The damaged leaves after HW3 were assessed carefully excluding previous damage.

### 3.4. Gas exchange

To examine photosynthetic rate and other physiological responses during the heatwaves, gas exchange measurements were made using the LI-6800 Portable Photosynthesis System (LI-COR® Biosciences, Nebraska), from here on called LI-COR. It is an open-system gas exchange measuring device, which means that the physiological measurements are taken based on differences in concentrations of carbon dioxide ( $\text{CO}_2$ ) and water vapor ( $\text{H}_2\text{O}$ ) of a reference and sample air flow. Focus lay on rapid measurements of net photosynthesis ( $A_{net}$ ), transpiration ( $E$ ) and stomatal conductance ( $g_{sw}$ ). Water use efficiency ( $WUE$ ) was attained dividing  $A_{net}$  by  $E$ . The LI-COR chamber conditions were set to mimic the phytochamber condition with a reference  $[\text{CO}_2]$  of  $420 \mu\text{mol mol}^{-1}$ , a flow of  $400 \mu\text{mol s}^{-1}$ , a temperature of  $23 \text{ }^\circ\text{C}/33 \text{ }^\circ\text{C}$  respective of the plants' treatment, a photosynthetic photon flux density (PPFD) of  $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , a relative humidity of  $50 \%/40 \%$  respective of the plants' treatment and the fan was set to 10,000 rpm. Three subsequent measurements were made per leaf after a stabilisation period of minimum 1 minute and maximum 2 minutes. This time frame was chosen to allow the system to reach technical stability but avoid gas exchange responses to chamber conditions. In the first and second heatwaves the measurements were done on two mature and healthy leaves per plant, that were picked in a PAR range of 750 to 1250  $\text{W/m}^2$ . Due to plant growth or leaf injury, different leaves had to be picked for some plants in the third heatwave, still maintaining a PAR range of 750 to 1250  $\text{W/m}^2$ . Two to four leaves were measured on in the last heatwave. The measurements were done between 10:00 and 17:00 on the third day of the heatwave each time.

Acclimation after heat exposure was evaluated by measuring the photosynthetic response to intercellular  $\text{CO}_2$  concentration ( $C_i$ ), also known as  $A/C_i$  curves. These were made four days after the heatwave treatment stopped over a course of three to four days. The measurement time frame was from 10:00 to 17:00 on each measuring day with plants from different species

and treatments measured in random order. The LI-COR was used for this as well with chamber conditions set to a flow of  $400 \mu\text{mol s}^{-1}$ , a temperature of  $23 \text{ }^\circ\text{C}$ , a PPFD of  $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , a relative humidity of 50 % and the fan was set to 10,000 rpm. For the  $\text{CO}_2$  response curve, the following concentrations were used (in  $\mu\text{mol mol}^{-1}$ ): 420, 60, 110, 180, 250, 420, 800, 1200, 1500, 2000, 420. To let the plants readjust physiologically to each of the periodically changing  $\text{CO}_2$  concentrations, and to ensure technical stability before each measurement that was taken, certain stability settings were set in the LI-COR that had to be fulfilled before logging a new measurement (Table 4). The minimum time to reach stability was set to 60 seconds and the maximum time was set to 240 seconds. The  $A/C_i$  measurements were made on one leaf per plant, using preferably one of the leaves that were used during the heatwave measurements.

Table 4: Stability settings for  $A/C_i$  measurements made with LI-COR.

Parameter	slope limit	period (sec)
$\Delta\text{H}_2\text{O}$	0.5	15
Flow	1	15
$\Delta\text{CO}_2$	0.5	15

The dark respiration ( $R_d$ ) measurements were made similarly to the “during heatwave” measurements, using the LI-COR to make measurements of  $A_{net}$ . Measurements were taken in a darkened room, where a PAR of near  $0 \text{ W/m}^2$  was measured. The plants were dark adapted for 30 minutes before the measurements.

### 3.5. Determining the maximum rates of carboxylation and electron transport

The biochemical model of photosynthetic  $\text{CO}_2$  assimilation in  $\text{C}_3$  plants’ leaves, by Farquhar et al. (1980), known as the FvCB model, explains that the photosynthetic assimilation of  $\text{CO}_2$  ( $A$ ) is limited if one of the following processes is slowed. Firstly, the Rubisco limited step, where  $A$  is limited by the maximum rate of Rubisco catalysed carboxylation. Secondly, the Ribulose-1,5-bisphosphate (RuBP) limited step, where the electron transport controlled regeneration of RuBP limits  $A$ . And lastly the triose phosphate utilization limited step, where RuBP regeneration is limited by the rate of triose phosphate utilization (Farquhar et al. 1980; Bernacchi et al. 2001). The latter was not observed in the collected data and will not be further discussed in this thesis. These limitation steps can be characterized mathematically.

The Rubisco limited photosynthesis' CO<sub>2</sub> response is described in the following equation (Eq 1):

$$A = V_{cmax} \left[ \frac{C_i - \Gamma^*}{C_i + K_c \left(1 + \frac{O}{K_o}\right)} \right] - R_d \quad (\text{Eq 1})$$

$V_{cmax}$  is the maximum carboxylation rate of RuBP (Haworth et al. 2018) or called the maximum velocity of Rubisco for carboxylation (Sharkey et al. 2007),  $C_i$  describes the intercellular CO<sub>2</sub> concentration,  $O$  is the partial pressure of O<sub>2</sub> at Rubisco,  $K_c$  is the Michaelis constant of Rubisco for CO<sub>2</sub>,  $K_o$  is the inhibition constant of Rubisco for oxygen, and  $\Gamma^*$  is the photorespiratory compensation point. With this the slope of the  $A/C_i$  curve can be used to estimate  $V_{cmax}$  using a regression approach (Sharkey et al. 2007).

The limiting of  $A$  by RuBP regeneration can be described in the following equation (Eq 2):

$$A = J \frac{C_i - \Gamma^*}{4C_i + 8\Gamma^*} - R_d \quad (\text{Eq 2})$$

$J$  is the rate of electron transport, and four electrons per carboxylation and oxygenation are assumed in this model. In saturating light, the maximum rate of  $J$ ,  $J_{max}$ , can be obtained (Sharkey et al. 2007). The  $C_i$  based formula is used for Eq 1 and 2 in this thesis instead of using  $C_c$  (partial pressure of CO<sub>2</sub> at Rubisco).

The following formula can be used for calculating  $J_{max}$  (Eq 3):

$$J = \frac{\alpha_e Q}{\left(1 + \left(\frac{\alpha_e Q}{J_{max}}\right)^2\right)^{0.5}} \quad (\text{Eq 3})$$

where  $J$  is the electron transport rate related to  $Q$ ,  $Q$  is the photosynthetic photon flux density, and  $\alpha_e$  is the apparent quantum yield of the electron transport, which is presumed to be 0.24 mol electrons mol<sup>-1</sup> photons (Harley et al. 1992, as cited in Tarvainen et al. 2016).

The  $A/C_i$  curves' CO<sub>2</sub> concentration line-up is arranged in a way that enables the retrieval of  $V_{cmax}$  and  $J_{max}$  by analysing the slopes of the curve. In the first part of the response curve, sub-ambient CO<sub>2</sub> concentrations were set. That is, because net photosynthesis ( $A_{net}$ ) is then limited by CO<sub>2</sub> availability and with rising  $C_i$  more and more CO<sub>2</sub> is available for Rubisco to use for carboxylation. That part of the curve is representing the Rubisco limited part (Bernacchi et al. 2001; Haworth et al. 2018). From the slope the  $V_{cmax}$  can be calculated. With increasing CO<sub>2</sub> concentration, the curve starts to level off and  $A_{net}$  is not limited by Rubisco anymore, because CO<sub>2</sub> is abundant. At that point the electron transport needed to regenerate RuBP limits  $A_{net}$ . This slope can be used to calculate  $J_{max}$  (Haworth et al. 2018).

