

Evaluation of Heat Tolerance in Foliar Tissue of *Acer* Genotypes

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Abstract. Background: Extreme summer heat events in which temperatures exceed 40 °C are expected to increase in duration and intensity worldwide. Consequently, selecting heat-tolerant trees for future predicted climatic conditions will be one of the significant challenges for urban landscape managers. Method: The effect of heat stress (44 °C for 4 hours) on chlorophyll variable:maximum fluorescence (Fv/Fm) ratios and Soil Plant Analysis Development (SPAD) values as measures of damage to the leaf photosynthetic system and leaf chlorophyll content was quantified in 8 *Acer* genotypes (*Acer pseudoplatanus* ‘Negenia’, *A. pseudoplatanus* ‘Spaethii’, *A. platanoides* ‘Royal Red’, *A. platanoides* ‘Princeton Gold’, *A. platanoides* ‘Emerald Queen’, *A. platanoides* ‘Drummondii’, *A. campestre*, *A. campestre* ‘Louisa Red Shine’). Results: Fv/Fm ratios proved to be a sensitive indicator of heat damage and positively identified marked differences in tolerance to and recovery from heat damage between the 8 *Acer* genotypes. Estimated leaf chlorophyll content (SPAD) was not sufficiently sensitive to distinguish differences in tolerance and recovery between genotypes. Conclusions: Chlorophyll fluorescence Fv/Fm ratios, but not estimated leaf chlorophyll content (SPAD), offers a rapid screening technique for assessing the tolerance to and recovery from heat stress in leaf tissue of trees.

Keywords. Heat Waves; Maple; Photosynthesis; Photosystem II; Species Selection; Urban Heat Island Effect; Urban Trees.

INTRODUCTION

Throughout Europe and the United States, extreme summer heat events in which temperatures exceed 35 to 38 °C are expected to increase in duration and intensity (IPCC 2013; Diem et al. 2017). During June 2021, for example, all-time maximum temperatures were recorded in 7 US states (California, Arizona, New Mexico, Utah, Colorado, Wyoming, and Montana). In Phoenix, Arizona, heat temperatures ranged between 46 to 47 °C, where typically mean summer temperatures range between 33 to 44 °C. Similar unprecedented heat waves have also been recorded in Canada (British Columbia), where summer temperatures in 2021 exceeded 49 °C. Within the United Kingdom, the hottest day since meteorological records began (40.3 °C) was recorded on 2022 July 19. According to the Climate Science Special Report, higher temperature extremes than those experienced to date are projected in the future (Vose et al. 2017). Fundamentally, all climatic predictions state that global warming will be characterized by more frequent and intense high-temperature events (Coumou and Robinson 2013).

Consequently, one of the significant challenges for urban landscape managers will be to select heat-tolerant trees for future predicted climatic conditions. To date, there have been few studies regarding the heat tolerance of urban tree species.

It is now recognized that leaves have reduced photosynthetic function when thermal tolerance limits are exceeded (Knight and Ackerly 2002; Curtis et al. 2014). Within photosynthesis, photosystem II (PSII) has been identified as one of the most thermally labile components of the photosynthetic electron transport chain and a critical site of damage caused by heat stress (Haque et al. 2014). Chlorophyll fluorescence (CF) is a non-destructive and non-invasive analytical system for detecting and quantifying damage to the leaf photosynthetic apparatus in deciduous and coniferous trees in response to environmental stress. CF measures changes in leaf chlorophyll fluorescence due to altered PSII activity, caused directly or indirectly by, for example, heat as well as freezing, chilling, and salinity stress. Importantly, alterations to the ratio of variable:maximum fluorescence (Fv/Fm)

have been shown to correlate with heat tolerance in plants (Allakhverdiev et al. 2008).

Portable equipment for the indirect/estimated measurement of leaf chlorophyll content (SPAD value) has been used in photosynthesis-related studies to demonstrate that heat stress damages thylakoids and results in a loss of chlorophyll (Liu and Huang 2000; Rosyara et al. 2010). Most heat-related studies where SPAD has been used to quantify the damage caused to the leaf photosynthetic system have been performed on plants such as wheat, tomato, and grass (del Blanco et al. 2000; Reynolds et al. 2007; Bhattara et al. 2021). Few studies exist evaluating the use of a SPAD meter to evaluate heat tolerance on tree leaf tissue, especially in urban environments.

While studies exist to quantify the effect of heat stress on primarily forest trees, few investigations have recorded the potential of leaves to recover from heat damage. The importance of rapid recovery from environmental stress has been shown elsewhere. Genotypes that rebound to original or near original physiological levels are most likely to survive and tolerate, for example, drought episodes compared to those that do not or are slower to recover (Aguilera et al. 1997; Bauerle et al. 2003).

