

Article

Genotypic Variation in Foliar Heat Tolerance Among 35 *Malus* Genotypes: Implications for Urban Tree Selection Under Climate Change

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Abstract

The frequency and intensity of heatwaves are increasing annually worldwide due to climate change. Combined with the urban heat island effect, elevated heat stress episodes threaten the survival and performance of urban trees, in turn reducing their ecosystem benefits. For this reason, the foliar heat tolerance of 35 *Malus* genotypes (two species, 32 cultivars, one variety, one hybrid) was evaluated under controlled laboratory assays. Heat injury to foliar tissue was quantified using chlorophyll fluorescence (Fv/Fm) to assess photosystem II (PSII) damage and an electrolyte leakage index (ELI) to evaluate cellular membrane integrity. A preliminary dose–response experiment using six genotypes exposed to a temperature gradient (40–50 °C) was conducted to establish thermal response curves and derive LT₅₀ values (temperature at 50% decline in Fv/Fm). These analyses confirmed substantial genotypic variation in thermal tolerance and identified 45 °C as an optimal discriminatory temperature for large-scale screening. This temperature was subsequently applied to assess heat injury across all 35 genotypes. Measurements were conducted in May (spring foliage) and August (summer foliage) to evaluate ontogenetic influences. In some instances, only one genotype was available for experimental purposes. Consequently, conclusions regarding genotypic differences in heat tolerance are based on replicated datasets, whereas genotypes represented by single-tree sampling are presented for descriptive purposes only. Heat stress significantly affected Fv/Fm and ELI, with strong genotype and seasonal effects recorded. In most genotypes, foliar damage was greater in spring than in summer. Good correlations between Fv/Fm and ELI confirmed their value as complementary physiological measures of heat tolerance in plants. Of the 35 genotypes evaluated, *Malus sargentii*, *M.* ‘Prairifire’, *M. baccata* ‘Jackii’, *M.* ‘Royal Fountain Huber’ and *M.* Donald Wyman were the most heat tolerant. The substantial variation in foliar heat tolerance detected across the 35 genotypes tested demonstrates potential for selecting *Malus* genotypes with superior foliar heat tolerance and highlights opportunities for identifying heat resilient candidates among other under-utilized urban tree taxa.



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Keywords: heat stress; foliar heat tolerance; urban trees; thermotolerance; seasonal acclimation; genotypic variation; tree selection

1. Introduction

Due to global climate change resulting in frequent and severe heat waves coupled with the urban heat island (UHI) effect which can further raise city temperatures by 4–9 °C compared to surrounding rural areas, trees planted within urban landscapes are increasingly exposed to heat stress episodes annually [1,2]. Thermal stress poses significant challenges

for urban forests, as prolonged heat episodes can seriously impair tree physiological processes, such as photosynthesis and respiration, reduce growth, and increase mortality rates, especially of newly planted trees if irrigation is limited or low precipitation levels exist within that geographical area [3–6]. Consequently, selecting tree species with high heat tolerance is increasingly important for sustaining tree canopy ecosystem services such as shading, cooling, absorption of atmospheric pollutants and carbon sequestration which can be achieved by evaluating physiological and molecular heat stress responses [6–8].

Apple trees (*Malus* spp., including hybrid and ornamental cultivars/varieties), traditionally cultivated in temperate regions, are extensively used for urban planting due to their aesthetic value, cultural significance, and potential for multifunctional landscapes [9]. However, *Malus* are highly sensitive to supra-optimal temperatures, which can disrupt photosynthesis, induce oxidative stress, and impair fruit quality [10,11]. Heat stress primarily affects foliar tissues by inhibiting photosystem II activity, reducing chlorophyll content, and enhancing cellular ion leakage due to damage to membrane stability, ultimately compromising tree vitality and productivity [11,12]. Differences among physiological thresholds for foliar damage have already been identified among a limited number of *Malus* genotypes, suggesting that genotypic differences in heat tolerance could be exploited for breeding programmes for heat tolerance [8,13].

Given the projected increase in frequency and intensity of heatwaves worldwide, quantifying the heat tolerance mechanisms in foliar tissue of *Malus* genotypes would be useful for guiding selection for urban landscapes. This knowledge will enable arborists, landscape designers and urban planners to identify resilient genotypes capable of maintaining physiological function under prolonged heat stress episodes, thereby ensuring long-term tree survival and the continual delivery of ecosystem services in warming cities.

The objective of this study was to quantify foliar heat tolerance across thirty-five selected apple (*Malus*) genotypes (two species (*M. sargentii* and *M. orientalis*), 31 cultivars, one variety (*M. baccata* var. *mandshurica*), one hybrid *M. × zumi* ‘Calocarpa’) and to compare genotypic-specific responses to identify those exhibiting superior heat resilience. These findings aim to inform *Malus* selection for urban forests and landscape planning based on robust heat-tolerance metrics. To achieve this aim, two physiological indicators—chlorophyll fluorescence as a measure of damage to the leaf photosynthetic system and leaf electrolyte leakage (ELI) as a measure of damage to cellular and membrane structural integrity—were assessed. Experiments were conducted under controlled laboratory heat stress conditions. Studies were conducted in May (spring foliage) and August (mature foliage) to evaluate the influence of ontogenetic variation, i.e., does heat tolerance ranking of spring foliage represent that of summer foliage. The reason for this is that while August tends to be the month when summer heatwaves predominantly occur in the US, Canada and Europe [5,14], heat stress episodes have also been recorded in springtime (May) coinciding with bud burst and leaf flush [15].

2. Materials and Methods

2.1. *Malus* Selection

All leaf material was taken from Bartlett Tree Research Arboretum (BTRL), Charlotte, North Carolina, US (Latitude 35.0840953, Longitude −80.9993747), hardiness zone 7b according to USDA Plant hardiness Zone Map. All trees used in the study had been established for over 10 years in unconstrained soil volume, growing in full sun with no visual symptoms of biotic (leaf fungal infection, leaf defoliation) or abiotic foliar stress (leaves yellowing, necrosis symptoms), indicating that the trees were exhibiting no visible stress symptoms under ambient conditions. A total of 35 *Malus* genotypes were evaluated (see Table 1); the number of replicates (number of individuals in the collection at Arboretum)

varied among genotypes by 1–8 trees. The mean annual temperature at the BTRL during 2024 when the study was conducted was 62.8 °F (17.1 °C) with the highest monthly mean temperature of 100 °F (37.8 °C) in July while the lowest mean monthly temperature was in January with 14 °F (−10.0 °C) recorded. These temperature extremes are relevant to *Malus* genotypes which typically perform best under moderate summer temperatures of 70–75 °F (21–24 °C), and exposure to elevated temperatures above this range can induce heat stress.

Table 1. Reductions in Fv/Fm ratios of *Malus* genotypes prior to and after heat stress (45 °C for 1 h). Species are ranked in order of tolerance based on percent reductions in Fv/Fm (mean of May and August values) as a measure of damage to the leaf photosynthetic system.

