



Thermal Regulation and Physiological Responses of Flowering Plants Under Heat Stress: Effects of Flower Colour on Heat Tolerance

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ABSTRACT

Flower colour is a poorly understood variable that can influence heat stress effects on floral function and reproductive performance, which poses an increasing threat. The colour-related differences in floral temperature, physiological responses and heat tolerance were assessed in this study by comparing dark-purple, light-purple and white flower morphs grown under different thermal environments. Thermal regulation was evaluated by measuring flower, petal and air temperature relationships and physiological responses were assessed by measuring transpiration, assimilation and boundary-layer conductance. Electrolyte leakage was used as a measure of heat tolerance across temperatures of 25 to 60°C, and nonlinear modelling was used to estimate the heat tolerance values (T50). The transpiration rate was highest for dark-purple flowers, the petal temperature was highest for purple flowers, and the white flowers showed almost no transpiration, indicating that the evaporative cooling was weakest. A reduction in assimilation occurred with increasing temperature, and this is thought to be due to metabolic impairment under heat stress. The progressive membrane damage was verified by the electrolyte leakage, which increased greatly with temperature. Cellular injury was most significantly related to temperature, with colour having a lesser effect. The estimated T50 values varied between the morphs, with the light-purple flower having the highest T50 value. In general, the colour of the flower influenced the thermal loading and physiological cooling, whereas cellular heat tolerance was largely dependent on absolute thermal exposure. The results shed light on the interactions of pigmentation with physiological regulation in determining floral responses to warming.

Keywords: Flower colour; Heat stress; Thermal regulation; Transpiration; Heat tolerance

1. Introduction

Global warming and more frequent heat waves are exacerbating thermal stress on plant systems and reproductive tissues are more vulnerable than vegetative tissues. Heat stress affects cellular homeostasis, alters metabolic activity, increases water loss and can impair reproductive success, even while vegetative organs are intact. Plants adapt in several physiological ways such as transpiration, membrane stabilization, photosynthetic regulation, antioxidant activity, and tissue temperature. But these responses are species, organ, and environment-dependent, and reproductive thermotolerance is an important factor in plant persistence when climate is warming up (Jagadish et al., 2021). It is particularly important in reproductive tissues since the development of flowers, the performance of pollen, fertilization and seed initiation are sensitive to narrow thermal windows (Chu et al., 2026).

The balance of absorbed radiation, convective heat exchange, latent heat loss and interactions with the atmosphere around the flower determines flower temperature. The temperatures in floral tissues can then vary significantly from ambient air. The humidity produced around flowers could change their microclimate and affect heat and water exchange (Harrap et al., 2020). An increase in vapor pressure deficit may increase the transpirational demand, leading to changes in plant water relations and reduce tissues' ability to cool by evaporation (Grossiord et al., 2020). Stress physiology suggests that the regulation of water status, maintenance of membrane integrity and metabolic activity are the key components of tolerance to environmental stress (Gupta et al., 2020). However, when heat happens in conjunction with drought or drying of the atmosphere, damage can be exacerbated as cooling is limited and tissue temperatures are still rising (Cohen et al., 2021).

The colour of the flower might be significant in this thermal balance as a result of the pigmentation affecting the absorption and reflection of incident radiation. Flowers that are darkly pigmented may have pigment combinations that affect optical properties, and thus the heat gain. Black and very dark floral phenotypes are extreme cases where pigment deposition could be linked to adaptive responses to specific environments (Ahmad et al., 2022). The floral colour is an evolutionarily dynamic character that is determined by pollinator selection, environment and other correlated floral characters (Koski, 2020). Functional trade-offs between attraction, protection and fitness (Assis, 2023) may emerge to floral traits as a result of a poly-functional response to ecologic stresses, such as pollinator interactions and abiotic conditions.

Colour variation may also be influenced by temperature during evolution and/or maintenance. There is evidence that floral colour affects thermal characteristics, and that the thermal effect on floral development and ability to set seed could favour different colour forms depending on local climate (Lacey, 2026). Furthermore, heat exposure can alter the pigment composition and colour expression, showing that the colour-temperature relationship is bi-directional (Narbona et al., 2026). This is especially relevant as floral development is very sensitive to the effects of concurrent heat and water stress, which can result in changes to floral morphology, fertility and reproductive timing (Kumari et al., 2024). Heat stress can affect multiple phases of plant reproduction, including floral organ development, gamete functionality and fertilization, and decrease plant reproductive fitness even prior to the appearance of visual damages to tissues (Resentini et al., 2023; Ahrens et al., 2021).

Fewer studies have combined aspects of flower colour with temperature regulation of the flower, gas-exchange patterns and direct measurement of cell-level heat injury in an analytical framework. The establishment of such relationships is significant because dark colours can absorb more radiant energy but can also be physiologically less cool or less tolerant of membranes. Similarly, lighter flowers can have a lower radiative heat gain but have contrasting other physiological properties affecting heat tolerance. Higher thermal tolerance and increased safety limits of plant tissues are induced by repeated exposure to heat; heat resistance is not a static property but rather a dynamic one. Knowledge of the interaction between colour-associated thermal loading and physiological regulation can therefore aid in the understanding of differences in the reactions of floral morphs to the increase in temperature.