The *Acer* genus consists of over 130 species of evergreen and deciduous trees and shrubs from Europe, North Africa, Asia, and North and Central America. Ornamental *Acer* genotypes are a popular choice for public and private urban landscape plantings throughout Europe and the USA. *Acer* trees are hardy, adaptable to varied soil conditions. They are available in a variety of sizes, ranging from 3 feet (0.9 m) (*A. palmatum* 'Coonara Pygmy') to 25 ft (7.6 m) (*A. palmatum* 'Sango-kaku'), and forms (upright, broom, vase, umbrella, weeping, cascading, dwarf, shrub, and ground cover). *Acer* spp. also possess aesthetic characteristics such as bark (snake bark maples, i.e., *A. davidii*, *A. rufrinerve* 'Winters Gold', and *A. conspicuum* 'Silver Vein') and leaf colour (*A. palmatum atropurpureum* 'Bloodgood', *A. palmatum dissectum* 'Crimson Queen' or 'Red Dragon', *Acer shirasawanum* 'Aureum'). Genotypes are suitable for urban gardens, courtyards, parking strips, public parks, and highway plantings (Santamour 1990; Raupp et al. 2006; Lahr et al. 2018).

This study aimed to identify whether differences in foliar heat tolerance and recovery from heat damage exist within the *Acer* genus and to evaluate the sensitivity of CF against SPAD values in quantifying

heat damage to provide information as to their usefulness for planting in urban areas where the frequency, intensity, and duration of heat stress episodes are predicted to increase. Studies were conducted in July and August as these months are when summer heat-waves predominantly occur in the US, Canada, and UK (Vose et al. 2017).

MATERIALS AND METHODS

Plant Material

In this study, 8-year-old 4.3-m (± 0.3) trees with a DBH of 50 to 53 mm (± 8.1) grown in 45-L Light Pots™ (white mypex woven grow bags) grafted onto their respective species type rootstocks were used for experimental purposes. The potting substrate consisted of a 50:50 green waste and pine bark compost mix with a pH of 7.7. Trees were potted during the winter of 2013/2014 and grown at Barcham Trees, Ely, Cambridgeshire, UK, before being planted at the Bartlett Tree Research Laboratory, Shinfield, Reading, UK in March 2017. Trees were arranged across 3 rows, with each measured genotype randomized within each row (Banks and Hirons 2019; Banks et al. 2019). Five trees from each of the following were used: *Acer pseudoplatanus* 'Negenia', *A. pseudoplatanus* 'Spaethii', *A. platanoides* 'Royal Red', *A. platanoides* 'Princeton Gold', *A. platanoides* 'Emerald Queen', *A. platanoides* 'Drummondii', *A. campestre*, *A. campestre* 'Louisa Red Shine'.

Fully developed mature leaves were collected from the 8 *Acer* genotypes on the morning of 2022 July 26 and 2022 August 8–9, a time when the leaf photosynthetic apparatus has completely developed and summer heat waves primarily occur within the US and Europe (Kitao et al. 1998; Vose et al. 2017). Visually healthy leaves were removed 20 to 30 cm below a terminal bud on the lower limbs. Leaves were collected between 08:00 to 08:30 AM on the morning of each sampling date. Immediately after removal, leaves were sealed in Ziploc® plastic bags. All material was prepared within 60 to 90 minutes of collection. Upon arrival at the laboratory, 50 leaf disks per *Acer* genotype (10 disks from 5 trees) were excised, and Fv/Fm and SPAD measurements were taken (i.e., 0 hours, prior to heat stress). Leaf disks were used in preference to entire leaves to ensure uniformity as leaf size differed between genotypes. Leaf disks were then placed abaxial surface down in a water bath set at 44 °C. After 4 hours, the same 50 disks per

genotype were removed, and damage to the leaf photosynthetic system was quantified using chlorophyll fluorescence (Greaves and Wilson 1987) and estimated leaf chlorophyll content (SPAD). Four hours at 44 °C was chosen as a heat stress response as previous research identified this temperature and duration sufficient to distinguish marked differences in tolerance between *Acer* genotypes (Percival and Percival 2022). Recovery from heat stress was then assessed. After the 4 hours heat exposure period, leaf disks were placed abaxial surface down in a water bath at room temperature (18 to 21 °C), and Fv/Fm and SPAD values were re-measured every 24 hours over a 72-hour period.

Chlorophyll Fluorescence

Measurements were performed with a plant efficiency analyser (Pocket PEA) chlorophyll fluorometer (Hansatech Instruments, King's Lynn, Norfolk, UK). During the measurement, a clip system shielded the sample from ambient light to reach a dark-adapted state (20 minute adaptation to the dark) and illuminated with 627 nm light from an LED source built into the fluorimeter sensor. Continuous light excitation (at 3500 $\mu\text{mol}/\text{m}^2\text{ s}$) was provided by a light-emitting diode focused on the leaf surface to provide homogeneous irradiation over a 4 mm diameter leaf surface. The ratio of variable ($F_v = F_m - F_o$) to maximal (F_m) fluorescence, i.e., Fv/Fm where F_o = minimal fluorescence of dark-adapted leaves (default Pocket PEA value), was used to quantify the detrimental effects of heat on leaf tissue. Fv/Fm is considered a quantitative measure of the maximal or potential photochemical efficiency or optimal quantum yield of photosystem II (Willits and Peet 1999). Likewise, Fv/Fm values are the most popular index used as a measure of plant vitality and early diagnostic of stress (Maxwell and Johnson 2001).