### 3.6. Data analysis

For the calculation of the  $V_{cmax}$  and  $J_{max}$  values, the “plantecophys” package by Duursma (2015) was utilized in the program R 4.3.0 (R Core Team, 2023). Using the “fitaci” function, estimates of  $V_{cmax}$  and  $J_{max}$  were fit based on the FvCB model, utilizing a non-rectangular hyperbolic model (Eq 4).

$$A_m = \frac{A_c + A_j - \sqrt{(A_c + A_j)^2 - 4\theta A_c A_j}}{2\theta} - R_d \quad (\text{Eq 4})$$

In this model the shape parameter ( $\theta$ ) is set to 0.9999.  $A_c$  describes gross photosynthesis rate when Rubisco activity is limiting, while  $A_j$  describes gross photosynthesis rate when RuBP-regeneration is limiting.  $A_m$  is the hyperbolic minimum of  $A_c$  and  $A_j$ .

### 3.7. Outlier detection

Stomatal closure affects photosynthesis, as the  $C_i$  is decreased consequently. Thus, to ensure good quality  $A/C_i$  curves, a threshold of 0.015 mol m<sup>-2</sup> s<sup>-1</sup> of  $g_{sw}$  was set. This threshold was set based on comparing the  $g_{sw}$  values of the whole dataset and comparing the  $g_{sw}$  values with their corresponding  $A/C_i$  curves. Additionally,  $A/C_i$  curves that could not be fit by the “plantecophys” R package were left out from analysis. Table 5 shows the number and percentage of  $A/C_i$  curves that were established as outliers and removed from data analysis.

Table 5: Number and percentages of  $A/C_i$  curve outliers per heatwave.

	<b>HW1</b>	<b>HW2</b>	<b>HW3</b>
<i>Apl</i>	0.0%	8.3%	8.3%
	0 of 11	1 of 12	1 of 12
<i>Bpe</i>	8.3%	8.3%	25.0%
	1 of 12	1 of 12	3 of 12
<i>Pav</i>	10.0%	0.0%	0.0%
	1 of 10	0 of 9	0 of 8
<i>Tco</i>	0.0%	0.0%	11.1%
	0 of 9	0 of 9	1 of 9

For the data measured during the heatwaves, the plants that displayed abnormality such as large variations in  $V_{cmax}$  or  $J_{max}$  between measurement occasions in control plants not exposed to heatwaves, which could allude to difficulties with rooting, etc., were treated as outliers and removed from the analysis (Table 6).

Table 6: Number and percentages of removed outliers from the during-heatwave experiments.

	<b>HW1</b>	<b>HW2</b>	<b>HW3</b>
<i>Apl</i>	16.7%	16.7%	9.1%
	2 of 12	2 of 12	1 of 11
<i>Bpe</i>	0.0%	0.0%	8.3%
	0/12	0 of 12	1 of 12
<i>Pav</i>	10.0%	0.0%	0.0%
	1 of 10	0 of 9	0 of 9
<i>Tco</i>	0.0%	0.0%	0.0%
	0 of 9	0 of 9	0 of 11

### 3.8. Statistical Analysis

To test the data for statistical significance non-parametric tests were performed. A non-parametric approach was chosen since large parts of the data followed non normal distribution and unequal variance and common transformations did not yield data fulfilling assumptions for an Anova. The “rstatix” package in R was used to analyse the statistical tests (Kassambra 2023).

The different plant species in their different treatments were analysed for significant differences in between the heatwave occasions using the Friedman test. It can be used to analyse a number of measurements of dependent variables that are taken on different occasions (Marshall and Marquier n.d.). If a test found a significant difference between values, the pairwise Wilcoxon rank sum test, implementing a Bonferroni correction, was conducted as a post hoc test. In addition to the differences between responses to the heatwaves, the difference between treatments within each species and heatwave was tested. The Mann-Whitney-U test was used to conduct this analysis. Lastly, the Kruskal-Wallis test was used to test for significant heat response differences in each heatwave between the different species. In HW1, this test was also conducted in control treated plants to assess inherent species differences. This test is based on rank sums like the Mann-Whitney U test, but it can be used for more than two groups. Here again, the pairwise Wilcoxon rank sum test, using a Bonferroni correction was used for post hoc testing.

## 4. Results

### 4.1. Leaf growth

The growth of new leaves and the loss of leaves between the experiment start and first heatwave as well as after each heatwave was analysed. The heat-treated *Bpe* plants lost most leaves after HW3 (~18%), but also experienced a large leaf growth in the period before. In terms of damage, *Pav* in either treatment experienced the highest percentage of damaged leaves after HW3, followed by *Apl* and *Tco*. In terms of number of damaged leaves *Pav* in both treatments and *Tco* in heat treatment show the highest numbers (Table 7).

Table 7: Mean change in leaves in percent and numerically (Mean  $\pm$  SD). Leaf numbers were counted at the start of the experiment and after each heatwave. After HW3, leaf number and damaged leaves were counted (Mean  $\pm$  SD). Negative values signify a loss of leaves, positive numbers signify new leaf growth. The initial leaf count is to be considered relative to the new leaf growth or loss within one species and treatment category, as entire trees' leaves were counted in some, and specific branches' leaves were counted in other plants.

Species	Initial leaf number	Start to HW1	HW1 to HW2	HW2 to HW3	% Damaged HW3
<i>Apl</i> C	14.8 $\pm$ 3.9	25,0% $\pm$ 28.1	36,6% $\pm$ 59.9	17,5% $\pm$ 18.5	34,0% $\pm$ 24.3
		4,5 $\pm$ 5.3	7,3 $\pm$ 13.3	5.8 $\pm$ 9.9	6,7 $\pm$ 2.7
<i>Apl</i> H	17.5 $\pm$ 12.6	30,6% $\pm$ 37.5	55,6% $\pm$ 75.5	2,4% $\pm$ 35.7	20,5% $\pm$ 13.3
		4,0 $\pm$ 4.8	10,5 $\pm$ 11.1	-4,8 $\pm$ 20.4	5,0 $\pm$ 3.7
<i>Bpe</i> C	68.3 $\pm$ 16.3	15,9% $\pm$ 7.9	-0,1% $\pm$ 16.8	0,1% $\pm$ 2.4	9,1% $\pm$ 8.0
		10,7 $\pm$ 5.0	1,2 $\pm$ 15.3	-0,2 $\pm$ 1.7	7,0 $\pm$ 5.7
<i>Bpe</i> H	71.8 $\pm$ 15.6	18,0% $\pm$ 9.4	45,5% $\pm$ 42.9	-18,3% $\pm$ 18.0	8,0% $\pm$ 5.6
		13,8 $\pm$ 8.6	36,2 $\pm$ 27.5	-21,7 $\pm$ 22.1	7,2 $\pm$ 3.9
<i>Pav</i> C	46.2 $\pm$ 12.4	-5,4% $\pm$ 16,3	33,7% $\pm$ 103.7	10,3% $\pm$ 8.6	35,7% $\pm$ 13.6
		-3,0 $\pm$ 7.5	6,4 $\pm$ 25.2	4,8 $\pm$ 5.1	18,4 $\pm$ 15.2
<i>Pav</i> H	56.4 $\pm$ 26.3	14,7% $\pm$ 32.0	14,2% $\pm$ 51.1	14,1% $\pm$ 27.9	36,9% $\pm$ 23.1
		8,2 $\pm$ 16.5	7,0 $\pm$ 15.9	8,3 $\pm$ 17.3	18,5 $\pm$ 14.1
<i>Tco</i> C	23 $\pm$ 17.1	18,5% $\pm$ 35.4	-1,1% $\pm$ 19.1	6,3% $\pm$ 11.1	22,6% $\pm$ 17.6
		1,5 $\pm$ 3.8	-1,3 $\pm$ 5,3	1,0 $\pm$ 2.1	5,5 $\pm$ 5.8
<i>Tco</i> H	30.3 $\pm$ 19.3	49,5% $\pm$ 48.1	22,4% $\pm$ 42.1	2,8% $\pm$ 10.1	28,7% $\pm$ 10.1
		7,7 $\pm$ 8.2	7,0 $\pm$ 12.0	1,5 $\pm$ 3.7	14,3 $\pm$ 2.5

#### 4.2. Gas exchange during the heatwaves

For HW1, a significant difference between the species has been detected in  $A_{net}$  in the control-treated plants ( $p < .005$ ) (Table A4). For the heat-treated plants a significant difference between species in  $A_{net}$  was only found in HW1 ( $p < .045$ ). For both treatments specifically between *Apl* and *Bpe* ( $p < .05$ ).

