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Running title: Leaf traits and temperature regulation

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Ability of plants to provide cooling in the urban environment is increasingly recognised. Plants use various mechanisms to regulate leaf temperature, so we investigated how several leaf traits (hairiness, colour, thickness) and processes (leaf water loss) rank in their contribution to the leaf temperature regulation. We showed that the relative importance of water loss and leaf traits for leaf temperature varied with plant genera. This can lead to different plant types having significantly different potentials for cooling in applications such as green roofs.

Abstract

Urban greening solutions such as green roofs help improve residents' thermal comfort and building insulation. However, not all plants provide the same level of cooling. This is partially due to differences in plant structure and function, including different mechanisms that plants employ to regulate leaf temperature. Ranking of multiple leaf/plant traits involved in the regulation of leaf temperature (and, consequently, plants' cooling 'service') is not well understood. We therefore investigated the relative importance of water loss, leaf colour, thickness and extent of pubescence for the regulation of leaf temperature, in the context of species for semi-extensive green roofs. Leaf temperature were measured with an infrared imaging camera in a range of contrasting genotypes within three plant genera (*Heuchera*, *Salvia* and *Sempervivum*). In three glasshouse experiments (each evaluating three or four genotypes of each genera) we varied water availability to the plants and assessed how leaf temperature altered depending on water loss and specific leaf traits. Greatest reductions in leaf temperature were closely associated with higher water loss. Additionally, in non-succulents (*Heuchera, Salvia*), lighter leaf colour and longer hair length (on pubescent leaves) both contributed to reduced leaf temperature. However, in succulent *Sempervivum*, colour/pubescence made no significant contribution; leaf thickness and water loss rate were the key regulating factors. We propose that this can lead to different plant types having significantly different potentials for cooling. We suggest that maintaining transpirational water loss by sustainable irrigation and selecting urban plants with favourable morphological traits is the key to maximising thermal benefits provided by applications such as green roofs.

Key words: Leaf colour; Leaf hairs; Leaf temperature; Leaf thickness; Water deficit; Water loss

Introduction

Green infrastructure (i.e. street trees, parks and gardens, green roofs and walls) in the urban environments is being increasingly recognised for a number of services it provides, including its role in regulation of air temperatures, particularly during periods of hot dry weather (Taha 1997; Wong *et al.* 2003; Bowler *et al.* 2010). Green, vegetated, roofs in particular are gaining prominence for their ability to improve residents' thermal comfort and building insulation (along with energy savings from the reduced use of air conditioning) (Saiz *et al.* 2006; Rowe 2011; Peng and Jim 2013). Plant species choice on extensive and semi-extensive green roofs, which are designed with lower maintenance in mind, usually revolves around low growing plants such as *Sedum* or grass mixes (Getter and Rowe 2006; Oberndorfer *et al.* 2007). Our previous work, however, suggested that by choosing an alternative to *Sedum*, substrate temperatures (and even air temperatures at times) can be consistently significantly lowered (Blanusa *et al.* 2013). More broadly, little is known about how different plants compare in their potential for these 'temperature regulation' services and what are the mechanisms/traits that underpin those differences.

Certain leaf traits and physiological processes can influence the amount of radiation absorbed by the leaf and how the absorbed heat is later dissipated. Individual morphological traits such as leaf colour, the extent of leaf hairiness and structure of leaf hairs (if leaves are pubescent) and leaf thickness, are known to affect leaf temperatures (Ansari and Loomis 1959; Ferguson *et al.* 1973; Ehleringer and Mooney 1978). Leaves, however, exhibit these multiple traits simultaneously (e.g. a *Stachys byzantina* leaf is light-coloured as well as pubescent), but the relative contribution of multiple traits to leaf temperature regulation, and how do they 'rank' in importance, in various types of leaves, is not understood.