Leaf Chlorophyll SPAD Measurements

A Konica Minolta chlorophyll meter SPAD-502 (Marunouchi, Chiyoda, Tokyo) was used at the mid-point of the leaf next to the central leaf vein. Previously the SPAD meter had been calibrated by measurement of absorbance at 663 and 645 nm in a spectrophotometer (PU8800 Pye Unicam) after extraction with 80% v/v aqueous acetone (regression equation = $5.68 + 0.066x$; r^2 adjusted = 0.95, $P < 0.001$) (Lichtenthaler and Wellburn 1983; Percival and Graham 2021).

Statistical Analysis

Damage to leaf tissue and recovery from heat stress was assessed by quantifying leaf chlorophyll fluorescence (Fv/Fm) and chlorophyll content (SPAD) values over time using linear regression, ($y = a + xT$), where y = chlorophyll fluorescence value; a = chlorophyll fluorescence of control value (calculated intercept); x = rate of fluorescence (slope of the linear regression line) with time (T). Plants were ranked in order of heat tolerance based on reductions in x of Fv/Fm (reference). ANOVA was used to determine whether each slope significantly differed between genotypes at the 95% confidence level ($P < 0.05$). The heat tolerance of each *Acer* genotype was assessed on 2022 July 26 and repeated on 2022 August 08 to 09. Recovery from heat damage was only recorded in August. All statistical analysis was performed using R version 4.2.1 (R Development Core Team, Vienna, Austria).

RESULTS

Heat Stress

A significant influence of sampling time (July/August) on leaf chlorophyll fluorescence Fv/Fm values ($P < 0.05$) in response to heat was observed. Results show that irrespective of the *Acer* genotype, damage to the leaf photosynthetic system, as assessed by Fv/Fm, was always greater in July compared to August. For example, concerning *A. pseudoplatanus* 'Negenia', 18.2% damage was recorded in July compared to 7.3% in August. Similarly, concerning *A. campestre*, 31.6% damage was recorded in July compared to 13.9% in August. Such a response indicates that foliar heat tolerance within genotypes can be significantly influenced by the time when a heat stress event occurs during the growing season.

A significant effect of the *Acer* genotype on leaf chlorophyll fluorescence Fv/Fm values ($P < 0.05$) in response to heat was observed (Table 1 and Figure 1). This is reflected by marked differences in the magnitude of the heat response (the slope value represented by the letter x recorded among genotypes (Table 1). For example, Fv/Fm values ranged from $-0.149x$ (July) and $-0.059x$ (August) for *A. pseudoplatanus* 'Negenia', representing 18.2% and 7.3% damage to the leaf photosynthetic system. Similarly, Fv/Fm values ranged from $-0.235x$ (July) and $-0.092x$ (August) for *A. pseudoplatanus* 'Spaethii', representing 29% and 11.4% damage to the leaf photosynthetic system (Table 1). Such a result indicates these genotypes are

Table 1. Percent reduction (–) in Fv/Fm compared to zero values (i.e., before heat stress was imposed). Species are ranked in order of tolerance based on percent reductions in Fv/Fm as a measure of damage to the leaf photosynthetic system.

Species	Fv/Fm	
	July	August
<i>A. pseudoplatanus</i> ‘Negenia’	–18.2d	–7.3c
<i>A. pseudoplatanus</i> ‘Spaethii’	–29.0c	–11.4c
<i>A. platanoides</i> ‘Royal Red’	–33.6bc	–12.8c
<i>A. campestre</i>	–31.6bc	–13.9c
<i>A. campestre</i> ‘Louisa Red Shine’	–34.4bc	–31.6b
<i>A. platanoides</i> ‘Princeton Gold’	–36.0bc	–26.4b
<i>A. platanoides</i> ‘Emerald Queen’	–40.8b	–32.0b
<i>A. platanoides</i> ‘Drummondii’	–53.0a	–47.9a

Numbers within a column followed by a common letter (taken from regression equations [Figure 1]) are not significantly different according to Tukey’s honest significance test ($P = 0.05$).