When comparing  $A_{net}$  between the control and heat-treatments within each species, only *Bpe* in HW1 showed a significant difference in  $A_{net}$  ( $p < .014$ ) (Table A3).

Looking across the measuring occasions, there was a general decline in  $A_{net}$  from the first and second to the third heatwave, in heat- as well as control- treated plants (Fig. 1). Generally, the third heatwave resulted in the lowest  $A_{net}$  values in all species, if not as clearly in *Apl* H plants. Over the course of the measuring occasions,  $A_{net}$  showed significant differences in all species, in the heatwave exposed plants and in the control plants ( $p < .041$ ) (Table A2). Post hoc analysis found that the difference between HW1 and HW3 was significant in *Apl* C, *Bpe* C and *Bpe* H

( $p < .038$ ), and also the difference between HW2 and HW3 in *Bpe* C ( $p < .016$ ). It should be noted, that the third heatwave was conducted on control as well as heat-treated plants.

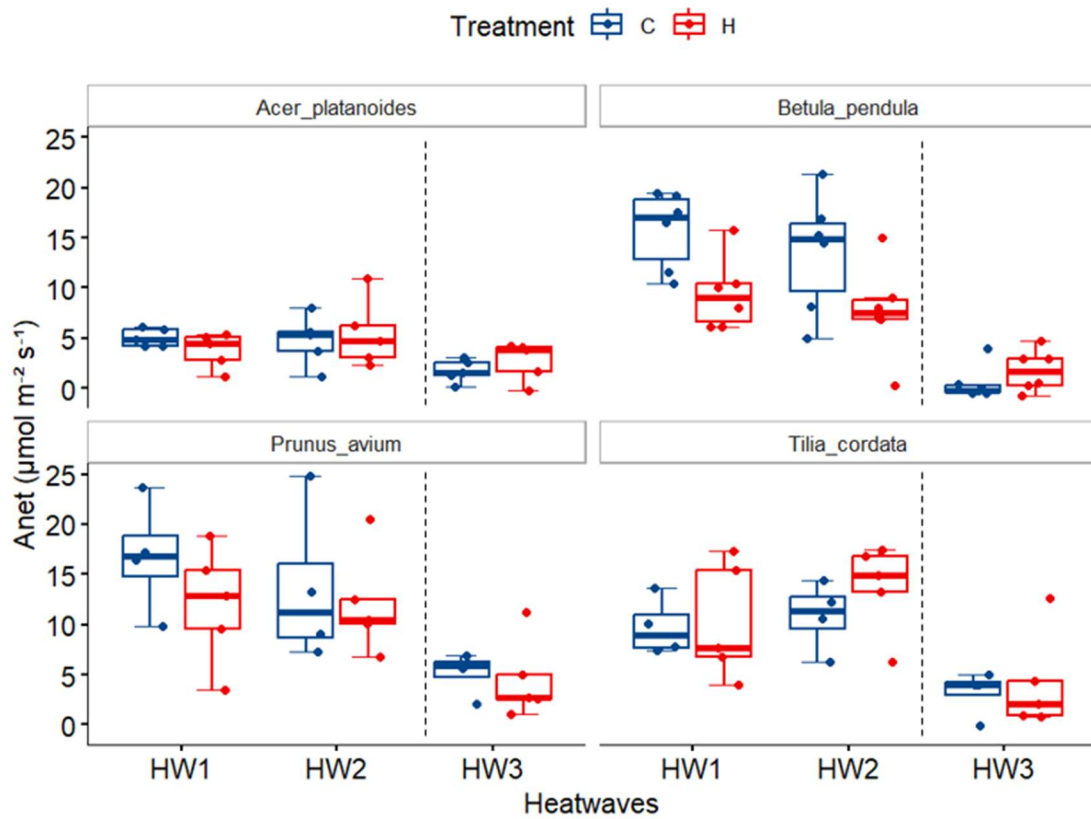


Fig. 1: Boxplot graph of the during-HW measurements of net photosynthesis ( $A_{net}$ ) ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), by species and by heatwave (HW1-3). The control data is coloured in blue, the heat-treated one is coloured red. The boxplots show the interquartile range (IQR; whole box) the median (central line in the box), the 75<sup>th</sup> percentile (upper line) and 25<sup>th</sup> percentile (lower line). The whiskers represent the largest value within 1.5 times IQR above the 75<sup>th</sup> percentile (upper whisker) and the smallest value within 1.5 times IQR below the 25<sup>th</sup> percentile (lower whisker). The jitter represents the individual data points. Heatwave treatment was applied only to the heat-treated plants during HW 1 and HW2, and on both control and heat-treated plants during HW3 (separated by the dashed line).

In HW1, the control-treated plants showed significant differences in  $g_{sw}$  between the different species ( $p < .012$ ) (Table A4). Like  $A_{net}$ , they also lay between *Apl* and *Bpe* ( $p < .05$ ). For the heat treatment, the  $g_{sw}$  was significantly different between the species in HW1 and HW2 ( $p < .042$ ).

Between the control- and heat-treated plants there were no significant differences in  $g_{sw}$  in any species during any of the heatwaves (Table A3).

Across the measuring occasions, the  $g_{sw}$  in both treatments showed very little variation in *Apl* compared to the other species (Fig. 2). The heat-treated plants'  $g_{sw}$  values are generally less dispersed and except in *Tco* lower than the  $g_{sw}$  values in the control plants (Fig. 2). Consistent with this, there is a significant difference between heatwaves in  $g_{sw}$  in *Bpe* C, H and in *Pav* C ( $p < .042$ ) (Table A2). For *Bpe* C the difference was significant between HW1 and 2 and between HW2 and 3 in ( $p < .026$ ).