Objectives:

- To compare the temperature patterns of the flowers and petals of dark purple, light purple and white flower under different thermal conditions.
- To find the effect of flower colour and temperature on transpiration, assimilation and relevant physiological reactions of flower cooling.
- To determine the amount of difference in heat-induced membrane damage between groups, based on colour of flowers, and to estimate the relative threshold for thermal tolerance among groups.

2. Materials and Methods

2.1 Study Design and Data Source

The quantitative secondary data research design was used in this study to assess the influence of flower colour on thermal regulation, physiological response and heat tolerance under various temperatures. Data are from the open-access Mendeley Data repository "Thermal regulation of flowers: Colour-driven differences in radiation absorption, cooling, and heat tolerance" version 2, DOI: 10.17632/4gm5hhhvt.2. The original data consisted of three sets of experimental files: one for heat tolerance, one for gas exchange physiology, and one for measurements of temperature of the flowers in the sun. In the present study, the variables directly related to research objectives were extracted and then aggregated in a structured master dataset, while keeping the experimental-component identifiers (Li et al., 2026b).

2.2 Plant Material and Dataset Structure

The data consisted of three morphs of the flower colour of *Vinca minor*, namely dark purple, light purple and white. A final analytical dataset was made up of 237 observations, 144 from the heat-tolerance experiment, 75 from the gas-exchange experiment, and 18 from the solar floral-temperature experiment. There were 79 observations provided by each flower colour group. The heat-tolerance experiment was conducted at temperatures ranging from 25 to 60°C and the physiological gas-exchange experiment at 16, 20, 25, 30, and 35°C.

2.3 Study Variables

Colour and temperature treatment of the flowers were taken as the major variables that would explain the results. Thermal regulation variables were air temperature, flower temperature, flower-centre temperature, petal temperature, flower minus air temperature and petal minus flower-centre temperature. Physiological variables measured were transpiration, assimilation, boundary-layer conductance, relative humidity, and incident radiation. The electrolyte-leakage response was used to evaluate heat tolerance as an indication of temperature-induced cellular membrane injury. The results of the electrolyte-leakage response were viewed as an indicator of greater damage to the membranes due to heat exposure.

2.4 Data Preparation and Thermal Indices

All three experimental components were standardized and placed in a single master dataset but retained as experimental components. The categories of flower colour and temperature were matched and replicates were left intact. Thermal regulation was further characterised by the difference in temperature between the flower and the air ($T_{\text{flower}} - T_{\text{air}}$) and between the petal and centre of the flower ($T_{\text{petal}} - T_{\text{centre}}$). Variables which were not measured in a specific experiment are represented in the table by blank cells and were not imputed.

2.5 Assessment of Thermal Regulation

Floral temperature measurement was used for thermal regulation in the experiments with flowers exposed to the sun and with gas exchange. The mean petal temperature, the flower-centre temperature, the flower temperature, and the temperature differentials were compared for the dark-purple flowers, the light-purple flowers and the white flowers. The temperature difference was compared between the different colour groups of flowers to see if there was any differential heat absorption or heat loss related to colour. In the gas-exchange part, the effect of flower colour and temperature treatment on the temperature of the flowers was also investigated.

2.6 Assessment of Physiological Responses

The outcome of the gas-exchange experiment was used to assess the physiological responses to rising temperature in terms of transpiration, assimilation, and boundary-layer conductance. Comparisons of changes in these variables were made between flower colour groups and temperature treatments. The use of transpiration as an indicator of evaporative cooling capacity was taken into account, as any extra loss of water from the floral tissues may help to cool the plant. Correlations between the physiological parameters of flower temperature, transpiration, assimilation and others were also investigated, to elucidate the relationship between thermal regulation and physiological performance.

2.7 Assessment of Heat Tolerance

Electrolyte-leakage responses from the three flower-colour groups were compared, in order to see how much more stable or unstable a cellular membrane was under increasing thermal stress. For each colour morph, a temperature-response curve was created, and for each morph where appropriate, the temperature corresponding to about 50% membrane injury (T_{50}) was estimated as a measure of the relative thermal tolerance.

2.8 Statistical Analysis

Continuous variables were analyzed using descriptive statistics such as mean, standard deviation, minimum, maximum, and confidence interval. The floral-temperature characteristics were compared among colour

groups using one-way analysis of variance (ANOVA) and Tukey's post-hoc test was performed when significant differences were found. The main and interaction effect of flower colour, temperature, and colour $\times$ temperature on the physiological and heat-tolerance responses were analysed using two-way ANOVA. Correlation analysis was carried out to explore correlations between thermal and physiological measurements within the same experimental component. Assumptions were checked prior to performing parametric testing and appropriate non-parametric alternatives were examined when an assumption was significantly violated. A p value of < 0.05 was considered as statistically significant.

2.9 Ethical Considerations

No primary data were collected in the study and the data were collected from a publicly available plant-based research study. The data used was obtained from Mendeley Data with appropriate acknowledgement of the original authors and source under a Creative Commons Attribution 4.0 International (CC BY 4.0) licence. No artificial observations were generated to replace structurally unavailable observations, and the original experimental structure was retained in order to ensure transparency, reproducibility and integrity of the data.