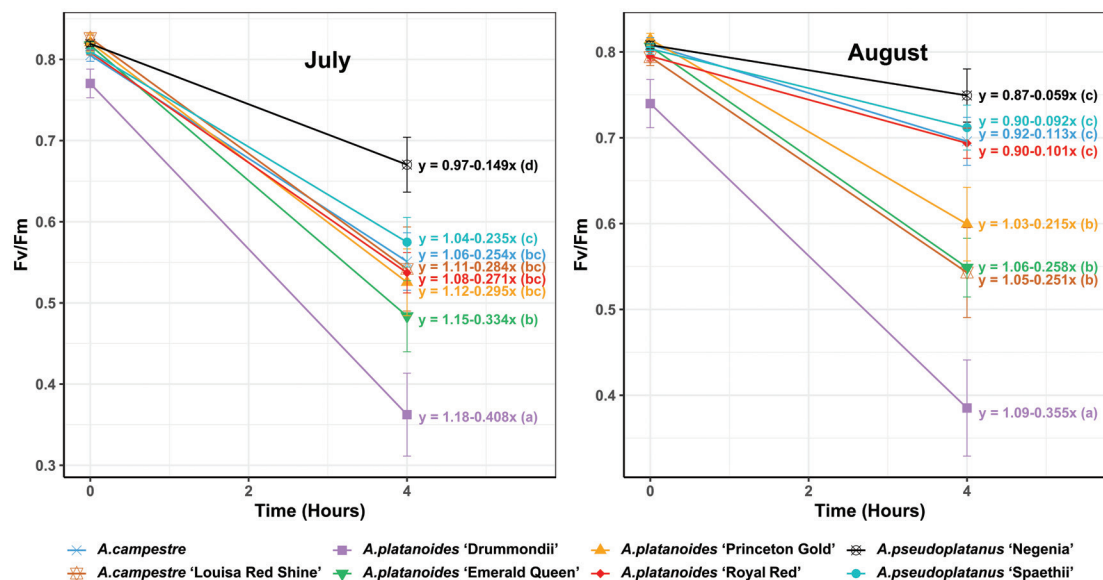


Figure 1. Regression of chlorophyll fluorescence value Fv/Fm after heat stress (44 °C, 20 mins) exposure in foliar tissue of 8 *Acer* species. y = chlorophyll fluorescence value; a = chlorophyll fluorescence of control value (calculated intercept); x = rate of fluorescence with heat (T).

more tolerant to heat stress compared to *A. platanoides* ‘Drummondii’, where Fv/Fm values ranged from $-0.408x$ (July) and $-0.355x$ (August), representing 53% and 47.9% damage to the leaf photosynthetic system respectively (Table 1). Importantly, results show that measurements of Fv/Fm values using chlorophyll fluorescence can distinguish marked differences in heat tolerance among genotypes of the same genus. Based on the damage to the leaf photosynthetic system as quantified by Fv/Fm, heat tolerance

ranking was in the order *A. pseudoplatanus* ‘Negenia’ > *A. pseudoplatanus* ‘Spaethii’ > *A. platanoides* ‘Royal Red’ > *A. campestre* > *A. campestre* ‘Louisa Red Shine’ > *A. platanoides* ‘Princeton Gold’ > *A. platanoides* ‘Emerald Queen’ > *A. platanoides* ‘Drummondii’ (Table 1).

Differences in the magnitude of damage to the leaf chlorophyll content (SPAD) were also recorded between the *Acer* genotypes (Table 2 and Figure 2). However, the degree of damage recorded was less

Table 2. Percent reduction (–) or increase (+) in leaf chlorophyll content (SPAD values) compared to zero values (i.e., before heat stress was imposed). Species are ranked in order of tolerance based on percent reductions in SPAD values as a measure of damage to the leaf chloroplast membrane.

Species	SPAD	
	July	August
<i>A. pseudoplatanus</i> ‘Spaethii’	+2.46a	–5.52a
<i>A. campestre</i> ‘Louisa Red Shine’	–1.57a	–3.90a
<i>A. pseudoplatanus</i> ‘Negenia’	–2.25a	–3.25a
<i>A. platanoides</i> ‘Royal Red’	–3.94a	–3.85a
<i>A. campestre</i>	–6.36a	–1.22a
<i>A. platanoides</i> ‘Emerald Queen’	–4.70a	–7.55a
<i>A. platanoides</i> ‘Princeton Gold’	–5.31a	–10.6a
<i>A. platanoides</i> ‘Drummondii’	–8.82a	–8.11a

Numbers within a column followed by a common letter (taken from regression equations [Figure 2]) are not significantly different according to Tukey’s honest significance test ($P = 0.05$).