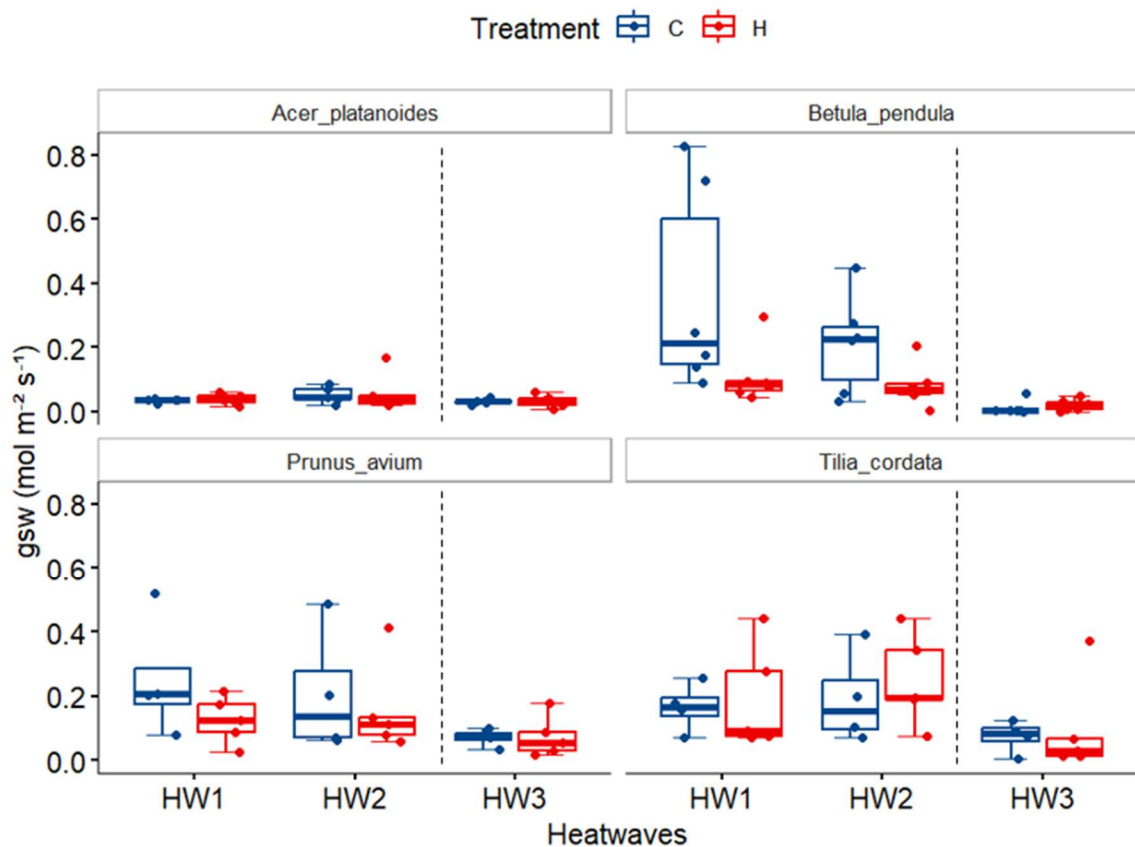


Fig. 2: Boxplot graph of the during-HW measurements of stomatal conductance ( $g_{sw}$ ) ( $\text{mol m}^{-2} \text{s}^{-1}$ ), by species and by heatwave (HW1-3). The control data is coloured in blue, the heat-treated one is coloured red. The boxplots show the interquartile range (IQR; whole box) the median (central line in the box), the 75<sup>th</sup> percentile (upper line) and 25<sup>th</sup> percentile (lower line). The whiskers represent the largest value within 1.5 times IQR above the 75<sup>th</sup> percentile (upper whisker) and the smallest value within 1.5 times IQR below the 25<sup>th</sup> percentile (lower whisker). The jitter represents the individual data points. Heatwave treatment was applied only to the heat-treated plants during HW 1 and HW2, and on both control and heat-treated plants during HW3 (separated by the dashed line).

In between species, there was a significant difference in water use efficiency (*WUE*) of the control plants in HW1 ( $p < .03$ ). For the heat-treated plants, no significant differences could be detected between the species in any heatwave (Table A4).

Between the treatments, a significant difference in *WUE* could be found in *Apl* and *Pav* in HW1 ( $p < .038$ ). Furthermore, a significant difference between the treatments could be found in HW2 in *Bpe* and *Pav* ( $p < .038$ ) (Table A3).

The *WUE* shows higher values in the control plants compared to heat-treated plants in all heatwaves except in HW3 for all species (Fig. 3). The differences of *WUE* in control treated plants over the course of the measuring occasions are significant in all species ( $p < .05$ ) (Table A2). It is important to note again, that the control plants were exposed to the third heatwave as well. For the heatwave exposed plants only *Pav* H shows significant difference between the heatwaves ( $p < .023$ ), specifically between HW1 and HW3 ( $p < .038$ ).

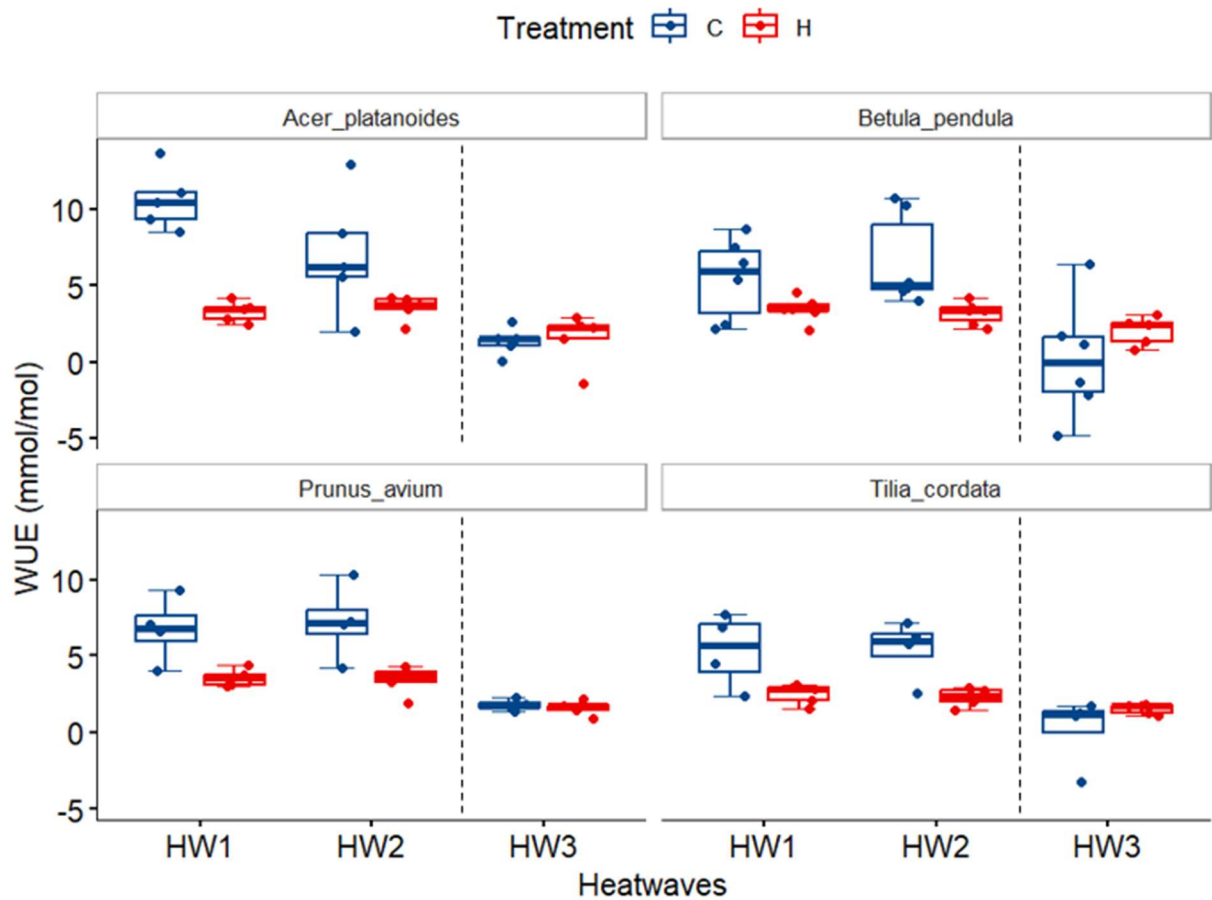


Fig. 3: Boxplot graph of the during-heatwave measurements of water use efficiency (WUE) (mmol/mol), by species and by heatwave (HW1-3). The control data (C) is coloured in blue, the heat-treated (H) one is coloured red. The boxplots show the interquartile range (IQR; whole box) the median (central line in the box), the 75<sup>th</sup> percentile (upper line) and 25<sup>th</sup> percentile (lower line). The whiskers represent the largest value within 1.5 times IQR above the 75<sup>th</sup> percentile (upper whisker) and the smallest value within 1.5 times IQR below the 25<sup>th</sup> percentile (lower whisker). The jitter represents the individual data points. Heatwave treatment was applied only to the heat-treated plants during HW 1 and HW2, and on both control and heat-treated plants during HW3 (separated by the dashed line).

### 4.3. Dark Respiration

The dark respiration ( $R_d$ ) values range on a similar level in all four species for control and heat-treatment, in all measuring occasions (Fig. 4). *ApI* displays the lowest measured values overall at around  $-2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ , however, no significant differences were detected for all species and treatments between the measuring occasions as well as in between the treatment differences (Table A2, A3).