Leaf colour is defined by leaf hue, chroma and lightness (Voss 1992); leaf lightness is directly linked to its reflectance. A lighter leaf colour of a similar hue (i.e. light *vs* dark green leaves) increases short-wave reflectance (Billings and Morris 1951) and thus reduces leaf temperature (Ferguson *et al.* 1973). Leaf pubescence too can be associated with higher visible reflectance (Billings and Morris 1951), but not in all cases as hairs can vary considerably in their structure and colour (Gausman and Cardenas 1969). Additionally, leaf hair density may affect leaf convection and transpiration (and thus leaf temperature) by affecting the leaf boundary layer resistance (Schuepp 1993) and/or by influencing the number of stomata present in a leaf (Skelton *et al.* 2012). Pubescence characteristics may also influence irradiance parameters, including the degree of shading on the epidermis, as these structures will act as a shield, reducing the radiation input onto the leaf itself (Lewis and Nobel 1977). Finally, an increase in leaf thickness (succulence) is linked to an increased capacity for leaf heat storage, but slower heat dissipation (Lewis and Nobel 1977) thus leading to increased leaf temperatures.

Leaf temperatures are also largely dependent on substrate moisture (Grant *et al.* 2007). Plants respond to periods of water deficit by closing their stomata and reducing transpiration loss (Hsiao 1973; Jones 1998; Chaves *et al.* 2002), consequently increasing leaf temperature. This might be of importance for plants grown on green roofs where summertime drying is routinely experienced (Nagase and Dunnett 2010). Not all plants respond to substrate drying in the same manner, however, with variations in stomatal behaviour during drying (Cameron *et al.* 2008; Campbell *et al.* 2010). Plants also employ a range of additional mechanisms to continue to function when subjected to long periods of water deficit. Plants/leaves with traits that promote reflectance adapt fairly well to prolonged water deficiency. For instance, the percentage of white, highly-reflective, hairs on certain xerophytes increases substantially

when they are experiencing prolonged water deficits (Ehleringer 1982). An increase in leaf hairiness augments reflectance and so leaf temperatures of those plants can be maintained close to the temperature of the air around them (Ehleringer and Mooney 1978). Other genera possessing thick and fleshy succulent leaves or stems have the ability to store water within specific water reserving cells and therefore can thrive in intense water deficit conditions. The effectiveness of these water reserves is evident from a study which showed that apical leaves of plants from *Sedum rubrotinctum* growing in a glasshouse environment were turgid for at least two years without supplemental water (Teeri *et al.* 1986). Many succulents are also facultative or compulsory Crassulacean Acid Metabolism (CAM) plants, and therefore significantly reduce CO_2 uptake during the day, and hence reduce stomatal opening, during periods of water deficiency without compromising their functioning (Kluge and Ting 1978). However, a strategy like this will not allow plants to remain cool, as heat storage within their leaves will also increase compared to thin-leaved plants.

The understanding of the relative importance of each of those morphological traits and physiological processes becomes relevant, when attempting to rank plant genotypes in their potential for ecosystem service delivery with respect to urban cooling. To elucidate this we have studied three plant genera, each with a number of genotypes with contrasting leaf attributes (dark *vs* light-coloured, thick *vs* thin-leaves, smooth *vs* pubescent, and pubescent leaves with short *vs* long hairs) when exposed to two contrasting water availability regimes. The following hypotheses were tested:

- Leaf water loss is key for leaf temperature regulation: a decrease in leaf stomatal conductance increases leaf temperature in all plant-types.

- Genotypes with light-coloured leaves, thin leaves and/or longer leaf hairs (in pubescent genotypes) have lowest leaf temperatures, even when subjected to water deficit.

Genera selected were all evergreen perennials or sub-shrubs which are commonly found in gardens. Although the key objective of this paper was to assess the relative contribution of multiple leaf traits to leaf temperature regulation, the choice of plants was based on their potential to also be used on semi-extensive green roofs. Low to medium growing perennials can be easily incorporated in such systems, providing cooling without occupying the restricted ground-level urban footprint.

Materials and methods

Plant material

Three plant genera, each with a number of genotypes, were selected for the experiments, carried out in a ventilated glasshouse located at the University of Reading (UK) experimental grounds. Genotypes were selected to include a range of contrasting leaf colour, pubescence (presence and length of hairs) and leaf thickness (Table 1/ Figure 1).