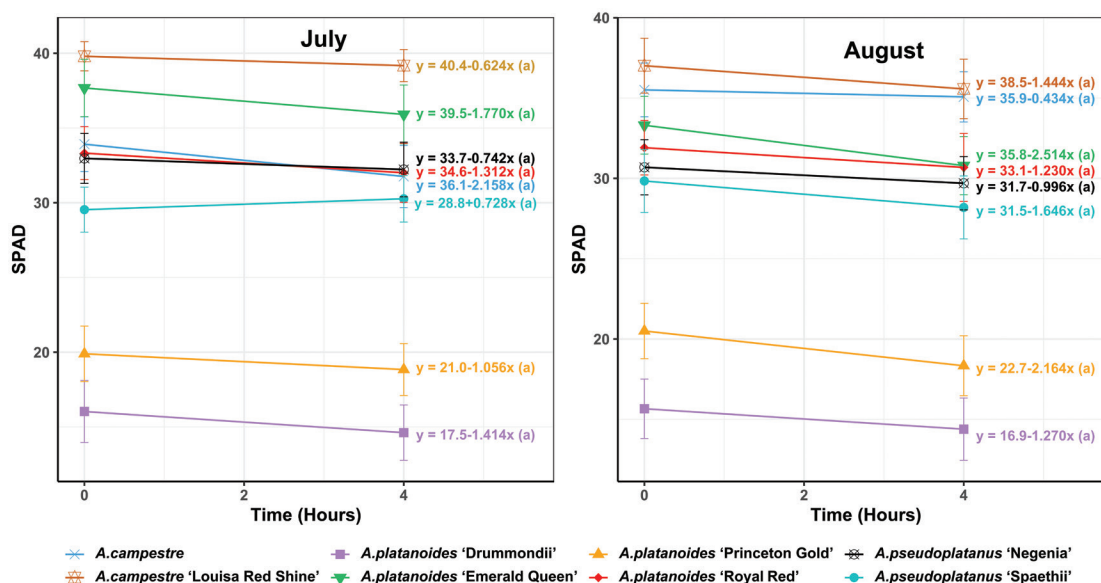


Figure 2. Regression of leaf chlorophyll content (SPAD values) after heat stress (44 °C, 20 mins) exposure in foliar tissue of 8 *Acer* species. y = leaf chlorophyll content (SPAD value); a = leaf chlorophyll content (SPAD values) of control value (calculated intercept); x = rate of leaf chlorophyll content (SPAD value) with heat (T).

than that recorded for Fv/Fm values. For example, following the July heat stress experiment, leaf chlorophyll content of leaf disks ranged from a 2.46% increase (*A. pseudoplatanus* ‘Spaethii’) to an 8.82% loss (*A. platanoides* ‘Drummondii’) compared to control leaf disks. Following the August heat stress experiment, leaf chlorophyll content of leaf disks ranged from a 1.22% (*A. campestre*) to a 10.6% loss compared to control leaf disks (*A. platanoides* ‘Princeton Gold’). Consequently, there was no statistically

significant difference ($P > 0.05$) between genotypes in terms of damage to leaf chlorophyll content (SPAD values). There was also no significant influence of time of sampling (July/August) on leaf SPAD values in response to heat. For example, concerning *A. pseudoplatanus* ‘Negenia’, 2.25% damage was recorded in July compared to 3.25% in August. Similarly, concerning *A. platanoides* ‘Royal Red’, 3.94% damage was recorded in July compared to 3.85% in August.

Recovery from Heat Stress

A significant effect of the *Acer* genotype on leaf chlorophyll fluorescence Fv/Fm values ($P < 0.05$) in recovery from heat was observed (Table 3 and Figure 3). With respect to Fv/Fm, at 72 hours after heat stress, 5 of the 8 genotypes began to recover, as shown by an increase in the rate of fluorescence (slope of the linear regression line, i.e., x -value) with time. Rates of

Table 3. Percent reduction (–) or increase (+) in Fv/Fm after 72 hours following heat stress (44 °C, 20 minutes) in foliar tissue of 8 *Acer* species. Species are ranked in order of tolerance based on percent increases in Fv/Fm as a measure of recovery from damage to the leaf photosynthetic system.

Species	Fv/Fm
<i>A. platanoides</i> ‘Royal Red’	+8.42b
<i>A. pseudoplatanus</i> ‘Spaethii’	+7.21ab
<i>A. campestre</i>	+6.36ab
<i>A. platanoides</i> ‘Princeton Gold’	+4.79ab
<i>A. pseudoplatanus</i> ‘Negenia’	+2.59ab
<i>A. platanoides</i> ‘Drummondii’	–1.17ab
<i>A. campestre</i> ‘Louisa Red Shine’	–1.76ab
<i>A. platanoides</i> ‘Emerald Queen’	–22.80a

Numbers within a column followed by a common letter (taken from regression equations [Figure 3]) are not significantly different according to Tukey’s honest significance test ($P = 0.05$).

recovery were in the order: *A. platanoides* ‘Royal Red’ > *A. pseudoplatanus* ‘Spaethii’ > *A. campestre* > *A. platanoides* ‘Princeton Gold’ > *A. pseudoplatanus* ‘Negenia’. Such a response indicates repair and increased functioning of the leaf photosynthetic system, although none of the genotypes fully recovered over the 72-hour period. However, 3 of the 8 genotypes continued to decline after heat stress, as shown by a continual decrease in the rate of fluorescence (x -value) in the order: *A. platanoides* ‘Drummondii’ > *A. campestre* ‘Louisa Red Shine’ > *A. platanoides* ‘Emerald Queen’. Increased recovery rates over the 72-hour recovery period ranged from 8.42% (*A. platanoides* ‘Royal Red’) to 2.59% (*A. pseudoplatanus* ‘Negenia’). Decreased rates over the 72-hour recovery period ranged from 1.17% (*A. platanoides* ‘Drummondii’) to 22.8% (*A. platanoides* ‘Emerald Queen’).

Although an increase in SPAD over the 72-hour recovery period was recorded for all *Acer* genotypes, there was no statistically significant difference in recovery rates between genotypes (Table 4 and Figure 4).