3. Results

3.1 Dataset Distribution and Experimental Structure

The heat-tolerance experiment was the largest and consisted of 144 observations over eight temperatures, ranging from 25 to 60°C as shown in Table 1. In the gas-exchange experiment 75 observations were made in 5 temperature treatments, while 18 observations were made in the solar-temperature experiment. The full data set incorporated 79 observations from each flower-colour group, which was adequate to represent dark-purple, light-purple and white floral morphs.

Table 1. Experimental structure of the analytical dataset

Experimental component	N	Temperature levels	N per flower colour	Principal responses
Heat tolerance/electrolyte leakage	144	25, 30, 35, 40, 45, 50, 55, 60°C	48	Electrolyte-leakage response
Gas-exchange physiology	75	16, 20, 25, 30, 35°C	25	Flower temperature, ΔT , transpiration, assimilation, gbw
Solar floral temperature	18	Solar exposure experiment	6	Flower-centre temperature, petal temperature, petal-centre ΔT
Total	237	—	79	Integrated thermal and physiological traits

3.2 Flower Colour and Solar Floral Temperature

There were significant differences between the three colour morphs in their floral thermal characteristics. As shown in Table 2, the mean petal temperature for the dark-purple flowers was $23.084 \pm 0.403^\circ\text{C}$, light-purple flowers $22.658 \pm 0.171^\circ\text{C}$ and white flowers $17.125 \pm 0.195^\circ\text{C}$.

The differences in the temperature of the petals as a result of flower colour were highly significant ($F(2,15) = 866.703$, $p < 0.001$). The dark- and light-purple flowers were significantly different ($p = 0.044$) and both morphs of purple flowers were significantly different than the white flowers ($p < 0.001$) from Tukey comparisons.

There was also significant difference between flower-centre temperatures within flower colour groups, $F(2,15) = 6.913$, $p = 0.007$. There was no significant difference between the dark-purple and light-purple flowers, but the centres of both were significantly warmer than the centres of white flowers.

The petal-to-centre thermal gradient had a particularly strong colour pattern. Positive gradients were seen in purple flowers, meaning that the petals were warmer than the floral centres, but not in white flowers, which had a negative gradient of $-2.835 \pm 0.643^\circ\text{C}$. This colour effect was significant, $F(2,15) = 145.965$, $p < 0.001$.

Table 2. Floral thermal characteristics according to flower colour

Flower	Flower-centre	Petal	Petal – centre
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colour	temperature, °C	temperature, °C	temperature, °C
Dark purple	21.107 ± 0.646	23.084 ± 0.403	1.978 ± 0.587
Light purple	20.953 ± 0.385	22.658 ± 0.171	1.705 ± 0.378
White	19.960 ± 0.666	17.125 ± 0.195	-2.835 ± 0.643
ANOVA p-value	0.007	<0.001	<0.001

Values are mean ± standard deviation.

Figure 1 shows that there is a significant difference between the white and purple flowers, especially in the petal temperature. For petals, however, the difference was much more pronounced than for flower centres, suggesting that there was marked variation in heating of flower surfaces associated with colour.

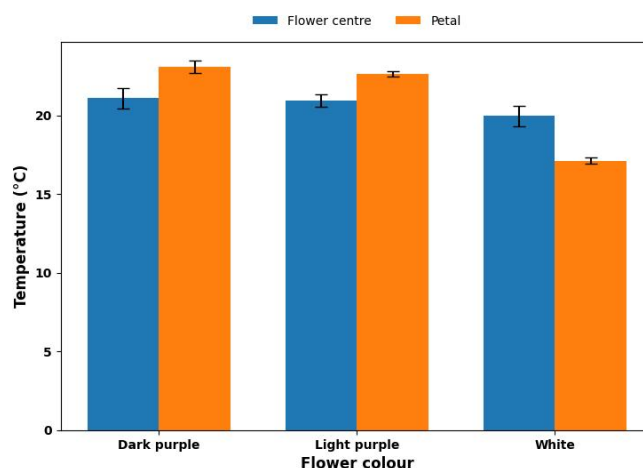


Figure 1. Mean flower-centre and petal temperatures of dark-purple, light-purple, and white flowers

3.3 Temperature-Dependent Floral Thermal Regulation

The gas-exchange experiment showed that floral temperature increased gradually with increasing treatment temperature from 16°C to 35°C and the principal thermal and physiological responses for each temperature treatment are shown in Table 3.

There was a definite colour dependent pattern in the difference between the flower temperature and air temperature. Dark-purple flowers changed from -0.028°C at 16°C to 0.334°C at 35°C, while light-purple flowers increased from 0.009°C to 0.342°C. White flowers, however, were cooler than ambient air throughout the temperature range with a maximum deviation of only 0.049°C at 35°C.