Genotype	May			August		
	Fv/Fm (0 h)	Fv/Fm (Post Heat Stress)	Percent Reduction	Fv/Fm (0 h)	Fv/Fm (Post Heat Stress)	Percent Reduction
<i>sargentii</i> ^{n = 3} (S)	0.838	0.458	45.3 ± 2.70 ^{ab}	0.840	0.564	32.9 ± 3.12 ^{abc}
‘Prairifire’ ^{n = 4} (C)	0.848	0.362	57.3 ± 4.92 ^{cde}	0.845	0.580	31.4 ± 2.43 ^{ab}
baccata ‘Jackii’ ^{n = 3} (C)	0.838	0.473	42.8 ± 3.81 ^a	0.839	0.512	39.0 ± 3.42 ^{abcd}
‘Royal Fountain Huber’ ^{n = 2} (C)	0.837	0.332	60.2 ± 2.49 ^{def}	0.842	0.591	29.4 ± 2.01 ^a
‘Donald Wyman’ ^{n = 3} (C)	0.824	0.309	62.3 ± 5.74 ^{fg}	0.831	0.477	42.6 ± 3.33 ^{bcd}
‘Winter Gold’ ^{n = 2} (C)	0.843	0.419	50.3 ± 3.44 ^{abc}	0.844	0.490	42.0 ± 5.64 ^{bcd}
‘Centurion’ ^{n = 3} (C)	0.852	0.424	50.2 ± 5.00 ^{abc}	0.845	0.440	47.9 ± 5.04 ^{de}
<i>orientalis</i> ^{n = 2} (S)	0.836	0.290	65.3 ± 6.22 ^{fg}	0.838	0.468	44.1 ± 3.88 ^{cd}
‘Red Jewel’ ^{n = 2} (C)	0.845	0.394	53.3 ± 4.76 ^{bcde}	0.848	0.330	61.2 ± 3.29 ^{fg}
‘Indian Summer’ ^{n = 2} (C)	0.850	0.362	57.4 ± 4.61 ^{cdef}	0.844	0.359	57.4 ± 4.56 ^{efg}
‘Adams’ ^{n = 3} (C)	0.852	0.329	61.3 ± 3.20 ^{ef}	0.850	0.379	55.4 ± 4.81 ^{ef}
‘Sparkling Sprite’ ^{n = 2} (C)	0.842	0.248	70.8 ± 6.18 ^{gh}	0.840	0.270	67.8 ± 7.01 ^{gh}
‘JFS-KW5’ (Royal Raindrops®) ^{n = 7} (C)	0.847	0.327	61.4 ± 3.69 ^f	0.848	0.180	78.8 ± 6.14 ^{hi}
‘Velvet Pillar’ ^{n = 3} (C)	0.852	0.332	61.1 ± 5.92 ^f	0.855	0.176	79.4 ± 5.11 ^{hi}
× <i>zumi</i> ‘Calocarpa’ ^{n = 3} (C)	0.848	0.204	75.9 ± 6.03 ^{hi}	0.850	0.272	68.0 ± 5.38 ^{gh}
‘Bob White’ ^{n = 2} (C)	0.852	0.295	65.4 ± 5.94 ^{fg}	0.844	0.151	82.1 ± 5.51 ^{ij}
‘Ralph Shay’ ^{n = 2} (C)	0.842	0.178	78.8 ± 5.27 ⁱ	0.846	0.263	68.9 ± 6.20 ^{gh}
‘Snowdrift’ ^{n = 2} (C)	0.854	0.403	52.9 ± 4.77 ^{bcd}	0.851	0.040	95.3 ± 5.70 ^k
‘Indian Magic’ ^{n = 2} (C)	0.842	0.063	91.4 ± 6.11 ^{kh}	0.841	0.132	84.3 ± 7.95 ^{ijk}
‘Sutyzam’ (Sugar Tyme®) ^{n = 2} (C)	0.835	0.018	97.8 ± 5.88 ^h	0.836	0.162	80.7 ± 7.72 ^{ij}
‘Coral Burst’ ^{n = 2} (C)	0.831	0.040	94.9 ± 3.59 ^{kh}	0.834	0.132	84.2 ± 4.05 ^{ijk}
<i>sargentii</i> ‘Roseglow’ ^{n = 3} (C)	0.846	0.106	87.5 ± 8.36 ^{jk}	0.845	0.057	92.1 ± 8.09 ^{jk}
baccata var. <i>mandshurica</i> ^{n = 3} (V)	0.834	0.091	89.1 ± 3.09 ^{jk}	0.839	0.058	93.2 ^k

Numbers within a column followed by a common letter are not significantly different according to Tukey’s honest significance test ($p = 0.05$). Significance of Species (S) = $p < 0.0001$; Time (T) = 0.005; S × T ≤ 0.0001. (C) = Cultivar; (S) = Species; (V) = Variety; n = number of replications.

2.2. Heat Stress Assays

2.2.1. Preliminary Experiment (2023): Temperature Optimization

To identify a temperature capable of differentiating heat tolerance among *Malus* genotypes, a preliminary experiment was conducted in 2023 using six representative genotypes. This experiment was designed to establish a single discriminatory temperature for use in future large-scale heat tolerance evaluation of a wider range of genotypes. Mature, fully expanded leaves were collected on the morning of 12 August 2023, when the photosynthetic apparatus is fully developed [16]. Leaves were sampled from south-facing, sun-exposed branches approximately 20–30 cm below a terminal bud. Collections were made between 08:00 and 09:30 h. Immediately after excision, leaves were sealed in plastic bags and processed within 60 min of collection. In the laboratory, 50 leaf discs (1 cm diameter) were

excised per genotype. Chlorophyll fluorescence (F_v/F_m) was measured prior to heat exposure (0 h) at room temperature (21 °C) and was considered an untreated control [17]. Leaf discs were used instead of whole leaves to minimize variability associated with differences in leaf size among genotypes. Leaf discs were subjected to controlled heat stress in a programmable water bath across a gradient of incrementally increasing temperatures (40–50 °C in 2 °C intervals) for 1 h per temperature treatment [18]. Following each exposure, samples were rapidly cooled to room temperature to arrest further thermal injury. Based on the response curves generated (Figure 1), 45 °C for 1 h was identified as the optimal temperature treatment to identify differences in F_v/F_m ratios among genotypes. This temperature was therefore selected for subsequent screening of a larger genotype set in 2024 studies.