DISCUSSION

Alterations to chlorophyll fluorescence Fv/Fm emissions as a measure of photosystem II (PSII) photochemical efficiency are sensitive indicators of damage to the leaf photosynthetic system caused by heat (Haque

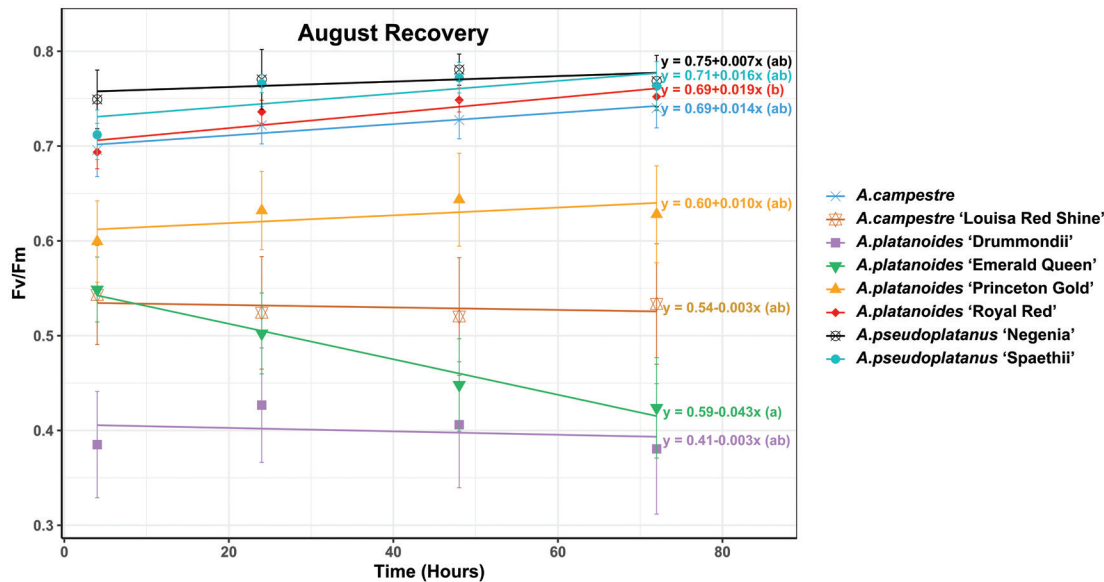


Figure 3. Recovery rates of chlorophyll fluorescence value Fv/Fm after 72 hours following heat stress (44 °C, 20 mins) in foliar tissue of 8 *Acer* species. y = chlorophyll fluorescence value; a = chlorophyll fluorescence of control value (calculated intercept); x = rate of fluorescence with heat (T).

et al. 2014). Consequently, temperatures inducing a rapid decline in Fv/Fm ratios are widely used to quantify heat tolerance in foliar tissue of trees (Dreyer et al. 2001; Knight and Ackley 2003; O'Sullivan et al. 2017; Zhu et al. 2018; Húdoková et al. 2022). Results of this study support that of others in that measurement of Fv/Fm ratios proved to be a sensitive measurement of foliar heat tolerance, distinguishing marked

Table 4. Percent increase (+) in leaf chlorophyll content (SPAD values) after 72 hours following heat stress (44 °C, 20 minutes) in foliar tissue of 8 *Acer* species. Species are ranked in order of tolerance based on percent increases in leaf chlorophyll content (SPAD values) as a measure of recovery from damage to the leaf chloroplast membrane.

Species	SPAD
<i>A. platanoides</i> 'Drummondii'	+22.8a
<i>A. platanoides</i> 'Princeton Gold'	+17.9a
<i>A. platanoides</i> 'Emerald Queen'	+11.7a
<i>A. platanoides</i> 'Royal Red'	+9.80a
<i>A. campestre</i> 'Louisa Red Shine'	+7.26a
<i>A. campestre</i>	+4.92a
<i>A. pseudoplatanus</i> 'Negenia'	+2.86a
<i>A. pseudoplatanus</i> 'Spaethii'	+2.28a

Numbers within a column followed by a common letter (taken from regression equations [Figure 4]) are not significantly different according to Tukey's honest significance test ($P = 0.05$).

differences in heat tolerance between the 8 *Acer* genotypes evaluated. *A. pseudoplatanus* 'Negenia', *A. pseudoplatanus* 'Spaethii', *A. platanoides* 'Royal Red', and *A. campestre* were identified as heat tolerant. Irrespective of when the heat stress was imposed (July or August), damage to the leaf photosynthetic system as assessed by chlorophyll fluorescence Fv/Fm ratios ranged from 7.3% to 33.6%. In the case of *A. platanoides* 'Emerald Queen' and *A. platanoides* 'Drummondii', damage to the leaf photosynthetic system ranged from 32% to 53% and consequently were identified as sensitive to heat stress. With respect to *A. campestre* 'Louisa Red Shine' and *A. platanoides* 'Princeton Gold', damage ranged from 26.4% to 36%, indicating these genotypes are moderate to tolerant to heat stress.