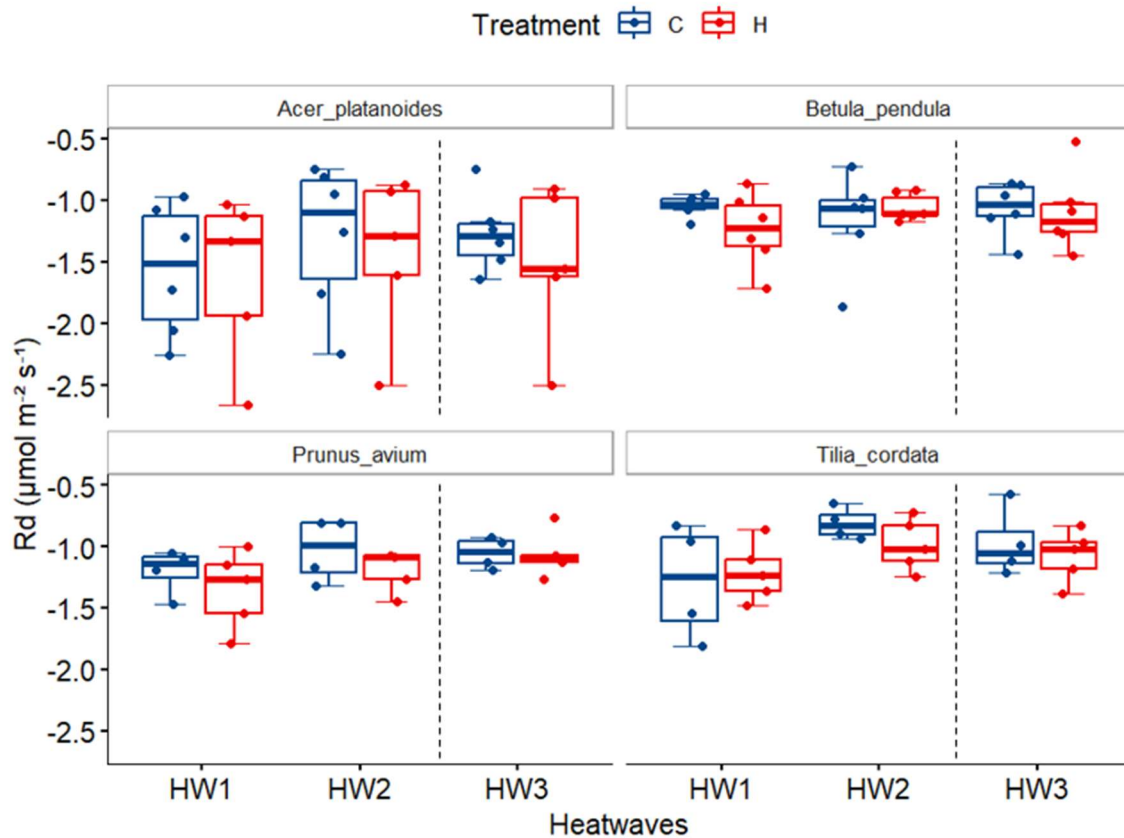


Fig. 4: Boxplot graph of the post-heatwave measurements of  $R_d$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), by species and by heatwave (HW1-3). The control data (C) is coloured in blue, the heat-treated (H) one is coloured red. The boxplots show the interquartile range (IQR; whole box) the median (central line in the box), the 75<sup>th</sup> percentile (upper line) and 25<sup>th</sup> percentile (lower line). The whiskers represent the largest value within 1.5 times IQR above the 75<sup>th</sup> percentile (upper whisker) and the smallest value within 1.5 times IQR below the 25<sup>th</sup> percentile (lower whisker). The jitter represents the individual data points. Heatwave treatment was applied only to the heat-treated plants during HW 1 and HW2, and on both control and heat-treated plants during HW3 (separated by the dashed line).

#### 4.4. Photosynthetic capacity post-heatwave

Between the species, significant differences of control-treated plants in HW1, could only be found for  $V_{cmax}$  ( $p < .045$ ), not  $J_{max}$ . In heat treatment, only for HW1 significant differences could be found between the species for  $V_{cmax}$ , and  $J_{max}$  ( $p < .01$ ) (Table A4). Post hoc testing could not show, between which species these differences lay.

Significant differences in between treatments could only be found for  $V_{cmax}$  in *Bpe* in HW2 ( $p < .015$ ) (Table A3).

For *Bpe* and partially for *Pav* in both treatments a slight pattern of decreasing  $V_{cmax}$  and  $J_{max}$

over the course of the experiment can be seen (Fig. 5,6). *Apl* and *Tco* show variation but the values range on approximately the same level in all three measuring occasions. Reflecting this, the  $V_{cmax}$  values show no significant differences between the heatwaves except in *Bpe* C and *Pav* H ( $p < .05$ ), and similarly so do the  $J_{max}$  values only in *Bpe* C, H and *Pav* H ( $p < .05$ ) (Table A2). For *Pav* it could be determined that the difference lay between HW1 and HW3 ( $p < .04$ ). Due to too little paired data across the heatwaves for *Tco* C, the Friedman test could not be performed, so no statement can be made about significance here.

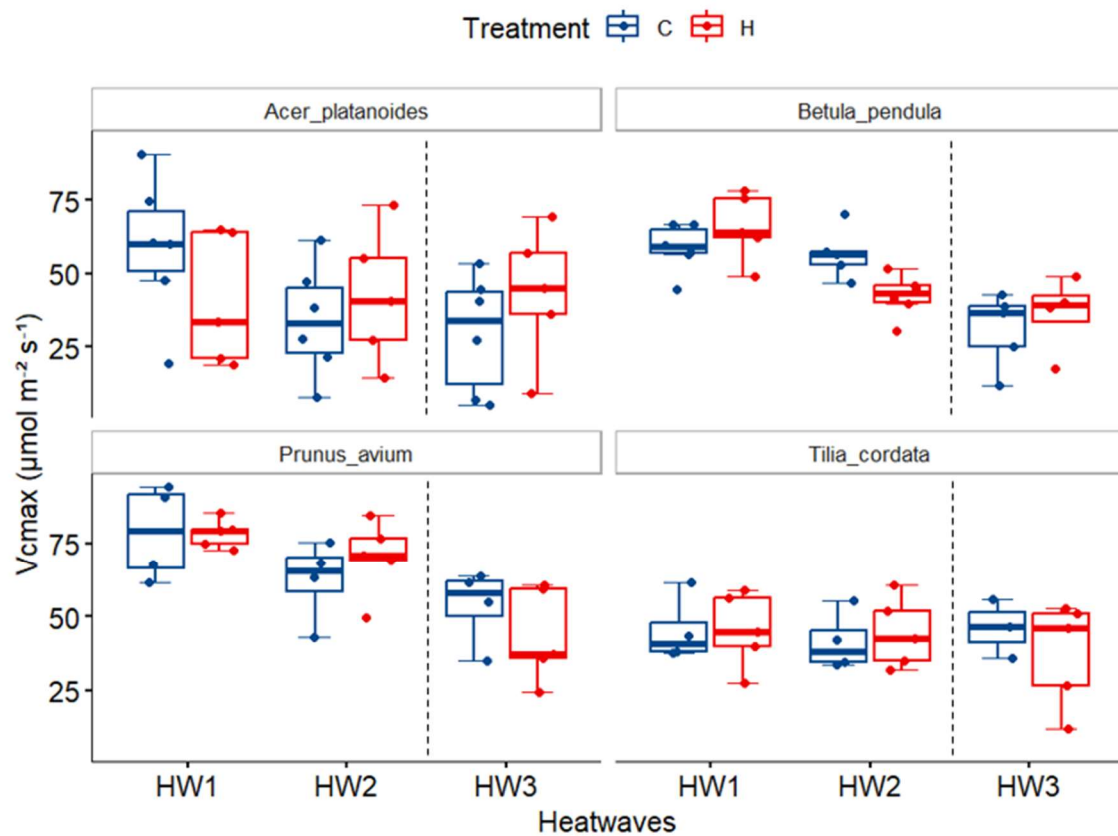


Fig. 5: Boxplot graph of the post-heatwave measurements of  $V_{cmax}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), by species and by heatwave (HW1-3). The control data (C) is coloured in blue, the heat-treated (H) one is coloured red. The boxplots show the interquartile range (IQR; whole box) the median (central line in the box), the 75<sup>th</sup> percentile (upper line) and 25<sup>th</sup> percentile (lower line). The whiskers represent the largest value within 1.5 times IQR above the 75<sup>th</sup> percentile (upper whisker) and the smallest value within 1.5 times IQR below the 25<sup>th</sup> percentile (lower whisker). The jitter represents the individual data points. Heatwave treatment was applied only to the heat-treated plants during HW 1 and HW2, and on both control and heat-treated plants during HW3 (separated by the dashed line).