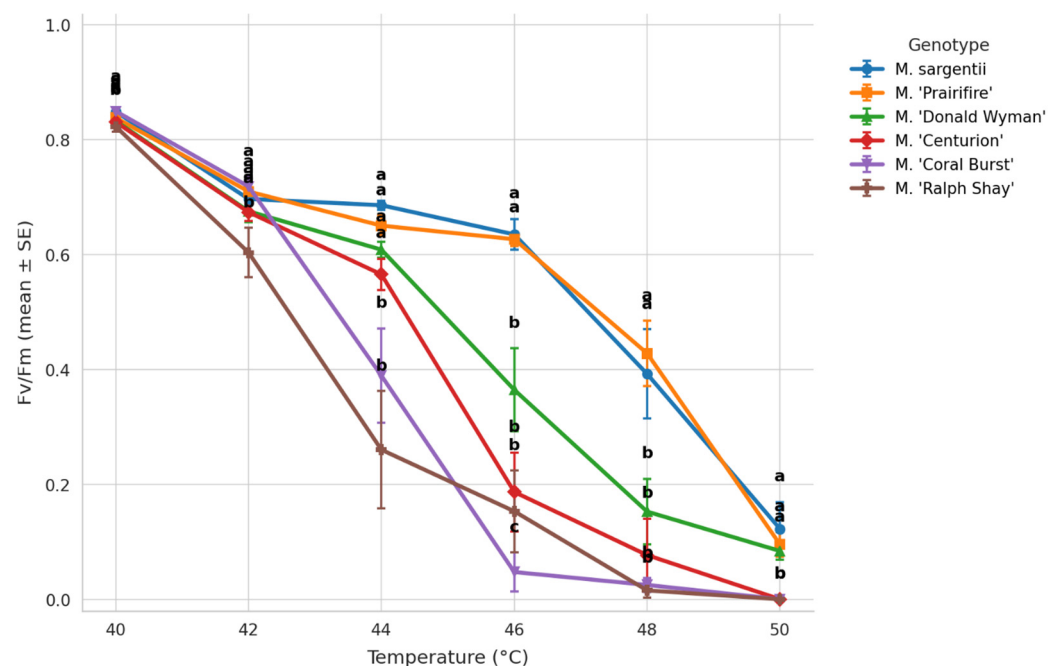


Figure 1. Decline in maximum quantum yield (F_v/F_m) with increasing temperature across six *Malus* genotypes. F_v/F_m values (mean $\pm$ SE) of six *Malus* genotypes measured following exposure to increasing temperatures (40–50 °C). Declines in F_v/F_m reflect impairment of photosystem II function under heat stress. Different lowercase letters above data points indicate significant differences among genotypes at each temperature based on Tukey's HSD test ($p \leq 0.05$). Significance of Species (S) = $p < 0.0001$; Temperature $p < 0.0001$ (T); $S \times T = p < 0.0001$.

2.2.2. Main Experiment (2024): Genotype Screening

The temperature determined in the 2023 experiment (45 °C for 1 h) was applied to evaluate heat tolerance across a broader set of 35 *Malus* genotypes. Leaf collection followed the same protocol as described for 2023. Sampling was conducted on 9–10 May and 8–9 August 2024 to capture seasonal variation (spring vs. summer foliage). For each genotype, 50 leaf discs (1 cm diameter) were prepared and placed abaxial side down in a water bath maintained at 45 °C. After 1 h of heat exposure, leaf discs were removed and immediately assessed for damage to the photosynthetic apparatus using chlorophyll fluorescence (F_v/F_m). Because chlorophyll fluorescence is a non-destructive technique, the same leaf discs were subsequently used to assess cellular membrane stability via an electrolyte leakage assay, providing an integrated measure of thermal damage at both the physiological and cellular levels.

2.3. Electrolyte Leakage Index (ELI)

Leaves were rinsed with deionized water, and two circular discs (1 cm diameter) were excised per leaf. For each pair, one disc was assigned to the heat treatment, while the second served as a non-heated control to quantify baseline electrolyte leakage associated with tissue excision and handling. Heat-treated discs were exposed to 45 °C for 1 h, whereas control discs were maintained at 22 °C (72 °F) for the same duration. This paired design enabled separation of heat-induced injury from background leakage. Following their respective treatments, discs were placed in 30 mL universal bottles containing 20 mL distilled water and incubated at 22 °C (72 °F) in darkness for 24 h. Conductivity of the bathing solution ($C_{(24\text{ h})}$) was then measured using a Jenway conductivity probe and M4070 m (BDH, Loughborough, UK). Total solute leakage (C_{total}) was determined by autoclaving samples at 121 °C (250 °F) and 0.103 MPa for 1 h to release all cellular electrolytes.

Electrolyte leakage index (ELI) was calculated as:

$$\text{ELI (\%)} = (C_{(24\text{ h})} / C_{\text{total}}) \times 100$$

where

- $C_{(24\text{ h})}$ = conductivity after 24 h incubation at 22 °C in darkness
- C_{total} = conductivity following autoclaving

To account for baseline leakage, ELI values from heat-treated discs were corrected using their paired non-heated controls. Final values therefore represent heat-induced electrolyte leakage, calculated as:

$$\text{Corrected ELI (\%)} = [(C_{(24\text{ h, heat})} - C_{(24\text{ h, control})}) / C_{\text{total}}] \times 100$$

For clarity ELI terminology used throughout this paper refers to the corrected ELI values.

2.4. Chlorophyll Fluorescence

Measurements were performed with a Plant Efficiency Analyser (Pocket PEA) chlorophyll fluorometer (Hansatech Instruments, King's Lynn, UK). During the measurement, a clip system shielded the sample from ambient light to reach a dark-adapted state (20 min adaptation to the dark) and was 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., F_v/F_m 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. F_v/F_m is considered a quantitative measure of the maximal or potential photochemical efficiency or optimal quantum yield of photosystem II [19]. Likewise, F_v/F_m ratios are the most popular index used as a measure of plant vitality and for early diagnosis of abiotic stress [20].

2.5. Statistical Analysis

2.5.1. Preliminary Experiment (2023): Temperature Optimization

F_v/F_m data were analyzed by two-way ANOVA with genotypes and temperature as fixed effects, including the genotype $\times$ temperature interaction. Six genotypes were evaluated across six temperature treatments (40, 42, 44, 46, 48, and 50 °C). Tukey's HSD test was used for post hoc separation of means. Model assumptions were assessed using Shapiro–Wilk and Levene's tests. To estimate LT_{50} , mean F_v/F_m values at each temperature were normalized to the corresponding mean at 40 °C and fitted with a four-parameter logistic model; LT_{50} was defined as the temperature at which relative F_v/F_m declined to 0.40.

2.5.2. Main Experiment (2024): Genotype Screening

Damage to leaf tissue caused by heat stress was quantified using chlorophyll fluorescence (Fv/Fm) and electrolyte leakage index (ELI). For each individual tree, 50 leaf discs were excised and used as subsamples to estimate the tree-level response, i.e., discs were not treated as independent replicates, and the individual tree was considered the experimental unit for all analyses. Although many cultivars used in the study are clonally propagated and genetically uniform, replication where possible was necessary to quantify any tree-level environmental and experimental variance. Percent reduction in Fv/Fm and percent increase in ELI were analyzed using linear mixed-effects models with genotype, sampling time (May vs. August), and their interaction as fixed effects, and tree nested within genotype as a random effect to account for unequal replication (1–8 trees per genotype). Models were fitted using restricted maximum likelihood following confirmation of normality and homoscedasticity. Post hoc comparisons among genotypes were performed using Tukey's honestly significant difference (HSD) test based on least-squares means. Statistical significance was accepted at $p < 0.05$. Genotypes represented by a single tree were retained to facilitate comparative screening; however, inference for these genotypes should be interpreted with caution because within-genotype variance could not be estimated. Pearson's correlation analysis was used to examine relationships between heat tolerance responses measured in May and August, and between Fv/Fm and ELI as complementary indicators of heat tolerance. All analyses were conducted using JMP version 19.0 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Preliminary Experiment (2023): Temperature Optimization

A significant influence of heat, species and species $\times$ temperature interaction on Fv/Fm was recorded, indicating that genotypes differed in the extent of Fv/Fm decline with increasing temperature (Figure 1). At a temperature of 40 °C, there was no significant difference in Fv/Fm values between genotypes. At 42 °C, however, Fv/Fm values in *M. 'Ralph Shay'* and *M. 'Coral Burst'* were significantly lower ($p < 0.05$) than all remaining genotypes. Greatest effects on Fv/Fm among genotypes occurred between 44 and 46 °C, where separation into tolerant and sensitive groups was evident. At temperatures ≥ 46 °C, *M. 'Coral Burst'* and *M. 'Ralph Shay'* showed near-complete loss of photosynthetic efficiency, whereas *M. sargentii* and *M. 'Prairifire'* retained comparatively higher Fv/Fm values. At 48–50 °C, all genotypes exhibited severe declines, with Fv/Fm values approaching zero. As temperatures between 44 and 46 °C provided the greatest resolution among genotypes, 45 °C was therefore identified as an appropriate screening temperature for differentiating heat tolerance across a wider range of *Malus* genotypes. Logistic estimates of LT₅₀ at Fv/Fm = 0.4 (50% reduction in Fv/Fm values) ranked genotypes as *M. 'Prairifire'* (48.1 °C) > *M. sargentii* (48.0 °C) > *M. 'Donald Wyman'* (45.8 °C) > *M. 'Centurion'* (44.9 °C) > *M. 'Coral Burst'* (44.0 °C) > *M. 'Ralph Shay'* (43.3 °C) indicating significant variation in heat tolerance based on the temperature response of Fv/Fm.