Regardless of *Acer* genotypes evaluated in this study, damage to the leaf photosynthetic system, when assessed by Fv/Fm, was always greater in July compared to August. Such a response indicates that foliar heat tolerance within the *Acer* genotypes evaluated was influenced by the time when heat stress was imposed during the growing season. Heat waves tend to be a summer phenomenon occurring during July and August within Europe and the USA. At this time of year, leaves are generally expanded with a fully developed and functional leaf photosynthetic system (Kitao et al. 1998). Consequently, if heat stress is

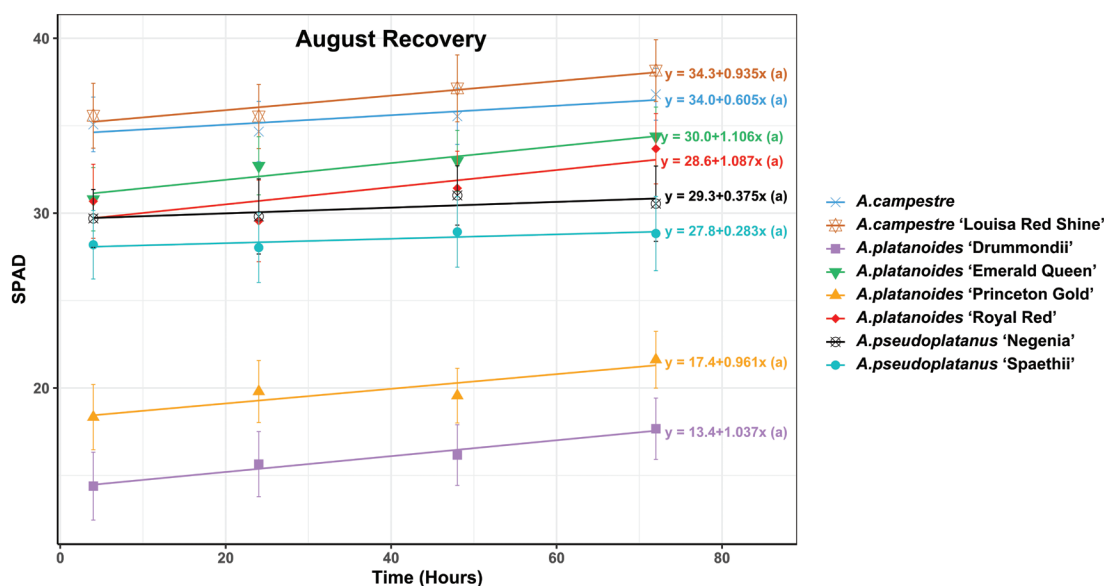


Figure 4. Recovery rates of leaf chlorophyll content (SPAD values) after 72 hours following heat stress (44 °C, 20 mins) in foliar tissue of 8 *Acer* species. y = leaf chlorophyll content (SPAD value); a = leaf chlorophyll content (SPAD values) of control value (calculated intercept); x = rate of leaf chlorophyll content (SPAD value) with heat (T).

imposed during this period, it is generally considered that an increase of 10 to 15 °C above ambient temperatures is required to cause heat-related damage (Wahid et al. 2007). The increase in heat tolerance from July to August recorded in this study may be explained by the fact that July 2022 was identified as a period of unusually hot weather leading to the National Severe Weather Warning Service issuing its first-ever extreme heat warning in the UK, resulting in the UK government declaring a national emergency on 2022 July 8, 15, 18, and 19. It has been shown that artificially subjecting trees to heat stress before a simulated heat wave results in a higher temperature required to, for example, disrupt PSII functioning (Hamerlynck and Knapp 1994; Ghouil et al. 2003; Daas et al. 2008). Likewise, previous research has shown that if excessive heat occurs in spring coinciding with bud burst and leaf flush rather than in summer when leaves are fully expanded, considerably lower temperatures can cause substantial damage to trees (Filewod and Thomas 2014; Teskey et al. 2015). In our study, the 2022 July 26 sampling occurred one week after a heatwave (2022 July 19) was recorded as the hottest day ever in the UK, where temperatures exceeded 40 °C. The July heatwave may have instigated an acclimation process within the *Acer* trees used in this study. The time taken for acclimation to occur and the duration of heat tolerance conferred has been studied mainly in crop plants and a few tree genotypes, with the acclimation process occurring 3 to 10 days after the heat event and lasting between 4 to 6 weeks (Charng et al. 2023) which would have provided sufficient time for the *Acer* spp. used in this study to acclimate.

Using a SPAD meter to quantify tree heat tolerance has received little attention. Abnormal heat stress episodes induce sunburn on leaves, leaf yellowing/necrosis, marginal tip, and peripheral leaf burn, which in turn lower leaf chlorophyll content due to damage to the leaf chlorophyll molecule (Teskey et al. 2015; O'Sullivan et al. 2017). A SPAD meter is a non-destructive, non-invasive, commercially available portable piece of equipment used to estimate foliar chlorophyll concentrations based on optical responses when a leaf is exposed to light (Uddling et al. 2007). Consequently, SPAD meters have successfully quantified heat damage regarding leaf chlorophyll loss in crops (del Blanco et al. 2000; Reynolds et al. 2007; Bhattara et al. 2021). Results of this study show that

while a SPAD meter could distinguish differences in heat responses between genotypes, it was not as sensitive a measure as Fv/Fm, as no statistically significant differences in SPAD values between genotypes were recorded. For example, following heat stress, effects on SPAD ranged from an increase in values of 2.25% (*A. pseudoplatanus* 'Negenia' [July]) to a 10.6% reduction in values (*A. platanoides* 'Princeton Gold' [August]). Because of this lack of sensitivity, estimated leaf chlorophyll content based on SPAD values appears to have a limited role in quantifying tree responses to abnormally high temperatures over a short time period (72 hours) using leaf disks. Concerning crop plants, effects on leaf chlorophyll content can be monitored over more extended time periods due to their smaller size and stature whole plants can be subjected to heat stress rather than leaf disks as used in this study.