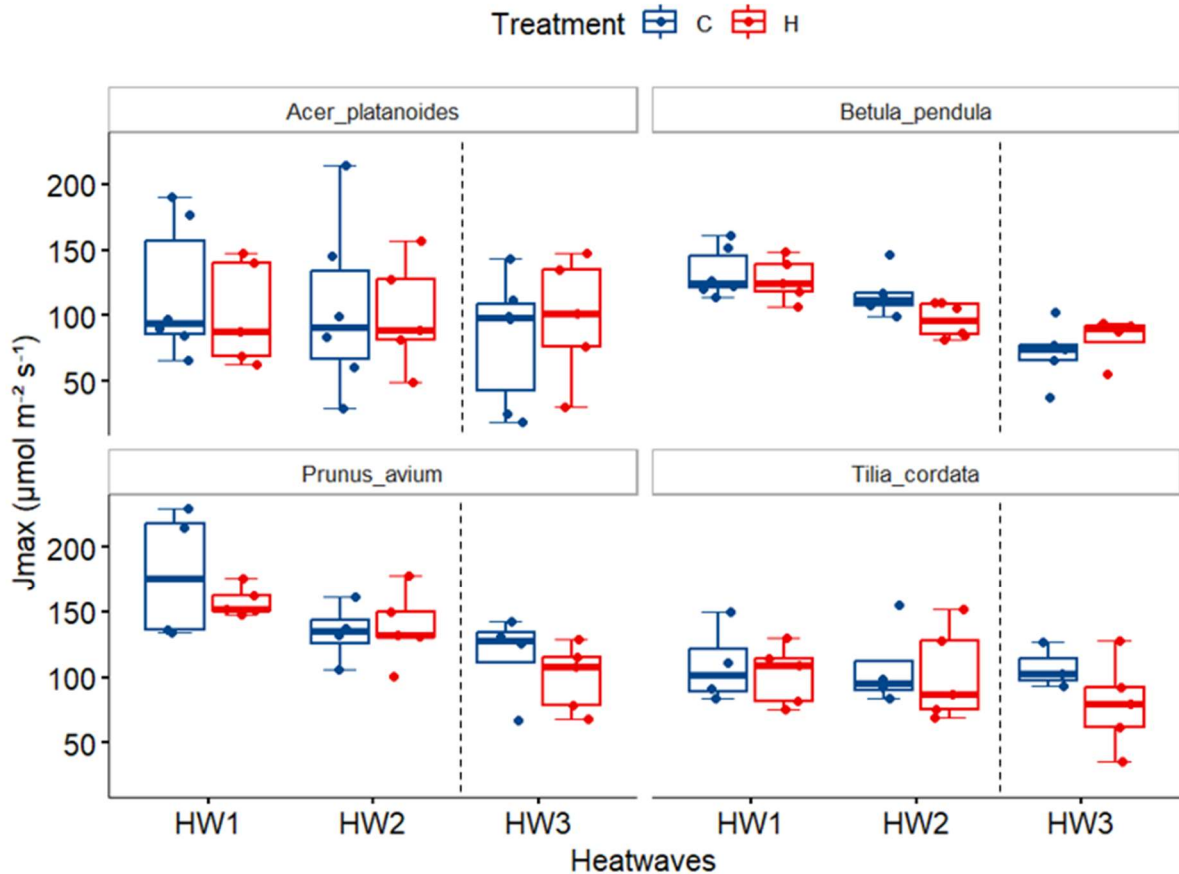


Fig.6: Boxplot graph of the post-heatwave measurements of  $J_{max}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), by species and by heatwave (HW1-3). The control data (C) is coloured in blue, the heat-treated (H) one is coloured red. The boxplots show the interquartile range (IQR; whole box) the median (central line in the box), the 75<sup>th</sup> percentile (upper line) and 25<sup>th</sup> percentile (lower line). The whiskers represent the largest value within 1.5 times IQR above the 75<sup>th</sup> percentile (upper whisker) and the smallest value within 1.5 times IQR below the 25<sup>th</sup> percentile (lower whisker). The jitter represents the individual data points. Heatwave treatment was applied only to the heat-treated plants during HW 1 and HW2, and on both control and heat-treated plants during HW3 (separated by the dashed line).

## 5. Discussion

### 5.1. Among Species differences in heat tolerance

When comparing the control treated plants' physiological responses in the first heatwave among each other, inherent differences in gas exchange are found. Looking at the different species' responses, for  $A_{net}$ , all species show significant decreases between the heatwaves. Since a significant difference between the first and second heatwave could not be found in any

species, the last strong heatwave is likely the cause for this significant difference. This indicates that all species experienced photosynthetic negative effects during the last heatwave. However, the post hoc testing could only verify this for *Bpe* H as well as for the control treated *Apl* and *Bpe* plants with significant differences between the first and last heatwave, and also between the second and last heatwave for *Bpe* C. This decrease in  $A_{net}$  in control plants can be explained by the heatwave exposure of both treatment types during the last heatwave. However, the  $A_{net}$  response compared in between the different treatments did not differ considerably in *Apl*, *Pav* and *Tco*, except for *Bpe* in HW1. *Bpe* was thus affected by the 33 °C heatwave, but only in the first heatwave. For *Apl*, *Pav* and *Tco* the notion that heat could be tolerated photosynthetically in the first two heatwaves, but not in the last one is thus underpinned. This is in line with previous findings of heat tolerance in plants. In broad-leaved temperate trees, a temperature breaking point ( $T_5$ ) starting from 38 °C was found by Kunert and Hajek (2022). Alternatively, findings with a  $T_5$  starting from 35.5 °C were also found in European ash (Münchinger et al. 2023). Okubo et al. (2023) found that in deciduous trees the quantum yield declined between 40 to 50 °C. This underlines that the short heatwaves of 33 °C might have been too low in temperature to stress the plants significantly. Since *Bpe* showed negative effects of a 33 °C heatwave only in the first one, it should be further investigated if lower temperature heatwaves could have stronger effects on *Bpe*.

The  $g_{sw}$  of *Bpe* in both treatments and in *Pav* C decreased significantly across the measuring occasions, and specifically in *Bpe* C between first and second heatwave and second and third heatwave. The treatments themselves did not differ significantly from another in any species and any measuring occasion. Generally, higher temperatures increase the vapor pressure deficit (VPD) in the air which can lead to stomatal closure (Will et al. 2013). Consistent with this, a slightly higher VPD was found in the heat-treated plants during heatwaves, however the stomatal closure did not react accordingly for all species and in all occasions. While that explains the significant differences in  $g_{sw}$  in *Bpe* H and *Bpe* C between HW2 and HW3, it does not explain why the  $g_{sw}$  of control treated plants does not differ significantly from those in heatwave treatment, or why *Bpe* C differs significantly between HW1 and HW2. Moreover, as one of the beforementioned ecosystem services of trees include cooling their surroundings by evapotranspiration, the  $g_{sw}$  is an important aspect to consider for optimal urban tree species selection. Keeping their  $g_{sw}$  levels up, even during heatwave events implies that they could exhibit better cooling effects in these cases. For the heat-treated plants no significant differences were found between the first two heatwaves and between the second and third only in *Bpe* H. Also no significant differences in between treatments were found. This could imply

that the tree species were able to keep up their  $g_{sw}$  levels in the 33 °C heatwaves, and 38 °C heatwave except *Bpe*. However, the control treated plants of *Bpe* and *Pav* showed decreases in  $g_{sw}$  even in 23 °C conditions, but only statistically significant in *Bpe* C. So especially for *Bpe* and *Pav* the  $g_{sw}$  response to heatwaves should be further investigated to prevent coming to a false conclusion here.