[Insert Table 1]

[Insert Figure 1]

Heuchera, Sempervivum and *Salvia* genotypes were tested in three separate phases starting on 21 March, 2 June and 21 June 2011, respectively; each phase lasting 15-17 days. Plants were purchased as six months old plugs. *Heuchera* and *Salvia* were transplanted into a peat-based

144 growing medium (SHL, 'William Sinclair', Lincoln, UK) one month before the start of each
145 experiment into 2 L containers (round, d = 17 cm, 10 cm of substrate). *Sempervivum* were
146 transplanted at the same time, but to 1 L containers (round, d = 13 cm, 8 cm of substrate);
147 here, the substrate was mixed with sand (v/v 50:50) to increase drainage and minimise risk of
148 root pathogens (*Pythium* and *Phytophthora* spp.) in this xerophytic genus.

149 Each irrigation treatment/genotype combination was represented by either seven (*Heuchera*
150 and *Salvia)* or eight (*Sempervivum)* replicate plants. For *Heuchera* and *Salvia*, containers
151 were arranged on two benches within a single glasshouse compartment using a randomized
152 two-block design (each bench contained three to four containers of each treatment). For
153 *Sempervivum*, all containers were arranged on one bench using a randomized design.

154 *Watering treatments*

155 On the morning of Day 0 of each experiment, containers were watered to full capacity. From
156 Day 1 onwards containers were either kept at full substrate water holding capacity (100%,
157 wet regime - 'WR') or subjected to regulated deficit irrigation (dry regime - 'DR') (Cameron
158 *et al.* 2006). Irrigation was carried out manually, based on a proportion of evapo-transpiration
159 (ET) over the preceding 24 h period; thereby accounting for daily variations in evapo-
160 transpirational demand. For *Heuchera* and *Salvia*, 'WR' plants received daily 100% of
161 moisture lost in the preceding 24 h period, whereas 'DR' plants received 50% of this volume.
162 For the succulent *Sempervivum*, due to naturally low ET rates, 'WR' plants received all the
163 water lost by evapotranspiration in 48 h cycles, rather than daily, and the 'DR' plants
164 received no irrigation for the duration of the experiment. Moisture loss was determined by
165 weighing containers on Adam CBK 32 Bench Scale (Scales and Balances, Thetford, Norfolk,
166 UK).

Plant and substrate measurements

The air temperature and relative humidity within the glasshouse compartment in each of the experiments was recorded every 30 minutes by a screened Tinytag logger Plus 2 – TGP-4500 (Gemini Data Loggers Ltd., Chichester, West Sussex, UK; -25 to 85 °C and 0-100% RH range and an accuracy of 0.4 ºC and 3.0% RH at 25°C). Air temperatures during the experiment are presented in the Results section; mean daily relative humidity in the glasshouse compartment was relatively constant within each experiment and averaged 68 % for the *Salvia* experiment and 70% for the *Heuchera* and *Sempervivum* experiments.

Substrate moisture content (SMC) was measured using a SM200 capacitance-type probe connected to a HH2 Moisture Meter (Delta-T Devices, Cambridge, Cambridgeshire, UK; 0 – 100% range and an accuracy of 3%). Measurements were made regularly throughout the experiment, as moisture availability decreased in the 'DR' treatment (with four dates that represent different phases of the drying process being shown - see Figures 3-5). Two measurements per container were made in *Heuchera* and *Salvia* and one measurement per container in *Sempervivum*, between 09:30 - 11:30 h on each date. Probes were inserted into the substrate vertically, as far away as possible from the container edge, to minimise edge effects.