Table 3. Thermal and physiological responses across flower colour and temperature treatments

Temperature	Flower colour	Flower temperature, °C	Flower – air ΔT , °C	Transpiration, E	Assimilation, A
16	Dark purple	16.843 ± 0.577	-0.028 ± 0.156	0.340 ± 0.127	-0.353 ± 0.333
16	Light purple	16.265 ± 0.276	0.009 ± 0.130	0.116 ± 0.101	-0.199 ± 0.145
16	White	15.870 ± 0.263	-0.297 ± 0.105	0.122 ± 0.051	-0.633 ± 0.605
20	Dark purple	20.036 ± 0.134	0.038 ± 0.135	0.315 ± 0.083	-0.565 ± 0.442
20	Light purple	20.088 ± 0.090	0.089 ± 0.089	0.161 ± 0.075	-0.428 ± 0.177
20	White	19.795 ± 0.125	-0.204 ± 0.123	0.161 ± 0.086	-0.855 ± 0.784
25	Dark purple	25.148 ± 0.134	0.151 ± 0.136	0.539 ± 0.148	-0.998 ± 0.692
25	Light	25.185 ± 0.078	0.185 ± 0.185	0.099 ± 0.058	-0.631 ± 0.631

	purple		0.079		0.256
25	White	24.889 ± 0.124	-0.109 ± 0.123	0.147 ± 0.076	-1.974 ± 1.691
30	Dark purple	30.219 ± 0.193	0.219 ± 0.195	0.714 ± 0.507	-1.800 ± 1.024
30	Light purple	30.262 ± 0.090	0.263 ± 0.092	0.225 ± 0.145	-0.921 ± 0.468
30	White	29.983 ± 0.131	-0.018 ± 0.129	0.183 ± 0.044	-3.705 ± 2.521
35	Dark purple	35.334 ± 0.184	0.334 ± 0.186	0.777 ± 0.486	-4.608 ± 3.339
35	Light purple	35.343 ± 0.136	0.342 ± 0.135	0.338 ± 0.259	-1.787 ± 1.520
35	White	35.054 ± 0.135	0.049 ± 0.138	0.487 ± 0.196	-6.057 ± 3.812

Values are mean $\pm$ standard deviation.

The factorial analysis in Table 4 did confirm that there were significant effects due to flower colour and temperature on both absolute flower temperature and flower-to-air temperature differential. Flower temperature showed significant effects of colour, $F(2,60) = 23.661$, $p < 0.001$, temperature, $F(4,60) = 18,789.346$, $p < 0.001$, and their interaction, $F(8,60) = 3.279$, $p = 0.004$.

Flower colour $\times$ flower-to-air temperature difference was significant ($F(2,60) = 35.925$, $p < 0.001$), the same was true for temperature $\times$ flower-to-air temperature difference ($F(4,60) = 16.090$, $p < 0.001$), but flower colour $\times$ temperature was not significant.

White flowers had a consistently lower flower-to-air thermal differential than the purple morphs throughout the experimental temperature range, as shown in Figure 2.

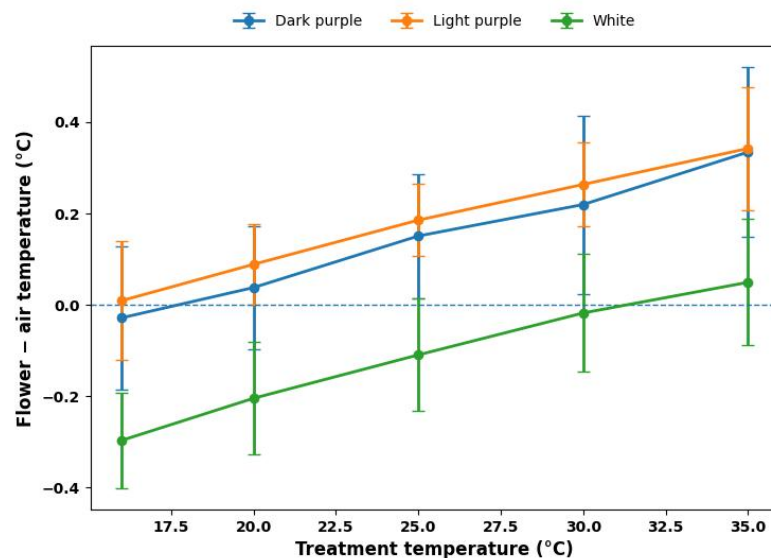


Figure 2. Changes in flower-to-air temperature difference across treatment temperatures for dark-purple, light-purple, and white flowers

3.4 Physiological Responses to Increasing Temperature

Transpiration was different for colour morphs and the different temperature treatments. When the complete gas-exchange experiment was repeated, the transpiration response of dark-purple flowers was the greatest at 0.537 ± 0.356 , while the light-purple flowers had a transpiration response of 0.188 ± 0.160 and white flowers a transpiration response of 0.220 ± 0.168 .

The results of the factorial analysis showed significant differences among mean transpiration by colour of flower ($F(2,60) = 19.892$, $p < 0.001$) and temperature ($F(4,60) = 6.395$, $p < 0.001$). The interaction between colour and temperature was not significant ($p = 0.533$). Dark-purple flowers had the higher transpiration rates, and this increased to 0.777 at 35°C , while white flowers had a higher mean transpiration rate than the light-purple flowers and this rose to 0.487 at 35°C . Figure 3 shows Mean transpiration response across

increasing treatment temperature in the three flower-colour morphs. Error bars indicate standard deviations.