For some species, namely *Apl*, *Bpe* and *Pav*, the heatwave had significant effects on  $WUE$ , as control and heat-treated plants were significantly different in one of the first two heatwaves or both. In line with this are the findings of Ruehr et al. (2015), stating a decline in  $WUE$  with higher leaf temperature and VPD in Douglas-fir and black locust trees.

The dark respiration ( $R_d$ ) stayed on similar levels in all three measuring occasions, for either treatment or either species. One can say that  $R_d$  was not significantly affected by the heatwave exposure. Previous research found that most plant species can acclimate respiration to temperature changes (Atkin and Tjoelker 2003, Atkin et al. 2005, as cited in Slot and Kitajima 2015). However, in growth temperatures of 10 and 20 °C, a range from complete to no acclimation of  $R_d$  was found in leaves of alpine and lowland species (Larigauderie and Körner 1995) demonstrating species specificity of acclimation responses. In addition, Tjoelker et al. (1999) found that in response to changing temperatures leaf  $R_d$  was greater in boreal conifers than broad-leaved tree species. Reich et al. (2016) showed that boreal and temperate trees strongly acclimated  $R$  to warming. This demonstrates variation of heat effects on  $R_d$  so in case of the studied species and the kind of treatment that was performed,  $R_d$  was not significantly affected by heatwaves.

Summarizing the findings of the heatwave measurements, the different species seemed, for the most part, to tolerate the heatwaves of 33 °C, by adjusting  $A_{net}$  and  $WUE$ , while the  $g_{sw}$  was not altered. The last heatwave of 38 °C seemed to have affected the plants the most negatively. Control and heat-treated plants showed no significant differences between treatments in that heatwave for all measured parameters, underlining that previous exposure to heat did not improve the tolerance of highly elevated temperatures.

In the post-heatwave measurements, *Apl* and *Tco* showed no significant differences in between treatments and in between the different measuring occasions. Even in the last heatwave, the control treated trees showed no significantly different  $V_{cmax}$  and  $J_{max}$  responses, despite this being their first heatwave exposure.

*Bpe* exhibited significant differences of  $J_{max}$  in both treatments in between the different measuring occasions, and for  $V_{cmax}$  only *Bpe* C showed significant differences. Post hoc testing could not prove where the difference lay, but a decline of the median  $V_{cmax}$  and  $J_{max}$  is visible (Fig. 5,6). Since only  $J_{max}$  was significantly different in heat-treated *Bpe* plants and not  $V_{cmax}$ , that could mean that the electron transport was affected more than the carboxylation rate by the heat exposure. Concerning *Pav*, both  $V_{cmax}$  as well as  $J_{max}$  declined significantly in the heat-treated plants from HW1 to HW3. This shows that *Bpe* and *Pav* were not able to deal well with the recurrent heatwave exposure and suffered long-term effects, especially after the last and strongest heatwave. Visible injury to the leaves after the third heatwave were highest in *Pav*. Although, the second highest heat damage was found in *Apl* and *Tco* leaves, species that showed a tendency to higher heat tolerance. The control treated *Pav* plants did not differ significantly across measuring occasions in  $V_{cmax}$  and  $J_{max}$ , showing that the 38 °C heatwave had no significant effect on photosynthetic capacity, however leaf damage was almost as high as in the heat-treated *Pav*.

Overall, post-heatwave the species *Apl* and *Tco* seem to have not been affected strongly by the heatwave exposure of 33 °C as well as 38 °C. *Pav* and *Bpe* showed, that there were long term negative effects of the heatwaves on their photosynthetic capacity.

As *Bpe* has previously been classified as “very tolerant” to heat by Brune (2016), the result is unexpected. Brune (2016) also states, that the high transpiration rate of *Bpe* makes it react to a sudden lower water availability, so additional watering for *Bpe* could be considered in follow up experiments to eliminate water loss effects. In the same review *Apl* is classified as “moderately tolerant”, which was more or less verified in this study concerning heat tolerance. A study of thermal tolerance found *Pav* to be the least heat sensitive among other broadleaved tree species (Münchinger et al. 2023). Additionally, it was found that *Pav* could withstand a maximum daily temperature of 41 °C (Gonin et al. 2013). This does not align with the findings of this study, suggesting species that show short-term heat tolerance are not necessarily able to handle heatwaves of higher temperatures. *Tco* has been found to have a high tolerance to heatwaves (De Jaegere et al. 2016), and an upper temperature limit of 44 °C (Radoglou et al. 2008). As *Tco* demonstrated heat tolerance to both heatwave temperatures, these studies support the findings. All of the species examined in this thesis have a similar natural origin or distribution, covering large parts of Europe. *Tco* ranges from southern Finland to southern Italy, and *Pav* ranges from southern Sweden to southern Italy (Eaton et al. 2016; Welk et al. 2016). While *Apl* ranges from Fennoscandinavia to the Balkans and northern Italy (Caudullo and de

Rigo 2016). *Bpe* as well as *Pav* are also native to parts of Asia, and *Pav* even to north Africa (Beck et al. 2016; Welk et al. 2016). The vast species occurrence shows that all of them can tolerate some amount of climate variability, however their provenance, the local genetic variation of a certain plant population, plays an important role on how this tolerance plays out in each individual habitat. Regarding the heatwave experiments, the respective provenances of the plants used, seemed to be suitable mostly in *Apl* and *Tco* and only partially in *Bpe* and *Pav*. All of the plants used are of Danish provenance, suggesting that they have adapted to climate conditions similar to those in south-western Sweden and thus are not adapted to very warm conditions. What is demonstrated in this study, is that even species of Nordic provenances can exhibit heat tolerance to heatwaves to some extent. *Apl* and *Tco*, could deal with 33 °C heatwaves and also withstood a 38 °C heatwave. *Bpe* and *Pav*, though also able to survive the heatwaves, showed lower heat tolerance, especially to a 38 °C heatwave.

## 5.2. Thermal acclimation and previous heat exposure effects

For  $A_{net}$ , no significant difference between the first and second heatwave were found in any species. Since the  $A_{net}$  levels remained the same in the second heatwave, after prior heatwave exposure (HW1) it shows that none of the species photosynthetically acclimated to heat. The large variation in  $g_{sw}$  in *Bpe* and *Pav* in the first two heatwaves, compared to the third, show a general decrease in  $g_{sw}$  dispersion (Fig. 4). Between HW1 and HW2 as well as HW2 and HW3 there was a significant decrease in  $g_{sw}$  in *Bpe* C. Other than that, the significant differences between heatwaves in *Bpe* H and *Pav* C could not be specified as to which heatwaves differed. This demonstrates that there was no acclimation of  $g_{sw}$  to heatwaves in any species. Regarding  $WUE$ , none of the heat-treated species were significantly different between the first two heatwaves, again underlining that no acclimation of  $WUE$  in the species to the heatwaves has occurred. As for  $R_d$ , no significant differences in any species and between any heatwave signify that no acclimation of  $R_d$  has occurred. As brought up before the findings of Larigauderie and Körner (1995), Tjoelker et al. (1999) and Reich et al. (2016), generally show that  $R_d$  seems to vary in acclimation ability depending on species or temperature treatment. Because effects of the 33 °C heatwaves were overall quite low, this could explain the lack of acclimation in  $R_d$  as well. Analysing  $V_{cmax}$  and  $J_{max}$ , again no significant differences between HW1 and HW2 were found in any species. Coming back to the second study question on thermal acclimation ability of the four species, there was no thermal acclimation in the species to short heatwaves of neither 33 °C nor 38 °C. In addition, between the control and heat-treated plants, no significant differences were found in HW3 for any of the physiological parameters, indicating that the

previous exposure to heatwaves did not result in a higher heat tolerance compared to unexposed plants to a strong heatwave of 38 °C. Similar results were found for boreal tree species in recurrent heatwave experiments, where no thermal acclimation occurred (Gagne et al., 2020). However, Okubo et al. (2023) found that deciduous species like *Acer buergerianum* were less affected by mid-summer heating, after surviving heating treatment, compared to early summer heating. In that study, the plants were kept in heated conditions for two to three months though, so the short heatwaves could explain why there was no thermal acclimation in this thesis' experiment.