Water loss in *Heuchera* and *Salvia* was inferred by the measurement of their leaf stomatal conductance (g_s, mmol m^{-2} s^{-1}) using an LCi infra-red gas analyser (ADC Bioscientific, Hoddesdon, Hertfordshire, UK) with ambient CO_2 concentration at 400 ± 10 mm^3 dm^{-3}. During measurements, photosynthetic photon flux density was supplemented to 2000 µmol m^{-2} s^{-1} by an external halogen source (50 W, 12 V). Stomatal conductance was measured at the four dates when SMC was measured too, reflecting the different phases of drying in 'DR'

treatments. At each date, two young, fully expanded leaves per container were measured between 11.00 - 13.00 h (with measurements made on different treatments being spread out evenly through the evaluation time on each date). In *Sempervivum*, however, the small leaf size precluded the use of the gas analyser, so transpiration rates were estimated at a plant level from container water loss between consecutive weight measurements instead. As at least 90% of the substrate was completely covered by the low growing *Sempervivum* plants (see Figure 1), we assumed that evaporation from the substrate surface was minimal and that the recorded water loss corresponded mainly to plant transpiration.

Leaf thickness was estimated using the methodology proposed by Vile *et al*. (2005):

$$\mathbf{LT} = \frac{\mathbf{1}}{\boldsymbol{\rho}} \frac{\mathbf{1}}{(\mathbf{SLA \times LDMC})} \quad (1)$$

Where: LT = Leaf thickness; ρ = Density of the leaf (assumed to be similar to water i.e. 1 g cm^{-3}); SLA = Specific leaf area (ratio of area to dry mass, m^2 kg^{-1}); LDMC = Leaf dry matter content (ratio of dry to fresh mass, mg g^{-1}).

SLA and LDMC were calculated based on the protocol of Garnier *et al.* (2001) with one young fully expanded leaf per plant being assessed at the beginning and end of experiments. Leaves were hydrated for 6 h at 4 ^{o}C in the dark, before fresh weight and area were determined (Leaf Area Meter, Delta-T Devices, Cambridge, Cambridgeshire, UK). Leaf dry weight was assessed after drying at 70 ^{o}C for 48 h.

Leaf colour was evaluated visually (Table 1) and the relative luminance parameter Y (here presented as 'leaf lightness') was measured with a SP52 portable sphere spectrometer (X-Rite, Poynton, Cheshire, UK), which measures the percentage of reflectance in the visual spectral range of 400 to 700 nm. This parameter was measured, on the upper side of on one

leaf per container, at the beginning and end of the experiments for *Heuchera* and *Salvia* and mid-experiment for *Sempervivum*.

In addition to the visual description of pubescence in all genera, length of leaf hairs was determined in *Salvia*. Three cross sections on three leaves per treatment (one each of young, medium and old leaves) were captured using an Axioskop 2 microscope (Carl Zeiss, Cambridge, Cambridgeshire, UK). Hair length was then measured using the software Image J (National Institutes of Health, Bethesda, Maryland, USA). Six fully visible hairs were measured in each cross section to obtain average hair length values.

Thermal images of all individual containers were recorded using an infrared imaging camera Thermo Tracer TH7800 (NEC San-ei Instruments Ltd., Tokyo, Japan; -20 to 250 ^{o}C range and an accuracy of 0.1 ^{o}C) at the four dates SMC was measured, within one hour in the early afternoon of each date. Containers were randomly selected for imaging to minimise the impact of air temperature differences within the measurement hour on leaf temperatures. Images were recorded from a consistent angle and distance on plants placed out of direct sunlight. Plants were kept in the shade for 5 minutes before being measured so that the effect of previous heat load differences on leaf temperature was minimized. For each individual plant, temperatures were calculated in four separate sections of the canopy covering approx. 10 cm^2 (*Heuchera* and *Salvia)* or 5 cm^2 (*Sempervivum*). Leaf emissivity was determined on a sub-sample of leaves in thin-leaved genotypes using the technique described by López et al. (2012). Emissivity of *Sempervivum* was not measured due to its leaf morphology not being conducive to the technique employed. Mean emissivity values ranged between 0.974 for purple *Heuchera* and 0.968 for grey *Salvia*. Therefore a standard emissivity of 0.97 was used for all genera when analysing the thermal images.

Statistical analysis

Data were analysed using GenStat (16th Edition, VSN International Ltd., Hemel Hempstead, Hertfordshire, UK). Analysis of variance (ANOVA) was used to assess the effect of watering regime and plant genotype on measured parameters; variance levels were checked for homogeneity (where necessary data were transformed – e.g. leaf lightness in the *Heuchera* experiment) and values are presented as means with associated least significant differences (LSD, $P = 0.05$). Data for each day of the experiment were analysed separately.