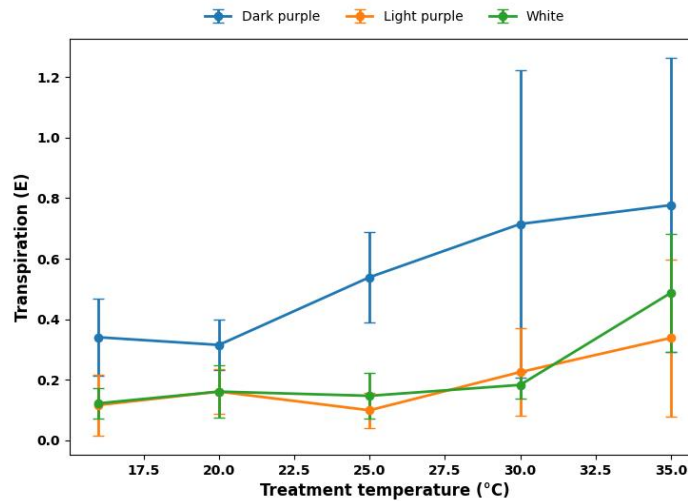


Figure 3. Mean transpiration response across increasing treatment temperature in the three flower-colour morphs

Assimilation response also differed significantly among flower colours, $F(2,60) = 7.973$, $p < 0.001$, and among temperature treatments, $F(4,60) = 13.004$, $p < 0.001$. Colour did not interact significantly with temperature $p = 0.212$. As temperature went up, the assimilation variable was increasingly negative especially for white and dark purple flowers. As shown in Figure 4, the mean values of the flower colour were -6.057 for white, -4.608 for dark-purple, and -1.787 for light-purple flowers at 35°C .

However, the value of boundary-layer conductance was not significantly affected by flower colour, temperature or their interaction, suggesting that this variable was relatively stable among the conditions studied.

Table 4. Two-way ANOVA results for thermal and physiological responses

Response	Effect	F statistic	p-value	Partial η^2
Flower temperature	Flower colour	$F(2,60) = 23.661$	<0.001	0.441
	Temperature	$F(4,60) = 18789.346$	<0.001	0.999
	Colour temperature $\times$	$F(8,60) = 3.279$	0.004	0.304
Flower – air ΔT	Flower colour	$F(2,60) = 35.925$	<0.001	0.545
	Temperature	$F(4,60) = 16.090$	<0.001	0.518
	Colour temperature $\times$	$F(8,60) = 0.034$	1.000	0.004
Transpiration	Flower colour	$F(2,60) = 19.892$	<0.001	0.399
	Temperature	$F(4,60) = 6.395$	<0.001	0.299
	Colour temperature $\times$	$F(8,60) = 0.887$	0.533	0.106
Assimilation Boundary-layer conductance	Flower colour	$F(2,60) = 7.973$	<0.001	0.210
	Temperature	$F(4,60) = 13.004$	<0.001	0.464
	Colour temperature $\times$	$F(8,60) = 1.407$	0.212	0.158
	Flower colour	$F(2,60) = 1.357$	0.265	0.043
	Temperature	$F(4,60) = 1.001$	0.414	0.063
	Colour temperature $\times$	$F(8,60) = 1.027$	0.426	0.120

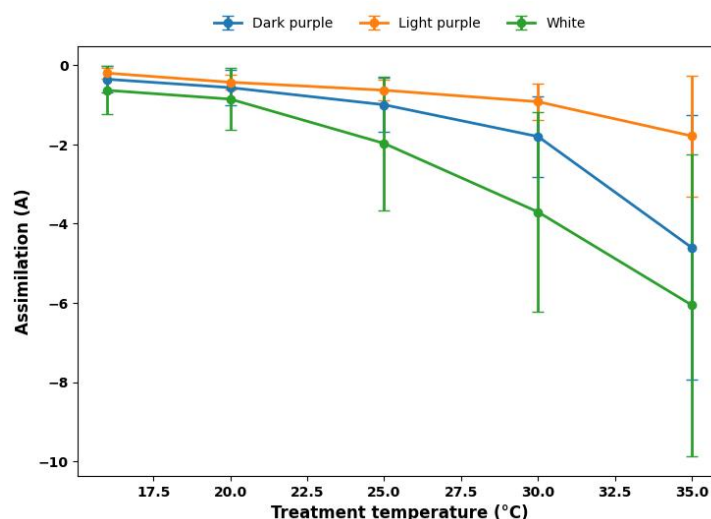


Figure 4. Assimilation response of dark-purple, light-purple, and white flowers across increasing temperature treatments

3.5 Cellular Membrane Response and Heat Tolerance

As treatment temperature rose, electrolyte leakage also rose significantly, which suggests that the membrane was damaged by heat stress. As can be seen in Table 5, the leakage was relatively low between 25°C and 35°C and became significantly higher at temperatures above around 40°C.

At 35°C, white flowers had the highest mean leakage response at 0.507 ± 0.202 , compared with 0.351 ± 0.129 in light-purple and 0.199 ± 0.073 in dark-purple flowers. The leakage of the electrolyte was found to be 0.858 ± 0.165 in dark-purple flowers, 0.941 in light-purple flowers and 0.941 in white flowers at 50°C.

Table 5. Electrolyte-leakage response across heat treatments

Temperature, °C	Dark purple	Light purple	White
25	0.199 ± 0.137	0.231 ± 0.200	0.235 ± 0.182
30	0.151 ± 0.137	0.131 ± 0.060	0.229 ± 0.074
35	0.199 ± 0.073	0.351 ± 0.129	0.507 ± 0.202
40	0.626 ± 0.148	0.381 ± 0.104	0.569 ± 0.223
45	0.745 ± 0.061	0.646 ± 0.293	0.775 ± 0.099
50	0.858 ± 0.165	0.941 ± 0.179	0.941 ± 0.079
55	0.722 ± 0.066	0.735 ± 0.145	0.727 ± 0.087
60	0.807 ± 0.058	0.696 ± 0.240	0.701 ± 0.064

Values are mean $\pm$ standard deviation.