### 5.3. Study limitations and research outlook

Due to the small replicate size of this study ranging from four to six trees per treatment, the physiological variation of individuals within species is little accounted for, making significant conclusions more difficult. It makes it clear, that this study serves for preliminary results instigating further research. Moreover, only four urban tree species were analysed, which does not account for tree species diversity used in urban environments. So, looking into each individual popular urban tree species' thermal acclimation and heat tolerance is needed to gain a bigger picture. Additionally, considering provenance experiments for analysing heat tolerance and thermal acclimation of the four tree species could prove important for urban planning, as previously unfavourable plant characteristics might become more favourable with the changing climate. Due to the small scale of this study, and the short period heatwaves a follow up study should be considered to fasten the findings made, ideally taking aspects like drought or flooding tolerance into account. Also longer heatwaves, with different temperature ranges should be considered, to further investigate the heat tolerance threshold to heatwaves of the four species.

## Conclusion

This study investigated the effect of heatwaves on the gas exchange of four common Swedish urban tree species. All studied species tolerated an exposure to 33 °C over two repeated heatwaves. In the last and strongest heatwave of 38 °C, all species suffered decreased rates of  $A_{net}$ , but could sustain life after. This indicates that heatwaves of 38 °C and above could bring the plants close to their limit. The second study question inquired about the species' ability to thermally acclimate to the heatwaves. In none of the physiological parameters, could a significant difference between the first and second heatwave be found, showing that thermal acclimation to short 33 °C heatwaves did not happen in any species. The third heatwave of

38 °C had strongest aftereffects on *Prunus avium* and *Betula pendula*, while *Acer platanoides* and *Tilia cordata* seemed to be able to recover from the high temperature, as post-heatwave measurements showed. As previous findings from short term heat exposure studies on *Prunus avium* and *Betula pendula* do not align with the findings of this study, the importance of heatwave, and especially recurrent heatwave studies is emphasized.

Summarizing these results, the four different species display different heat tolerances, of which some temperatures can be expected to occur in Swedish cities in the south-west, over the coming decades. The correct selection of tree species able to face future challenges for urban planning is an indispensable part of a healthy urban forest and community.

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## Appendix

Table A1: Temperature (T, °C), relative humidity (RH, %) and light gradient (PPFD,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) setting for the plant growth chambers in Control and Heatwave treatment chambers. In the third heatwave both chambers experience elevated temperatures. (Table prepared by Astrid Fridell, modified)

Time	Control		Heatwave 1 & 2		Heatwave 3		Light (15 cm from lamps)
	T	RH	T	RH	T	RH	PPFD
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

Table A2: Results of the Friedman test to test for difference between each heatwave in each species and respective treatment. For *Tco* C not enough paired data was available to perform this test (/). Green marked cells signify statistical significance.

	<i>Apl</i>		<i>Bpe</i>		<i>Pav</i>		<i>Tco</i>	
	C	H	C	H	C	H	C	H
<i>Anet</i>	0.022	0.022	0.009	0.041	0.039	0.022	0.039	0.022
<i>g<sub>sw</sub></i>	0.247	0.449	0.009	0.041	0.039	0.549	0.105	0.091
<i>WUE</i>	0.007	0.247	0.042	0.074	0.039	0.022	0.050	0.074
<i>V<sub>cmax</sub></i>	0.074	0.549	0.050	0.097	0.174	0.015	/	0.105
<i>J<sub>max</sub></i>	0.819	0.549	0.039	0.050	0.105	0.015	/	0.174
<i>R<sub>d</sub></i>	0.311	0.165	0.311	0.607	0.779	0.449	0.105	0.074

Table A3: Results of the Mann Whitney U test to test between Control and Heat treated plants for each species in all three heatwaves. Blue marked cells signify statistical significance.

	<i>Apl</i>			<i>Bpe</i>			<i>Pav</i>			<i>Tco</i>		
	HW1	HW2	HW3	HW1	HW2	HW3	HW1	HW2	HW3	HW1	HW2	HW3
<b>Anet</b>	0.403	1.000	0.296	0.013	0.128	0.083	0.270	0.903	0.540	0.903	0.178	1.000
<b>g<sub>sw</sub></b>	1.000	1.000	1.000	0.066	0.174	0.083	0.391	0.903	0.713	0.903	0.713	0.713
<b>WUE</b>	0.012	0.144	0.403	0.230	0.008	0.235	0.037	0.037	0.713	0.111	0.066	0.540
<b>V<sub>cmax</sub></b>	0.411	0.648	0.235	0.315	0.014	0.540	1.000	0.270	0.391	0.903	0.713	0.551
<b>J<sub>max</sub></b>	0.523	1.000	0.523	0.648	0.083	0.540	1.000	0.903	0.391	0.713	0.540	0.540
<b>R<sub>d</sub></b>	0.927	0.648	0.648	0.298	1.000	0.575	0.540	0.540	0.903	0.903	0.270	0.903

Table A4: Results of the Kruskal-Wallis test testing for significant differences between the species in heat treatment in the three heatwaves (1) and in the Control treatment in the first heatwave (2). Pink marked cells signify statistical significance.

1				2	
Heated	HW1	HW2	HW3		HW1
<b>Anet</b>	0.044	0.051	0.832	<b>Anet</b>	0.004
<b>gsw</b>	0.041	0.039	0.421	<b>gsw</b>	0.011
<b>WUE</b>	0.082	0.115	0.635	<b>WUE</b>	0.025
<b>Vcmax</b>	0.007	0.058	0.944	<b>Vcmax</b>	0.044
<b>Jmax</b>	0.009	0.171	0.680	<b>Jmax</b>	0.114

## Abbreviations

%	percent
°C	degree Celsius
μmol	micromole
<i>A/Ci</i>	Photosynthetic response curve to intercellular CO <sub>2</sub> concentration
<i>A<sub>net</sub></i>	Net Photosynthesis
<i>Apl</i>	<i>Acer platanoides</i>
<i>Bpe</i>	<i>Betula pendula</i>
C	Control treatment
C <sub>i</sub>	Intercellular CO <sub>2</sub> concentration
C <sub>o</sub>	Partial pressure of CO <sub>2</sub> at Rubisco
CO <sub>2</sub>	Carbon dioxide
<i>E</i>	Transpiration
FvCB	Farquhar, von Caemmerer and Berry biochemical model of photosynthetic CO <sub>2</sub> assimilation in C <sub>3</sub> plants' leaves
<i>g<sub>sw</sub></i>	Stomatal conductance
H	Heat treatment
H <sub>2</sub> O	Water
HW	heatwave
IQR	Interquartile range
<i>J</i>	Rate of electron transport

$J_{max}$	Maximum rate of $J$
$K_c$	Michaelis constant of Rubisco for CO <sub>2</sub>
$K_o$	Inhibition constant of Rubisco for oxygen
LI-COR	LI-6800 Portable Photosynthesis System
m <sup>2</sup>	Square meter
mL	milliliter
$O$	Partial pressure of O <sub>2</sub> at Rubisco
PAR	Photosynthetic Active Radiation
$P_{av}$	<i>Prunus avium</i>
PPFD	Photosynthetic Photon Flux Density
$Q$	Photosynthetic photon flux density
$R_d$	Dark respiration
rpm	revolutions per minute
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP	Ribulose-1,5-bisphosphate
s	Second
SD	Standard deviation
$T_5$	Temperature breaking point
$T_{co}$	<i>Tilia cordata</i>
$V_{cmax}$	Maximum carboxylation rate of RuBP
VPD	Vapor pressure deficit
W	Watt
$WUE$	Water use efficiency
$\alpha_e$	Apparent quantum yield of electron transport
$\Gamma^*$	Photorespiratory compensation point
$\Delta$	Delta (representing a change in a variable)

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