In addition to ANOVA analyses, multiple regressions were performed to identify which leaf factors contributed the most to leaf temperature differences in the three genera for the selected four experimental days representing different phases of drying in 'DR' treatments. Each daily regression had leaf temperature (averaged at the container level) as dependent variable and the mean container´s g_s/water loss, leaf lightness and leaf thickness as independent variables. In *Salvia*, hair length was also included as an independent variable. When more than one plant factor was significant for the regression model, their measure of importance was established using a dominance analysis, as described by Budescu (1993).

Results

Heuchera: The influence of genotype and substrate moisture on leaf temperature, stomatal behaviour, leaf lightness and leaf thickness

Heuchera plants were evaluated on Days 0, 7, 12 and 16 of the experiment. Maximum air temperatures within the glasshouse on Days 0 and 16 were above 30 ºC. On the remaining days, maximum air temperature was approximately 25 ºC (Figure 2.A).

Leaf temperatures were lowest for the yellow genotype throughout the experiment. 'WR' yellow plants had significantly cooler leaves than all other treatments, and 'DR' yellow plants had significantly cooler leaves than all purple and purple-white plants on all selected dates (e.g. plant differences on Days 0 and 16, both $P < 0.001$) (Figure 2.D). On the last day of the experiment, yellow plants were on average 2.8 ^{o}C cooler than purple plants under 'WR' and 1.9 ^{o}C under 'DR'. Additionally, substrate moisture content (SMC) influenced leaf temperatures significantly once the difference in watering regimes was introduced (e.g. moisture differences on Days 7 and 16, both $P < 0.001$). From Day 7, leaf temperatures in the 'DR' plants were significantly higher than their respective 'WR' controls (Figure 2.D).

Leaf stomatal conductance (g_s) also appeared to be strongly linked to the genotypes' leaf colour (e.g. differences on Days 0 and 16, both $P < 0.001$). In the 'WR', plants mean values were: 286 (yellow), 248 (green), 191 (purple/white) and 187 mmol m^{-2} s^{-1} (purple). Yellow and green foliage plants had significantly higher g_s values than purple or purple/white genotypes on all days when g_s was measured (Figure 2.C). Water deficits too had a dramatic effect on g_s, with all 'DR' plants bar the yellow demonstrating significant reductions in g_s by Day 7 (e.g. moisture differences on Days 7 and 16, both $P < 0.001$) (Figure 2.C). On that day the g_s of the 'DR' purple plants had declined by 27% compared to the ´WR´ ones, whilst for the yellow one the g_s reduction was 13%. However, by Day 12, SMC was < 0.20 m^3 m^{-3} across all the 'DR' treatments (Figure 2.B), and g_s correspondingly was significantly lower for each genotype in comparison to their 'WR' controls. On the last day, the ´DR´ yellow and purple plants were both showing a 45-50% reduction in their g_s values.

As expected, leaf lightness was highest in the yellow foliage, being approximately 4-fold greater than the other foliage colours (plant differences: Day 0 (data not shown) and Day 16,

279 (Table 2), both $P < 0.001$). Furthermore leaves from green *Heuchera* were 0.08 mm thicker
280 than those from the other genotypes (plant differences: Day 0 (data not shown) and Day 16
281 (Table 2), $P < 0.001$).

282 [Insert Figure 2]

283 [Insert Table 2]

284 *Salvia: The influence of genotype and substrate moisture on leaf temperature, stomatal*
285 *behaviour, leaf lightness and leaf thickness*

286 *Salvia* plants were evaluated on Days 0, 6, 13 and 17 of the experiment. Maximum air
287 temperature within the glasshouse on Days 6 and 13 was approximately 35 ^{o}C, whilst
288 maximum air temperatures on Days 0 and 17 were approximately 30 ^{o}C (Figure 3.A).