Temperature exerted a strong overall effect on electrolyte leakage, $F(7,120) = 60.787$, $p < 0.001$, partial $\eta^2 = 0.780$. The overall flower-colour effect was near significance ($F(2,120) = 2.880$, $p = 0.060$) as was the colour $\times$ temperature interaction ($F(14,120) = 1.698$, $p = 0.064$). Therefore, the heat treatment itself was the best statistical measure of membrane injury.

The curves of temperature response are presented in Figure 5 and show that injury to the membrane is rapid in the intermediate to high temperature range, with relatively high injury at higher temperatures.

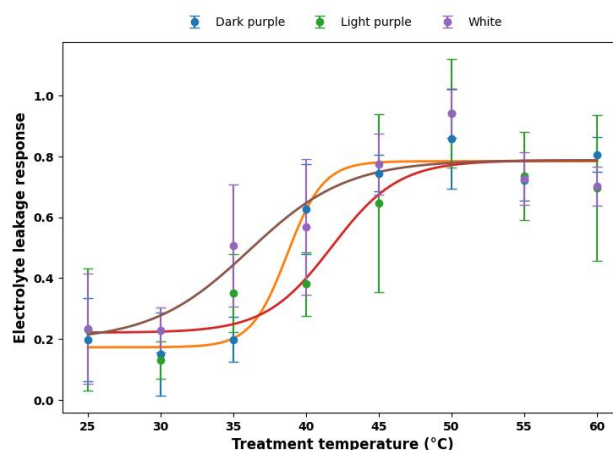


Figure 5. Observed mean electrolyte-leakage responses and fitted sigmoidal temperature-response curves

3.6 Estimated Thermal-Tolerance Thresholds

The electrolyte-leakage data were analysed separately for each colour morph using four-parameter logistic models. The estimated temperature for an electrolyte-leakage response of ~ 0.50 was highest for light-purple flowers at 41.72°C, and similar for the dark-purple flowers (38.88°C), and white flowers (36.28°C) as reported in Table 6.

The R^2 values obtained for model fit were highest for dark-purple flowers ($R^2 = 0.865$), moderate for white and light-purple flowers. These fitted thresholds give a model-based description of relative membrane thermal tolerance but need to be interpreted in conjunction with the factorial analysis, which showed only a sub-marginal (not significant at $p < 0.05$) overall colour effect and colour $\times$ T interaction.

Table 6. Nonlinear temperature-response model and estimated thermal-tolerance threshold

Flower colour	Lower asymptote	Upper asymptote	Estimated T50, °C	Slope	R^2
Dark purple	0.173	0.784	38.88	1.244	0.865
Light purple	0.221	0.787	41.72	2.296	0.636
White	0.193	0.788	36.28	3.370	0.689

In general, the result shows a clear correlation between flower colour and thermal behaviour of flowers. Compared to white flowers, the purple flowers also had significantly more heat accumulation at the flower surface and dark-purple flowers had relatively high transpiration. All colour group cells showed a significant increase in cellular membrane injury in the presence of heat exposure. There was some suggestion of variation among colour morphs in model-derived thermal thresholds, however differences in electrolyte leakage were not as statistically strong as the direct thermal and physiological effects of colour.

4. Discussion

The present study illustrates that flower colour is related to the thermal behaviour, physiological control and heat-response pattern of flowers. Petal temperatures of purple morphs were greater than petal temperatures of white flowers. Therefore, the ability of floral pigmentation to alter the absorption of radiant energy and the thermal environment of reproductive tissues may occur. Heat accumulation and thermal stability in the floral microenvironments are influenced by floral colour and morphology (Apland et al., 2026). Colour-associated radiation absorption and cooling have also been reported directly in flowers, with darker morphs having a higher thermal loading, but a different ability to dissipate heat (Li et al., 2026a).

The positive gradients from petal to centre found in purple flowers versus the negative gradient in white flowers further suggest that colour influences the spatial distribution of heat in the flower. If floral tissues are known to have varying temperature sensitivity, then such thermal heterogeneity could be significant. Humidity, radiation and boundary-layer effects in flowered plants can create distinct microclimates around the plants which in turn can affect their reproductive performance (Dahake et al., 2022). Optical characteristics also matter due to the fact that alterations in tissue reflectance and emissivity can affect tissue temperature in ways that differ from ambient air temperature (Richardson et al., 2021).

In addition, physiological responses showed an interaction between flower colour and thermal regulation. The dark-purple flowers always had higher transpiration rates than the light-purple and white flowers, especially at the warmer treatments. This implies that the evaporative cooling is offsetting part of the radiative heat gain. The cooling due to transpiration has been reported to be effective under extreme heat events for reducing tissue temperatures (Bachofen et al., 2025). Furthermore, flowers may also have distinct hydraulic properties and water loss rates from leaves, suggesting that the cooling properties of flowers are limited by water relations of the flower itself (An et al., 2023). Water loss regulation seems to be a key factor in floral stress avoidance during hot conditions that raise evaporative demand (Li et al., 2025).