3.2. Genotypic Variation in Foliar Heat Tolerance

A significant influence of genotype and sampling time (May/August) as well as interactions between these factors on leaf chlorophyll fluorescence (Fv/Fm) and ELI values ($p < 0.005$) was observed in genotypes where biological replication was available (Tables 1 and 2). These replicated data provide the basis for all inferential statistical comparisons presented herein. In contrast, a subset of genotypes represented by a single tree was evaluated without replication and is presented separately for descriptive comparison

only (Tables 3 and 4). These data provide preliminary observations of foliar heat injury but were not subjected to statistical analysis and should be interpreted cautiously.

Table 2. Percent increase (+) in cellular electrolyte leakage (ELI) compared to zero values, i.e., before heat stress was imposed. Species are ranked in order of tolerance based on percent increases (mean of May and August values) in ELI as a measure of leaf cell membrane damage and loss of membrane integrity.

Genotype	May	August
<i>sargentii</i> ^{n = 3} (S)	40.5 ± 3.28 abc	30.3 ± 3.37 a
‘Prairifire’ ^{n = 4} (C)	37.6 ± 3.42 ab	34.4 ± 3.58 ab
baccata ‘Jackii’ ^{n = 3} (C)	37.0 ± 3.71 a	36.4 ± 2.94 a
‘Royal Fountain Huber’ ^{n = 2} (C)	40.7 ± 2.99 abcd	33.2 ± 2.76 a
‘Donald Wyman’ ^{n = 3} (C)	40.1 ± 3.56 abc	33.8 ± 4.02 a
‘Winter Gold’ ^{n = 2} (C)	38.2 ± 2.93 ab	36.5 ± 3.15 a
‘Centurion’ ^{n = 3} (C)	39.9 ± 3.09 abc	44.4 ± 3.27 bc
<i>orientalis</i> ^{n = 2} (S)	46.3 ± 5.19 abde	48.0 ± 4.47 bcde
‘Red Jewel’ ^{n = 2} (C)	50.3 ± 3.67 bcdef	45.6 ± 4.79 bc
‘Indian Summer’ ^{n = 2} (C)	51.3 ± 4.30 cdef	46.2 ± 3.91 bcd
‘Adams’ ^{n = 3} (C)	46.9 ± 4.50 abcde	58.3 ± 3.62 cdefg
‘Sparkling Sprite’ ^{n = 2} (C)	53.6 d ± 3.81 ef	61.2 ± 5.71 efghi
‘JFS-KW5’ (Royal Raindrops®) ^{n = 7} (C)	55.1 ± 6.07 efg	59.8 ± 4.69 defgh
‘Velvet Pillar’ ^{n = 3} (C)	62.2 ± 7.17 fgh	54.5 ± 4.78 cdef
× <i>zumi</i> ‘Calocarpa’ ^{n = 3} (C)	57.6 ± 4.29 eg	62.2 ± 6.12 fghi
‘Bob White’ ^{n = 2} (C)	60.7 ± 5.53 fg	59.2 ± 5.14 defgh
‘Ralph Shay’ ^{n = 2} (C)	60.2 ± 5.75 fg	68.3 ± 5.77 fghij
‘Snowdrift’ ^{n = 2} (C)	77.5 ± 4.81 i	69.8 ± 4.95 ghij
‘Indian Magic’ ^{n = 2} (C)	75.3 ± 6.34 hi	72.7 ± 6.72 hij
‘Sutyzam’ (Sugar Tyme®) ^{n = 2} (C)	66.9 ± 5.87 ghi	82.3 ± 8.36 j
‘Coral Burst’ ^{n = 2} (C)	74.3 ± 6.52 hi	77.2 ± 5.96 j
<i>sargentii</i> ‘Roseglow’ ^{n = 3} (C)	79.2 ± 7.25 i	75.3 ± 6.44 ij
baccata var. <i>mandshurica</i> ^{n = 3} (V)	78.8 ± 7.38 i	81.9 ± 6.38 j

Numbers within a column followed by a common letter are not significantly different according to Tukey’s honest significance test ($p = 0.05$). Significance of Species (S) = $p < 0.0001$; Time (T) = 0.005; S × T ≤ 0.0001. (C) = Cultivar; (S) = Species; (V) = Variety; n = number of replications.

Table 3. Descriptive foliar heat injury (percent reduction in Fv/Fm following heat stress) in selected *Malus* genotypes based on single-tree sampling. Values represent observed responses in May and August and are presented for qualitative comparison only.

Genotype	May			August		
	Fv/Fm (0 h)	Fv/Fm (Post Heat Stress)	Percent Reduction	Fv/Fm (0 h)	Fv/Fm (Post Heat Stress)	Percent Reduction
‘Cardinal’s Robe’ (C)	0.853	0.455	46.7 ± 4.39	0.857	0.520	39.3 ± 4.01
‘Weeping Candied’ (C)	0.849	0.271	68.1 ± 6.26	0.850	0.532	37.5 ± 5.11
‘Orange Crush’ (C)	0.854	0.309	63.9 ± 5.47	0.852	0.489	42.7 ± 4.28
‘New Centennial’ (C)	0.864	0.408	52.7 ± 3.93	0.859	0.387	54.9 ± 4.25
‘Moonglow’ (C)	0.853	0.367	57.1 ± 4.22	0.855	0.385	55.0 ± 4.18
‘Radiant’ (C)	0.852	0.384	55.0 ± 5.15	0.850	0.258	69.7 ± 6.13
‘Liset’ (C)	0.833	0.249	70.1 ± 4.65	0.840	0.379	54.9 ± 3.87
‘Emerald Spire’ (C)	0.843	0.315	62.7 ± 5.72	0.844	0.098	88.4 ± 4.83
‘Strawberry Parfait’ (C)	0.828	0.067	91.9 ± 3.93	0.831	0.144	82.7 ± 4.10
‘Dolgo’ (C)	0.844	0.129	84.7 ± 4.05	0.840	0.043	94.9 ± 4.38
‘Woven Gold’ (C)	0.836	0.071	91.5 ± 4.42	0.839	0.099	88.2 ± 3.96
‘Mary Potter’ (C)	0.826	0.014	98.3 ± 3.95	0.830	0.093	88.8 ± 4.12

Because only one tree per genotype was sampled ($n = 1$), no true biological replication exists. Consequently, inferential statistical comparisons (e.g., significance testing or ranking) were not performed. Values are presented descriptively as observed responses for each genotype, and differences should be interpreted as preliminary patterns rather than statistically supported effects; (C) = Cultivar.

Table 4. Descriptive foliar heat injury (percent increase (+) in cellular electrolyte leakage (ELI) following heat stress) in selected *Malus* genotypes based on single-tree sampling. Values represent observed responses in May and August and are presented for qualitative comparison only.