289 Throughout the experiment, leaf temperatures of 'WR' plants were significantly higher in the
290 purple genotype compared to the grey and green ones (e.g. plant differences on Days 0 and
291 17, both $P < 0.001$) (Figure 3.D). At the end of the experiment the difference between purple
292 and grey genotypes´ temperatures was on average 1.5 ^{o}C under 'WR' and 2.1 ^{o}C under 'DR'
293 (Figure 3.D). Water deficit increased temperature, with leaf temperatures of all 'DR'
294 treatments becoming significantly higher than their respective 'WR' controls from Day 6
295 onwards (e.g. moisture differences on Days 6 and 17, both $P < 0.001$). In the 'WR', plants of
296 the green and grey genotypes had similar temperatures, but from day 6 onwards in the 'DR'
297 the grey was significantly cooler (e.g. 0.8 ^{o}C on the last day of the experiment) than the green
298 genotype (Figure 3.D).

299 When well watered, g_s values in the green genotype were significantly greater than those in
300 the purple ones, with the g_s values of grey plants being intermediate at all dates tested (e.g.

301 plant differences on Day 0, $P < 0.001$ and Day 17, $P = 0.006$) (Figure 3.C). Water deficit
302 reduced g_s, and from Day 6 onwards all genotypes in the 'DR' treatments (where SMC was
303 reduced to around 0.2 m^3 m^{-3} – Figure 3.B) had significantly lower g_s compared to the
304 respective 'WR' controls (e.g. moisture differences: Day 6, $P = 0.013$ and Day 17, $P < 0.001$)
305 (Figure 3.C). However not all genotypes showed a similar rate of g_s decrease as on the last
306 day the g_s of the 'DR' green plants were reduced by 45% compared to their ´WR´ control,
307 whilst for the grey, the g_s reduction was 26%.

308 No differences in leaf thickness were detected, but genotypes with different leaf colour
309 differed significantly in their leaf lightness (plant differences: Day 0, (data not shown) and
310 Day 16, (Table 3), both $P < 0.001$). At the end of the experiment, leaf lightness of the grey
311 genotype was around 4% greater than that of the purple genotype. Leaf hair length was
312 significantly longer with the grey genotype too (0.96 mm) as compared to green or purple
313 genotypes (both averaging 0.63 mm) ($P < 0.001$, data not shown).

314 [Insert Figure 3]

315 [Insert Table 3]

316 *Sempervivum: The influence of genotype and substrate moisture on leaf temperature, plant*
317 *water loss, leaf lightness and leaf thickness*

318 *Sempervivum* plants were evaluated on Days 0, 7, 11 and 15 of the experiment. Maximum air
319 temperatures within the glasshouse on Days 0, 7 and 11 were approximately 30 ^oC and on
320 Day 15 maximum air temperature was approximately 25 ^oC (Figure 4.A).

321 Leaf temperature was highest with the green genotype, when plants were well watered (e.g.
322 plant differences: Day 0, $P < 0.001$ and Day 15, $P = 0.01$) (Figure 4.D). Imposing water

deficiency increased temperatures most markedly in the hairy genotype in the first instance, and by Day 11 temperature differences between 'DR' and 'WR' hairy plants of this genotype reached 2.8 ^{o}C. Water status also had a significant effect on temperature of the other two genotypes by this time (Day 11, $P < 0.001$).

Differences in plant water use between 'WR' and 'DR' were significant from Day 7 for all genotypes (Figure 4.C) (Day 7, $P = 0.008$), when all 'DR' treatments had a mean SMC of around 0.10 m^3 m^{-3} (Figure 4.B). When well watered, hairy plants lost the highest amount of water, but when water was withdrawn, the daily water loss of the hairy genotype plants was similar to the other ones (Figure 4.C).

There were significant genotype differences in both leaf thickness (plant differences: Day 0, $P < 0.001$ (data not shown) and Day 15, $P = 0.002$ (Table 4)) and leaf lightness ($P < 0.001$ (Table 4)). Green leaves were on average at least 0.3 mm thicker and had around 10% greater leaf lightness than the red leaves.