As temperatures increased, the assimilation response became increasingly negative, being the most negative for white flowers at the highest temperature. This pattern reflects limitation due to metabolic stress under thermal stress. Photosynthetic processes are sensitive to temperature because the activity of enzymes, respiration, and stomatal regulation change away from the thermal optimum as tissues deviate from optimum temperature (Crous et al., 2022). Similar changes in photosynthetic rates and stomatal conductance with temperature have been reported in crop species (Huang et al., 2021). Acclimation can also lead to a reduction in net carbon uptake, even in cases of partial acclimation, as demonstrated by Deluigi et al. (2025).

The results of the heat tolerance analysis indicated that the leakage of the electrolytes was markedly raised with the rise in temperature, supporting the progressive loss of integrity of the cellular membrane under severe thermal exposure. Membrane injury is consistent with the observation that combined heat and water stress can result in localized hydraulic failure and cellular damage in reproductive tissues (Carins-Murphy et al., 2023). Increased temperatures might also reduce thermal safety margins, resulting in tissues being nearer to damaging thresholds during extreme events (Kullberg et al., 2024). The present analysis has shown that the overall effect of colour and the interaction of colour and temperature were only marginal, while temperature was the most important factor in determining electrolyte leakage. Hence, flower colour should not be the only indicator of membrane damage when assessing the potential damage without taking thermal stress into account.

However, according to the nonlinear modelling, there were some differences in the estimated thermal-tolerance thresholds: light-purple flower had the highest T50, dark-purple flower second, and white flower last. Such differences are also consistent with the idea that colour polymorphism may be supported by differential environmental and/or physiological advantages and disadvantages of the different phenotypes, instead of a single universally advantageous colour (Grossenbacher et al., 2026). Darker flowers can absorb more energy, but compensate by having a higher rate of transpiration, while lighter flowers can have lower rates of heating by radiation but different physiological constraints. Additionally, nonevaporative heat dissipation can occur when evaporative cooling is limited, highlighting that there are multiple heat loss pathways that occur at the same time (Muller et al., 2021).

The tolerance to floral heat is a combination of optical, hydraulic, metabolic and cellular properties, and not just flower colour. The high colour-temperature-dependency of petal transpiration and temperature, and lesser absolute-temperature-dependency of cell injury, imply that melting and cooling of petals is more heavily influenced by colour than is cell injury, and that pigmentation plays a greater role than absolute temperature exposure. This integrated interpretation is in line with the recent finding that the mechanisms and the physiological impacts of flower colour, radiation absorption, transpiration and heat tolerance are not synonymous and are mechanistically coupled aspects of reproductive thermoregulation.

5. Conclusion

The data of this study confirm that flower colour plays a significant role in the thermal management and physiological response of flowers when exposed to heat stress, however, it does not play as significant a role as temperature on cellular heat injury. Overall, dark-purple and light purple flowers tended to have higher petal temperatures than white flowers, consistent with the effects of floral pigmentation on the radiant heat absorption and tissue warming. Water status in plants also was found to increase with dark-purple flowers, suggesting that the plant can counterbalance the higher thermal loads by improved evaporative cooling. The assimilation on the other hand showed a decrease with increasing temperature, reflecting the metabolic sensitivity of the floral tissues to heat exposure. The leakage of electrolytes became significantly higher at higher temperatures, which again indicates a progressive destabilisation and damage of the membrane and the cells under severe thermal stress. Colour-related differences in the extent of electrolyte leakage were relatively small but nonlinear modelling revealed that there were differences among the three colour morphs in estimated thermal-tolerance thresholds, with the light-purple flowers exhibiting the highest T50. In general, the findings suggest that heat tolerance in flowers depends on

combinations of a pigmentation – thermal loading – transpiration – metabolic response – and membrane stability rather than on the colour per se. The results deepen the understanding of the effect of floral traits on reproductive thermoregulation and will be useful for future research on climate resilience, floral adaptation and selection for heat-tolerant plants in a future climate with more extreme heat.