Genotype	May	August
‘Cardinal’s Robe’ (C)	35.6 ± 3.94	34.0 ± 4.51
‘Weeping Candied’ (C)	46.2 ± 5.05	37.5 ± 4.36
‘Orange Crush’ (C)	43.3 ± 4.22	46.5 ± 4.19
‘New Centennial’ (C)	49.6 ± 5.19	42.8 ± 3.86
‘Moonglow’ (C)	48.7 ± 5.56	50.1 ± 5.29
‘Radiant’ (C)	56.3 ± 3.78	48.7 ± 4.59
‘Liset’ (C)	50.1 ± 4.01	58.3 ± 6.11
‘Emerald Spire’ (C)	57.8 ± 4.48	73.3 ± 7.83
‘Strawberry Parfait’ (C)	74.5 ± 6.39	70.1 ± 8.28
‘Dolgo’ (C)	73.4 ± 6.91	77.9 ± 8.05
‘Woven Gold’ (C)	80.4 ± 4.25	76.2 ± 4.63
‘Mary Potter’ (C)	80.9 ± 3.82	83.0 ± 3.89

Because only one tree per genotype was sampled ($n = 1$), no true biological replication exists. Consequently, inferential statistical comparisons (e.g., significance testing or ranking) were not performed. Values are presented descriptively as observed responses for each genotype, and differences should be interpreted as preliminary patterns rather than statistically supported effects; (C) = Cultivar.

With respect to time, results show that in the majority of *Malus* genotypes, damage to the leaf photosynthetic system as assessed by chlorophyll fluorescence Fv/Fm ratios and cellular ion leakage (ELI) was greater in May compared to August (Tables 1 and 2). For example, in the case of *M. sargentii*, *M.* ‘Prairifire’, *M. baccata* ‘Jackii’, *M.* ‘Royal Fountain Huber’ and *M.* ‘Donald Wyman’, identified as the top five heat-tolerant *Malus* genotypes, damage to the leaf photosynthetic system ranged from 45.3 to 62.3% in May compared to 32.9–42.6% in August. Although not quite as pronounced with respect to ELI, May values ranged between 37.0 and 40.7% compared to 30.3–36.5% in August for these genotypes. Such a response indicates that foliar heat tolerance within *Malus* genotypes can be influenced by ontogenetic variation, with greater damage across most, but not all, genotypes inflicted during a spring compared to a summer heat stress episode. Descriptive data from non-replicated genotypes showed similar responses, i.e., greater heat sensitivity in May compared to August (Tables 3 and 4).

Across replicated genotypes, heat stress markedly reduced Fv/Fm values and increased ELI values, indicating substantial impairment of PSII function and cellular membrane damage. Indeed, a significant effect of genotype on leaf chlorophyll fluorescence Fv/Fm values and ELI ($p < 0.0001$) was observed (Tables 1 and 2). This is reflected by marked differences in the magnitude of the heat response recorded between genotypes (Tables 1 and 2). For example, damage to the leaf photosynthetic system (Fv/Fm ratios) ranged from 45.3 and 32.9% (*M. sargentii*, May and August data, respectively) to 65.3 and 44.1% (*M. orientalis*, May and August data, respectively) to 87.5 and 92.1% (*M. sargentii* ‘Roseglow’, May and August data, respectively). Similar responses were observed with respect to ELI, where values ranged from 40.5 and 30.3% (*M. sargentii*, May and August data, respectively) to 51.3–46.2% (*M. orientalis*, May and August data, respectively) to 78.8–81.9% (*M. sargentii* ‘Roseglow’, May and August data, respectively). Similar seasonal trends were observed descriptively among non-replicated genotypes (Tables 3 and 4). Importantly, these results show that measurements of Fv/Fm ratios using chlorophyll fluorescence or ELI can distinguish marked differences in heat tolerance among genotypes within the same genus. In addition, wide genotypic variation in response to heat stress indicates that potential exists in the selection of foliar heat tolerance within the *Malus* genus.

3.3. Influence of Ontogenetic Variation on Heat Tolerance Ranking

Although the magnitude of the heat stress response differed between May (spring) and summer (August) foliage (damage to foliar tissue was, in most genotypes, higher in May compared to August), this did not influence the ranking of heat tolerance and sensitivity between genotypes, i.e., the genotypes identified as the most heat tolerant and sensitive in May were also identified as heat tolerant and sensitive in August. A strong relationship between May and August responses was observed ($p = 0.001$; Pearson's $r = 0.72$ for Fv/Fm and 0.91 for ELI), indicating consistency in genotypic responses, suggesting that heat tolerance rankings derived from spring data are broadly representative of summer performance and are not strongly influenced by ontogenetic variation (Figures 2 and 3). Consequently, the heat tolerance ranking used in this study was based on the mean values of Fv/Fm and ELI from May and August data. Descriptive observations from non-replicated genotypes followed broadly similar seasonal patterns, with most exhibiting reduced damage in August relative to May.

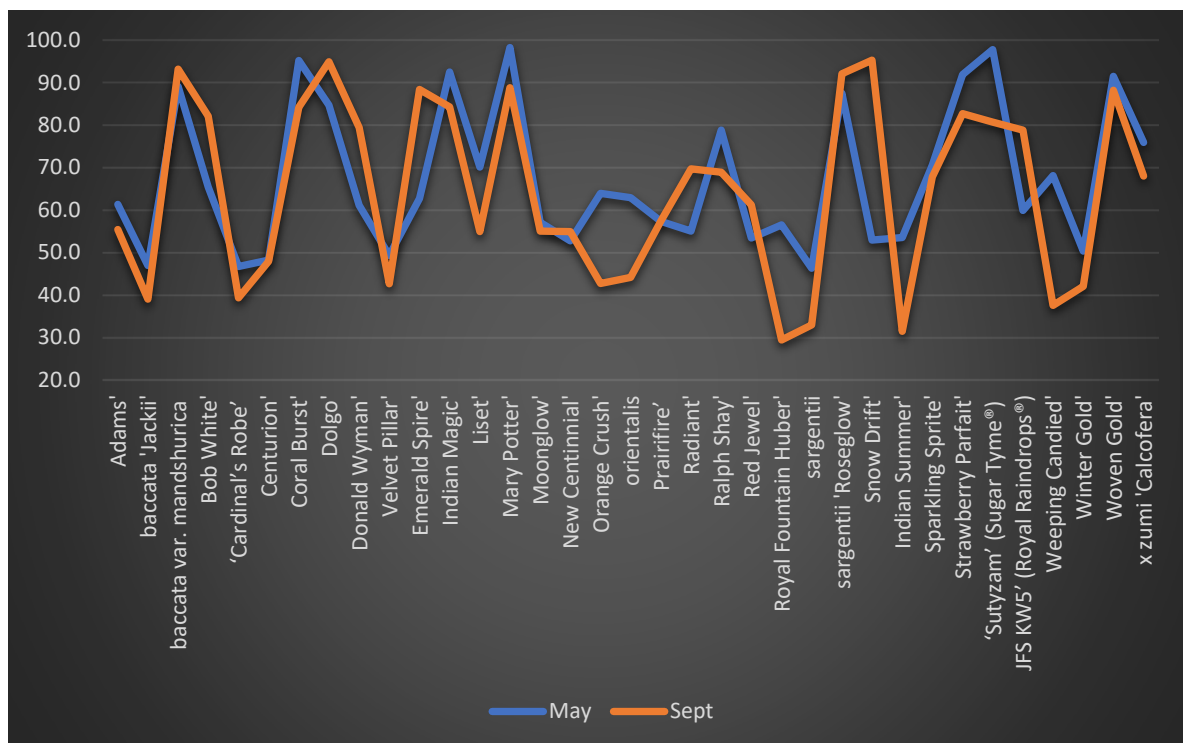


Figure 2. Relationship between chlorophyll fluorescence (Fv/Fm, %) following heat stress (45 °C for 1 h) in May and August across 35 *Malus* genotypes. Each point represents the mean Fv/Fm value for an individual genotype measured after heat stress exposure. A significant positive linear relationship was observed between seasonal measurements (adjusted $R^2 = 0.46$; Pearson's $r = 0.72$; $p = 0.001$), indicating consistency in relative foliar heat tolerance among genotypes across seasons.