While studies exist to quantify the adverse impact of heat stress on growth and physiological activities, this information has been gained primarily from forest tree studies. Much less is known about the potential of leaves to recover from heat damage. For example, the importance of rapid recovery from drought stress has been shown elsewhere. Genotypes that rebound to original or near original physiological levels are more likely to survive and tolerate drought episodes than those that do not or are slower to recover (Aguilera et al. 1997; Bauerle et al. 2003). Indeed, some authors argue that selecting traits for recovery may be of greater importance than selecting for growth during a heat stress episode (Wang and Huang 2004; Galmes et al. 2007). Although it is appreciated that results were obtained from detached leaf disks, clear differences in recovery rates over 72 hours were identified between the *Acer* genotypes evaluated. An increase in Fv/Fm values was recorded 72 hours after heat stress in *A. platanoides* 'Royal Red', *A. pseudoplatanus* 'Spaethii', *A. campestre*, *A. platanoides* 'Princeton Gold', and *A. pseudoplatanus* 'Negenia', indicating regeneration and improved functioning of the leaf photosynthetic system. Recovery rates over the 72-hour recovery period ranged from 8.42% (*A. platanoides* 'Royal Red') to 2.59% (*A. pseudoplatanus* 'Negenia'), indicating a genotypic-specific response. However, Fv/Fm ratios in *A. platanoides* 'Drummondii', *A. campestre* 'Louisa Red Shine', and *A. platanoides* 'Emerald Queen' continued to decrease over the 72-hour recovery

period, ranging from -1.17% (*A. platanoides* ‘Drummondii’) to -22.80% (*A. platanoides* ‘Emerald Queen’), indicating heat aftereffects still detrimental to leaf photosynthetic integrity.

It is recognized that trees possess physiological and biochemical mechanisms that allow them to survive extreme heat events. Although not investigated in this study, important ones include the ability to reduce thermal inactivation of RuBisCO activity, an essential carboxylase in the C3 carbon reaction of photosynthesis (Hu et al. 2020), rapid synthesis of heat shock proteins to protect against heat-induced protein denaturation (Tian et al. 2021), and synthesis of osmolytes to include sugars and sugar alcohols (polyols), proline, and tertiary and quaternary ammonium compounds (glycine betaine) (Sairam and Tyagi 2004). The ability to keep stomata open during heat stress episodes to allow for evaporative cooling (Hasan et al. 2020) and synthesis of volatile organic compounds (isoprene, monoterpene) (He et al. 2000; Kleist et al. 2012) have also been identified as significant physiological traits associated with survival against abnormally prolonged heat stress episodes. These findings, however, are based on data derived from a limited number of genera (*Quercus*, *Pinus*, *Eucalyptus*, *Acer*, *Picea*), and within those genera, only a small number of genotypes which in turn are predominately planted for forestry, not urban landscape purposes (Teskey et al. 2015).

In practical terms, the results of this investigation indicate that *A. pseudoplatanus* ‘Negenia’, *A. pseudoplatanus* ‘Spaethii’, *A. platanoides* ‘Royal Red’, and *A. campestre* possess good foliar heat tolerance and should be planted in urban areas subject to heat stress events such as within urban infrastructure while genotypes such as *A. platanoides* ‘Emerald Queen’ and *A. platanoides* ‘Drummondii’ should be avoided. Planting with more appropriate genotypes offers a simple and effective means of reducing tree mortality. Consequently, genotypes planted will remain healthier and live longer, reducing labour and replacement costs.

Results should, however, be interpreted with some degree of caution regarding providing *Acer campestre* with an absolute foliar heat tolerance ranking. *A. campestre* can be propagated from seed, and subsequent progeny may possess wide genetic variation. Likewise, *A. campestre* has a broad ecological range and a natural distribution covering most of Europe. This potentially offers an abundance of largely untapped genetic resources to select for heat tolerance. As *A. campestre*

is the only recognised UK native maple, then most likely, the provenance in this study would be of UK origin. However, most of the *Acer* genotypes evaluated here are propagated clonally and therefore possess a narrow genetic base, meaning it would be likely that there would be less variation among individual clones in response to heat stress.

In conclusion, wide genotypic variation in response to heat stress indicates that potential exists in the selection of foliar heat tolerance in urban trees. Further investigations are in progress to evaluate a broader range of genotypes and genera.

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The authors reported no conflicts of interest.