[Insert Figure 4]

[Insert Table 4]

Multiple regressions

For *Heuchera*, g_s and leaf lightness (unlike leaf thickness) were significantly related with leaf temperature at all times (Table 5.A). When plants were under well watered conditions (Day 0), leaf lightness contributed 9% more than g_s to the overall temperature variation. However, when differences in g_s between 'WR' and 'DR' plants became significant, g_s was the largest determinant of leaf temperature (accounting for 19% more of the variation than leaf lightness on the last day) (Table 5.A).

In *Salvia*, only leaf lightness was significantly related with leaf temperature on Day 0, when all plant factors (i.e. leaf lightness, hair length, leaf thickness as well as g_s) were considered simultaneously (Table 5.B). However, on Day 6, g_s and hair length also contributed significantly to leaf temperature, with g_s being the greatest determinant (54% more than leaf lightness). On Days 13 and 17, leaf lightness was no longer significantly related with leaf temperature when considered simultaneously with g_s and hair length. On the last day, g_s was a more significant determinant of leaf temperature than hair length, with g_s contributing 6% more to the overall variation in temperature (Table 5.B).

Unlike the other genera, in *Sempervivum*, leaf thickness was the only factor significantly related with temperature on Days 0 and 7 (Table 5.C). Plant water loss played a significant role in the leaf temperature variation as well but only when the SMC differences between 'WR' and 'DR' treatments became apparent. By Day 13, the contribution of water loss accounted for 10% more of the temperature variation than that of leaf thickness and by Day 15 it was the only significant factor (Table 5.C).

[Insert Table 5]

Discussion

All the leaf traits and physiological processes considered here (leaf lightness, extent of pubescence, leaf thickness and stomatal conductance/water loss) influenced significantly leaf temperature. This led to significant differences in leaf temperature between genotypes of the same genera. Additionally, the extent of each factor's contribution varied between genera and was also dependent on substrate moisture content.

It is well established that leaf temperature and g_s are strongly linked. This relationship has been shown in numerous studies on a range of species under different substrate moisture conditions, in glasshouses or in the field. For example, in a glasshouse experiment with *Phaseolus vulgaris*, g_s was accurately predicted from leaf thermal images using reference surfaces with known water vapour conductance (Jones 1999). Furthermore, in an experiment with *Fragaria ×ananassa* cultivars analysed under wet and dry conditions, g_s estimated from thermal images of leaves placed horizontally were strongly related with direct g_s measurements made with a porometer (Grant *et al.* 2012).

In our experiments, lower g_s (or lower plant water loss, in *Sempervivum*) was also always strongly related with higher leaf temperatures. The increase in temperature was largely controlled by the watering regime implemented. Leaf temperature differences between 'WR' and 'DR' plants became significant as soon as g_s/water loss decreased, due to less water being given to the dry treatments. The only exception was *Sempervivum*, where the red and green genotypes´ water losses were significantly reduced by Day 7 but a significant increase in their leaf temperature was only apparent later, on Day 11. A study comparing thick, succulent *Graptopetalum* leaves to other thinner leaves (in which the leaf mass of *Graptopetalum* was at least 472 mg cm^{-2} greater than the leaf mass of all other leaves considered), identified that *Graptopetalum* leaves took the longest to heat up or cool in response to changes in environmental conditions (in this case changes in sun/shade light intensities) (Ansari and Loomis 1959). This suggests that succulent leaves' temperatures are more decoupled from environmental conditions than thinner leaves and this could explain why some of the *Sempervivum* genotypes reacted more slowly to a significant change in their daily water losses. Nevertheless, even for *Sempervivum*, water loss was related with leaf temperature at the end of the experiment, when SMC was substantially reduced.

390 Inherent g_s/water losses differences between the genotypes of the same genera, however, also
391 contributed to differences in leaf temperature on some occasions. *Heuchera* and *Salvia*
392 genotypes with yellow or green leaves had higher g_s than genotypes with purple leaves
393 (Figures 2, 3). Consequently, and particularly in the *Heuchera* genotypes, differences in g_s
394 contributed to leaf temperature differences between genotypes even before SMC was reduced
395 in the dry treatments.