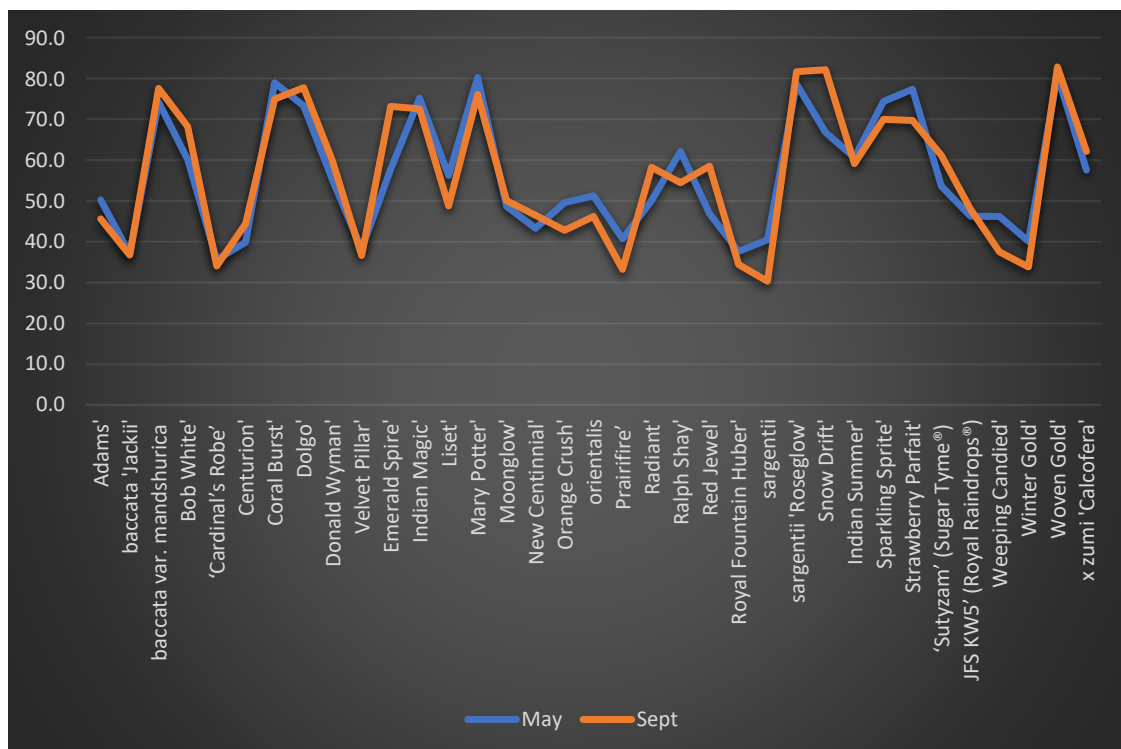


Figure 3. Relationship between ELI (%) following heat stress (45 °C for 1 h) in May and August across 35 *Malus* genotypes. Each point represents the mean ELI value for an individual genotype measured after heat stress exposure. A significant positive linear relationship was observed between seasonal measurements (adjusted $R^2 = 0.83$; Pearson's $r = 0.91$; $p = 0.001$), indicating consistency in relative foliar heat tolerance among genotypes across seasons.

3.4. Chlorophyll Fluorescence (F_v/F_m) and Electrolyte Leakage (ELI) as Physiological Measures of Heat Tolerance

A strong correlation between F_v/F_m ratios and ELI as physiological indicators to quantify genotypic-specific heat tolerance was recorded in May ($p = 0.001$; correlation = 0.92) and August ($p = 0.0001$; correlation = 0.94), indicating that a positive relationship exists between declines in leaf photosynthetic efficiency, which coincides with increased cellular damage (Figures 4 and 5). However, while chlorophyll fluorescence and electrolyte leakage function as complementary physiological indicators for quantifying genotypic heat tolerance, a high correlation does not necessarily prove they measure independent aspects but may reflect the same underlying heat-induced damage.

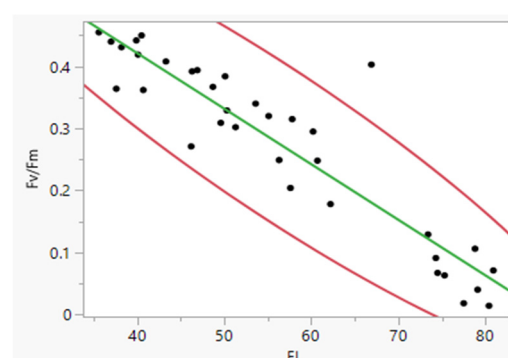


Figure 4. ELI, measured as % ELI increase, and the corresponding F_v/F_m ratio (maximum efficiency of photosystem II) decrease following heat stress (45 °C for 1 h) in May across 35 *Malus* genotypes. $R^2 = 0.84$, Pearson's $r = 0.92$, $p \leq 0.001$.

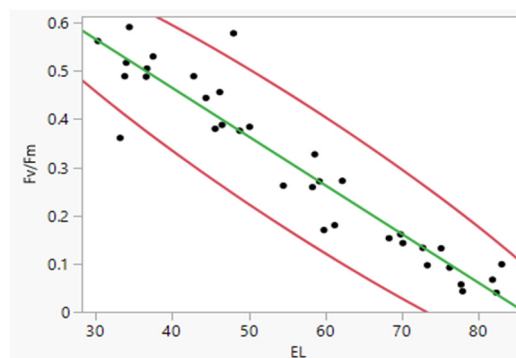


Figure 5. ELI, measured as % cellular electrolyte leakage increase, and the corresponding Fv/Fm ratio (maximum efficiency of photosystem II) decrease following heat stress (45 °C for 1 h) in August across 35 *Malus* genotypes. $R^2 = 0.89$, Pearson's $r = 0.94$, $p \leq 0.0001$.