References

1. Ahmad, S., Chen, J., Chen, G., Huang, J., Zhou, Y., Zhao, K., ... & Peng, D. (2022). Why black flowers? An extreme environment and molecular perspective of black color accumulation in the ornamental and food crops. *Frontiers in Plant Science*, 13, 885176.
2. Ahrens, C. W., Challis, A., Byrne, M., Leigh, A., Nicotra, A. B., Tissue, D., & Rymer, P. (2021). Repeated extreme heatwaves result in higher leaf thermal tolerances and greater safety margins. *New Phytologist*, 232(3), 1212-1225.
3. An, Y. D., Roddy, A. B., Zhang, T. H., & Jiang, G. F. (2023). Hydraulic differences between flowers and leaves are driven primarily by pressure-volume traits and water loss. *Frontiers in Plant Science*, 14, 1130724.
4. Aplant, J. S., Riddle, K. E., & Koski, M. H. (2026). Floral shape and color impact heat accumulation and thermal stability of the floral microenvironment in a subalpine meadow. *American Journal of Botany*, 113(1), e70070.
5. Assis, L. C. (2023). Pollination syndromes and the origins of floral traits. *Annals of Botany*, 132(6), 1055-1072.
6. Bachofen, C., Peillon, M., Meili, N., Bourgeois, I., & Grossiord, C. (2025). High transpirational cooling by urban trees despite extreme summer heatwaves. *Urban Forestry & Urban Greening*, 107, 128819.
7. Carins-Murphy, M. R., Cochard, H., Deans, R. M., Gracie, A. J., & Brodribb, T. J. (2023). Combined heat and water stress leads to local xylem failure and tissue damage in pyrethrum flowers. *Plant Physiology*, 193(1), 356-370.
8. Chu, J. W., Chang, S., & Li, H. J. (2026). Reproductive thermotolerance: from flower development to seed initiation. *New Phytologist*.
9. Cohen, I., Zandalinas, S. I., Huck, C., Fritschi, F. B., & Mittler, R. (2021). Meta-analysis of drought and heat stress combination impact on crop yield and yield components. *Physiologia plantarum*, 171(1), 66-76.
10. Crous, K. Y., Uddling, J., & De Kauwe, M. G. (2022). Temperature responses of photosynthesis and respiration in evergreen trees from boreal to tropical latitudes. *New Phytologist*, 234(2), 353-374.
11. Dahake, A., Jain, P., Vogt, C. C., Kandalaft, W., Stroock, A. D., & Raguso, R. A. (2022). A signal-like role for floral humidity in a nocturnal pollination system. *Nature Communications*, 13(1), 7773.
12. Deluigi, J., Bachofen, C., Didion-Gency, M., Gisler, J., Mas, E., Mekarni, L., ... & Grossiord, C. (2025). Prolonged warming and drought reduce canopy-level net carbon uptake in beech and oak saplings despite photosynthetic and respiratory acclimation. *New Phytologist*, 246(5), 2015-2028.
13. Grossenbacher, D. L., Lo, M. S., Waddington, M. E., O'Dell, R., & Kay, K. M. (2026). Soil and climate contribute to maintenance of a flower color polymorphism. *American Journal of Botany*, 113(1), e70018.
14. Grossiord, C., Buckley, T. N., Cernusak, L. A., Novick, K. A., Poulter, B., Siegwolf, R. T., ... & McDowell, N. G. (2020). Plant responses to rising vapor pressure deficit. *New phytologist*, 226(6), 1550-1566.
15. Gupta, A., Rico-Medina, A., & Caño-Delgado, A. I. (2020). The physiology of plant responses to drought. *Science*, 368(6488), 266-269.
16. Harrap, M. J., Hempel de Ibarra, N., Knowles, H. D., Whitney, H. M., & Rands, S. A. (2020). Floral humidity in flowering plants: a preliminary survey. *Frontiers in Plant Science*, 11, 249.
17. Huang, G., Yang, Y., Zhu, L., Peng, S., & Li, Y. (2021). Temperature responses of photosynthesis and stomatal conductance in rice and wheat plants. *Agricultural and Forest Meteorology*, 300, 108322.
18. Jagadish, S. K., Way, D. A., & Sharkey, T. D. (2021). Plant heat stress: Concepts directing future research. *Plant, cell & environment*, 44(7), 1992-2005.
19. Koski, M. H. (2020). Macroevolution of flower color patterning: biased transition rates and correlated evolution with flower size. *Frontiers in Plant Science*, 11, 945.
20. Kullberg, A. T., Coombs, L., Soria Ahuanari, R. D., Fortier, R. P., & Feeley, K. J. (2024). Leaf

- thermal safety margins decline at hotter temperatures in a natural warming ‘experiment’ in the Amazon. *New Phytologist*, 241(4), 1447-1463.
21. Kumari, S., Basu, S., & Kumar, G. (2024). A systematic review on the implications of concurrent heat and drought stress in modulating floral development in plants. *Plant Science*, 349, 112248.
 22. Lacey, E. P. (2026). Temperature and the evolution of flower color: A review. *American Journal of Botany*, 113(1), e70106.
 23. Li, Y., Grossiord, C., Ke, X., & Bachofen, C. (2026). Thermal regulation of flowers: Color-driven differences in radiation absorption, cooling, and heat tolerance (Version 2) [Data set]. Mendeley Data. <https://doi.org/10.17632/4gm5hhhvt.2>
 24. Li, Y., Zhang, Z., & Tang, Y. (2025). Drought avoidance: a shared strategy for perennial herbaceous flowers and leaves through distinct mechanisms. *Physiologia Plantarum*, 177(5), e70602.
 25. Muller, J. D., Rotenberg, E., Tatarinov, F., Oz, I., & Yakir, D. (2021). Evidence for efficient nonevaporative leaf-to-air heat dissipation in a pine forest under drought conditions. *New Phytologist*, 232(6), 2254-2266.
 26. Narbona, E., Perfectti, F., González-Megías, A., Navarro, L., Del Valle, J. C., Armas, C., & Gómez, J. M. (2026). Heat drastically alters floral color and pigment composition without affecting flower conspicuousness. *American Journal of Botany*, 113(1), e70096.
 27. Resentini, F., Orozco-Arroyo, G., Cucinotta, M., & Mendes, M. A. (2023). The impact of heat stress in plant reproduction. *Frontiers in plant science*, 14, 1271644.
 28. Richardson, A. D., Aubrecht, D. M., Basler, D., Hufkens, K., Muir, C. D., & Hanssen, L. (2021). Developmental changes in the reflectance spectra of temperate deciduous tree leaves and implications for thermal emissivity and leaf temperature. *New Phytologist*, 229(2), 791-804. earch, 242, 126626.