4. Discussion

4.1. Analytical Methodology to Quantify Heat Tolerance

It is now widely recognized that rising global temperatures will exacerbate thermal stress within urban landscapes, potentially changing the suitability of commonly planted tree species [21,22]. Consequently, heat-tolerant species are desirable to increase the biodiversity [23] and resilience of urban forests [24]. To aid in the selection of suitable tree genotypes, quantitative tools to identify and rank the tolerance to future hotter urban climates are required [25]. Leaves have thermal limits that, if passed, lead to damage and loss of photosynthetic function [26,27]. This thermal limit has been identified to range between 42 and 45 °C for a wide range of tree species [6,28,29]. Within leaves, photosystem II (PSII), in the thylakoid membrane of chloroplasts, has been shown to be a highly sensitive physiological process to heat stress [30]. Consequently, the temperature at which the Fv/Fm ratio declines by 50% (LT₅₀), as a measure of photosynthetic efficiency, has been used extensively to quantify the thermal tolerance of woody plants [26,28,31–33], because this is the value at which irreversible damage to PSII occurs [27]. In this study, a preliminary experiment using a subset of six *Malus* genotypes exposed to a temperature gradient (40–50 °C) and logistic modelling of data enabled estimation of LT₅₀ values, which ranged from 43.3 °C in heat-sensitive genotypes (*M. 'Ralph Shay'*) to 48.1 °C in more tolerant genotypes (*M. 'Prairifire'*), confirming genotypic variation in thermal tolerance. The temperature response curves that demonstrated the greatest separation among genotypes occurred between 44 and 46 °C, with Fv/Fm values falling to near-zero Fv/Fm at temperatures ≥ 48 °C. Consequently, 45 °C for 1 h was selected as a temperature for evaluating a larger population of genotypes. This approach provided a pragmatic compromise between physiological rigour and experimental scalability. Importantly, genotypes identified as heat tolerant based on reduced Fv/Fm decline at 45 °C (*M. sargentii*, *M. 'Prairifire'*) were also those exhibiting higher LT₅₀ values, while heat-sensitive genotypes showed both steeper declines in Fv/Fm and lower LT₅₀ thresholds.

Electrolyte leakage (ELI), in contrast, quantifies cellular membrane stability by measuring ion efflux from leaf tissues following thermal injury (high leakage = reduced cellular membrane integrity). Likewise, ELI has also been used extensively as a measure to quantify heat tolerance in plants [34]. For example, ELI, along with leaf relative water content, was used to develop a comprehensive screening platform for identifying woody plant species tolerant to sequential drought and heat stress among 27 native species growing in Korea. ELI clearly distinguished differences in cellular damage among species under sequential drought and heat stress conditions [35]. Further work used ELI combined with cellular staining to establish a selection system for heat-tolerant herbaceous plant species

among 27 different plants [36]. Other studies using ELI as a measure of heat tolerance in woody plants include [34,37,38]. Previous studies have demonstrated a strong negative correlation between Fv/Fm ratios and ELI (ELI rises, Fv/Fm falls) caused by heat and other abiotic stressors in leaf tissue of woody plants, indicating that declines in photosynthetic efficiency due to oxidative stress or structural damage in chloroplasts coincide with increased cellular damage due to loss of ions from cells [39]. In support of previous research, a significant inverse relationship was recorded between ELI and Fv/Fm ratios following damage to *Malus* foliage in May (spring; Pearson's $r = 0.92$, $p = 0.001$) and August (summer; Pearson's $r = 0.94$, $p = 0.0001$) in this investigation. Such a strong relationship between these parameters further validates the opinion that these two complementary physiological indicators provide rapid screening tools to evaluate heat tolerance and identify genotypes with superior resilience for urban landscape applications.

4.2. Identification of Heat-Tolerant Genotypes and Implications for Tree Selection

Results of this study also support that of others in that Fv/Fm ratios proved to be a sensitive measurement of foliar heat tolerance, distinguishing significant differences in heat tolerance between the 35 *Malus* genotypes evaluated. *M. sargentii*, *M.* 'Prairifire', *M. baccata* 'Jackii', *M.* 'Royal Fountain Huber' and *M.* 'Donald Wyman', for example, were identified as the top five heat-tolerant *Malus* genotypes, with damage to the leaf photosynthetic system ranging from 45.3 to 62.3% in May and 32.9–42.6% in August. Alternately, *M.* 'Indian Magic', *M.* Sutyzam' (Sugar Tyme®), *M.* 'Coral Burst', *M. sargentii* 'Roseglow', and *M. baccata* var *mandshurica* were identified as genotypes that were extremely sensitive to heat stress, with damage to the leaf photosynthetic system ranging from 87.5 to 97.8% in May and 80.7–93.2% in August.

Evaluation of heat tolerance using ELI also proved to be a highly sensitive measurement of foliar heat tolerance, proving a complimentary analytical system to Fv/Fm to quantify heat-induced cellular membrane damage. Indeed, four of the top five heat-tolerant *Malus* genotypes identified using Fv/Fm in May and August were the same ones identified using ELI, with *M.* 'Winter Gold' replacing *M.* 'Donald Wyman'. ELI also provided a similar tolerance ranking of the five most heat-sensitive *Malus* genotypes, identifying four of the same five genotypes as Fv/Fm. The only difference is that using ELI *M.* 'Indian Summer' replaced *M.* Sutyzam' (Sugar Tyme®), as a more heat-sensitive genotype. In practical terms, the results of this study indicate that *M. sargentii*, *M.* 'Prairifire', *M. baccata* 'Jackii', *M.* 'Royal Fountain Huber' and *M.* 'Donald Wyman' would be better suited for planting in urban areas subject to heat stress episodes compared to genotypes such as *M.* 'Indian Magic', *M.* Sutyzam' (Sugar Tyme®), *M.* 'Coral Burst', *M. sargentii* 'Roseglow', and *M. baccata* var *mandshurica*. Planting with more appropriate genotypes offers an effective means of reducing tree mortality. Consequently, genotypes planted will remain healthier and live longer, reducing labour and replacement costs.

4.3. Spring vs. Summer Heat Tolerance

In the majority of *Malus* genotypes evaluated, damage to the leaf photosynthetic system, when assessed by Fv/Fm ratios or ELI, was greater in May compared to August. Exceptions to this rule included *M.* 'New Centennial', *M.* 'Red Jewel', *M.* 'Snow Drift', *M.* 'Emerald Spire', *M.* 'Dolgo', *M. baccata* var *mandshurica*, *M.* 'Radiant', *M.* 'Bob White', *M.* 'JFS-KW5' (Royal Raindrops®), and *M.* 'Donald Wyman', where heat damage to the leaf photosynthetic system was marginally greater in summer compared to spring. Interestingly, in the genotypes where a reversal was recorded between summer and spring foliage, most species were identified as heat intermediate to sensitive (*M.* 'Emerald Spire', *M.* 'Dolgo',

M. baccata var *mandshurica*, *M.* ‘Radiant’, *M.* ‘Bob White’, *M.* ‘JFS-KW5’ (Royal Raindrops®) rather than tolerant.

Multiple studies have demonstrated that foliar heat tolerance (e.g., critical temperature where PSII efficiency declines) tends to be higher during summer than in winter or early in the growing season. Pertinent examples include Endris and Rehm [40] who conducted a community-level assessment of thermal safety margins in 11 hardwood tree species native to eastern North America using chlorophyll fluorescence to determine the critical heat tolerance of PSII across 2 years in central Tennessee, USA. They concluded that the temperature at which PSII first starts to decline (T_{crit}) varied within the season and between years, being higher in July than June and in 2022 than 2023, indicating seasonal differences to peak summer heat. T_{crit} also varied among species, with *Ulmus rubra* and *Ostrya virginiana* showing consistently lower heat tolerances. Work by Grossman [41] summarized research on seasonal patterns in physiological stress tolerance in primarily woody plants native to temperate climates, including thermotolerance. He concluded that thermotolerance typically increases through spring to summer, mirroring the onset of heatwaves. Work by Sastry and Barua [42] quantified leaf thermotolerance in 41 tree species growing in a seasonally dry tropical region of the Indian subcontinent. Foliar thermal tolerance based on Fv/Fm ratios was significantly higher for most species during the hot dry season compared to the cool wet season, where thermotolerance ranged from 45.5 °C to 50.5 °C among species. Similar to the results of this study, however, a small number of species evaluated exhibited an opposite trend of higher thermotolerance during the cool wet monsoon season. Previous research has also shown that excessive heat episodes in spring that coincide with bud burst and leaf flush rather than in summer when leaves are fully expanded, considerably lower temperatures can cause substantial damage to trees [15,43]. Okubo et al. [44] compared tolerance to high temperatures among nine urban tree species in Japan during the growing season under greenhouse conditions. Their work showed that leaves heated in early summer adapted over time, showing increased LT₅₀ compared to unheated controls, reflecting phenological acclimation to heat. It has been shown that artificially subjecting trees to heat stress before a simulated heat wave generally results in a higher temperature required to disrupt PSII functioning [45–47]. Reasons for a higher summer heat tolerance in foliar tissue include a more well developed leaf cuticle to reduce water loss, higher basal levels of heat-responsive proteins, antioxidants, and protective enzymes, combined with a superior ability to modulate transpiration for evaporative cooling through responsive signalling for stomatal closure to prevent desiccation during midday heat spikes [34,41,44,48,49].

However, other studies highlight exceptions where this seasonal trend is absent, reversed, or highly species-specific. Work by Kullberg and Feely [50] measured leaf traits and thermal tolerances of seven focal tree species across steep thermal gradients in Miami’s urban heat island. While they found that some functional traits varied significantly across air temperatures within species, only two exhibited any adjustment in leaf thermal tolerance across warmer conditions, with most species showing little evidence of enhanced thermotolerance in summer versus spring. In another study by Húdoková et al. [33], the impact of supra-optimal temperatures on PSII in five tree species (*Abies alba*, *Fagus sylvatica*, *Picea abies*, *Pinus sylvestris* and *Quercus petraea* agg.) was quantified. With respect to *F. sylvatica*, they found that PSII heat tolerance decreased from early to peak season, though its overall tolerance remained relatively high (comparable to *Quercus petraea*) with the authors concluding that this response represents a rare seasonal decline in heat tolerance. Despite some discrepancies in heat tolerance between spring and summer foliage recorded in this study, a strong correlation between spring and summer foliage tolerance ranking based on Fv/Fm (correlation = 0.72, $p = 0.001$) and ELI correlation = 0.91, $p = 0.001$) was

recorded. This indicates that selection of heat-tolerant *Malus* genotypes used in this study in spring strongly reflects that of heat tolerance in summer and is not strongly influenced by ontogenetic variation. This finding supports the consensus of research in that while seasonal increases in heat tolerance are common, they are not universal between genotypes. As heatwaves are expected to become more frequent and severe due to global warming, this gives credence to the fact that tree species should be screened individually to assess their ability to tolerate or acclimate to high temperature.

4.4. Value and Limitations of Using Arboreta to Identify Heat-Tolerant Genotypes

This study was conducted within a botanical tree collection near Charlotte, North Carolina, comprising more than 2000 tree species, many of which are seldom incorporated into public plantings. Consequently, empirical data on the heat tolerance of these under-utilized taxa are limited or absent. Although the study included a few commonly planted *Malus* genotypes used in urban landscapes (*M.* ‘Donald Wyman’, *M.* $\times$ *zumii* ‘Calocarpa’, and *M. baccata* varieties), the primary focus was on lesser-known *Malus* species to (i) contrast conventional and unconventional genotypes and (ii) develop a broader comparative framework for identifying promising candidates for urban landscape plantings. The use of rare or unique plant material provided important research opportunities but also introduced methodological constraints. Botanical collections emphasize taxonomic diversity, which inherently restricts the number of individuals available per genotype due to spatial limitations within the arboretum, thereby limiting replication across the evaluated taxa. A further challenge was to implement research methods that preserved the integrity of this distinctive collection while still producing robust physiological data. This necessitated a minimally invasive approach. Chlorophyll fluorescence and ELI are well-established, low-impact analytical methods because measurements rely primarily on leaf tissue. Fluorescence can be measured directly on intact leaves, whereas ELI typically requires leaf detachment followed by laboratory-based heat assays, procedures that impose minimal whole-tree damage aside from leaf excision. As noted by Sjöman et al. [51], an alternative approach would involve propagating target species from seed or vegetative material for controlled greenhouse experimentation, enabling more detailed assessments of stress tolerance. However, this strategy is time-intensive, requiring several years to cultivate genotypes to a suitable size for evaluation. Thus, the methodology conducted in this study was essential for identifying genotypes with sufficient promise to merit continued investigation. Without such an initial assessment, there is a risk of investing resources into species with limited potential while overlooking candidates better suited for future urban deployment.

Although previous research has demonstrated intraspecific variation in heat tolerance within many tree species, it is important to acknowledge that the results of this study may have differed had alternative genetic material from the same species been evaluated. Numerous species occupy broad ecotypic ranges, representing a largely untapped reservoir of genetic diversity that could provide valuable sources of heat tolerance for future urban plantings. However, most botanical collections maintain only a limited number of individuals per species, which constrains the ability to assess ecotype-level differences and restricts the scope of genotype-by-environment investigations. This limited representation of individual ecotypes poses a significant long-term challenge for botanical collections seeking to contribute to research on climate-resilient urban trees. A notable strength of the present study, however, is that most of the material evaluated consisted of cultivars (31 of 35 genotypes). Because cultivars are clonally propagated and genetically uniform, minimal variation among individuals in their response to heat stress would be expected. This genetic consistency enhances the precision of identifying heat-tolerant genotypes for specific urban applications under future climate scenarios.

In contrast, the two species (*M. sargentii* and *M. orientalis*) and the *M. baccata* var *mandshurica* can be propagated from seed, resulting in progeny with broader genetic variability that may influence their relative heat tolerance rankings. Such intraspecific variation may help explain why *M. baccata* ‘Jackii’ exhibited strong heat tolerance in this study, whereas *M. baccata* var *mandshurica* was identified as heat sensitive.

5. Conclusions

This study employed two physiological metrics, namely chlorophyll fluorescence (Fv/Fm ratios) and electrolyte leakage (ELI), to evaluate genotype-specific heat tolerance in 35 *Malus* genotypes. The Fv/Fm ratio, a widely used indicator of maximum quantum efficiency in PSII, provided a sensitive, non-destructive measure of photochemical impairment under thermal stress. ELI, by contrast, quantifies loss of membrane integrity through ion efflux following heat injury, thereby capturing a distinct but related dimension of cellular stability. A strong correlation between reductions in Fv/Fm ratios and increases in ELI following heat exposure was observed, consistent with prior research findings that declines in PSII efficiency often reflect the onset of membrane disruption [39,52]. Together, these results confirm that Fv/Fm and ELI serve as effective, rapid screening tools for distinguishing heat-tolerant genotypes.

The substantial variation in heat tolerance detected across the collection demonstrates useful potential for selecting *Malus* genotypes with superior foliar heat tolerance and highlights opportunities for identifying resilient candidates among other under-utilized urban tree taxa. Consequently, ongoing efforts are expanding this work to a broader range of species and genera to support climate-adaptive tree selection frameworks. Because most *Malus* genotypes evaluated in this study are clonally propagated and therefore genetically uniform, minimal within-genotype variation in heat response would be expected.

Collectively, these results contribute valuable evidence toward identifying suitable genotypes and resilient genetic material for future urban environments to aid in the development of climate-resilient planting schemes.

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