396 Leaf lightness was used to quantify genotype differences in leaf colour. Some studies
397 recognized the importance of light leaf colour to achieve high visible reflectance and
398 decrease plant temperature (Ferguson *et al.* 1973). In our study, the contribution of leaf
399 lightness to temperature regulation was significant only among the thin-leaved non-succulent
400 genera (*Heuchera* and *Salvia*) (Table 5). In both genera, leaf lightness was the factor that
401 contributed to temperature regulation most strongly before water deficit was introduced.
402 Furthermore, even when water deficit developed, leaf lightness significantly influenced leaf
403 temperature on some occasions, although less than g_s. More specifically, in the *Heuchera*
404 experiment the yellow genotype had lowest leaf temperature, even though its g_s was similar
405 to that of darker genotypes (e.g. 'WR' yellow *vs* 'WR' green or 'DR' yellow *vs* 'WR' purple
406 – Figure 2). With *Salvia*, a lighter leaf colour also led to lower leaf temperatures, even when
407 there were no differences in g_s (e.g. 'DR' green and purple genotypes, on the last day of the
408 experiment, with green genotype being cooler – Figure 3).

409 Similarly, leaf hair length also contributed to temperature differences in thin, pubescent
410 *Salvia* leaves, but only in water deficit conditions. When comparing the grey to the green
411 genotype, the 'DR' grey genotype – which has longer hairs - was always cooler than 'DR'
412 green (Figure 3). This supports earlier work arguing that the presence of leaf hairs may

increase the leaf's time-scale of response to water deficit, compared to other non-hairy or less hairy leaves (França *et al.* 2012; Blanusa *et al.* 2013). This may be linked to the effect that the size and density of leaf pubescence can have on the leaf boundary layer thickness (Schuepp 1993). Hairs in *Salvia* are relatively sparse (Table 1), so a small increase in their length may enhance air turbulence (via an increased roughness) close to the leaf surface leading to reduced boundary layer resistance to heat and water vapour transfer. This could reduce leaf temperature, even when substrate moisture (and thus g_s) is restricted. It can also be linked to the fact that highly pubescent leaves can have a higher number of stomata per leaf area than glabrous/less pubescent leaves (Skelton *et al.* 2012). The number of stomata was not assessed in this study but a possible increase in stomatal density could explain why, on the last day, g_s of 'DR' grey *Salvia* was still only marginally lower than g_s of 'WR' purple *Salvia*; this uncharacteristically small difference in g_s, along with the greater visible reflectance of the grey leaves, may have contributed to 'DR' grey *Salvia* having slightly lower leaf temperatures than 'WR' purple *Salvia* on Day 17.

Leaf thickness was only important for leaf temperature differences in succulent genera/genotypes (Table 5). Thick leaves store more heat than thin leaves and consequently have typically higher leaf temperatures (Lewis and Nobel 1977). In extreme cases, as for thick desert cacti such as *Opuntia*, surface plant temperatures can rise up to 13 ^{o}C above surface leaf temperatures shown by other surrounding desert plants with smaller thinner leaves (Gates *et al.* 1968). Temperature differences between different *Sempervivum* genotypes were not as large but still green *Sempervivum* – with thicker leaves - had higher leaf temperature than the red, despite its highest visible reflectance among *Sempervivums* (Table 4). In *Sempervivum*, along with leaf thickness, only differences in water loss between the genotypes influenced leaf temperatures.

437 These results suggest therefore that different plant genera may depend on different
438 processes/traits to effectively regulate the temperature of their leaves and this is also
439 dependent on substrate moisture availability (summarized in Figure 5). Under water deficit
440 conditions, maintenance of transpiration (here approximately determined by leaf g_s or plant
441 water loss) was the key process for temperature regulation in all genera considered.
442 Temperature of thin leaves, however, was additionally dependent on leaf colour and, in
443 pubescent leaves, the length of leaf hairs (with lighter leaf colour and longer hair length being
444 associated with lower temperatures). Conversely, in succulent leaves, temperature was mostly
445 controlled by leaf thickness, with other simultaneously measured factors (such as leaf
446 hairiness and darker colour) not being significant.

447 [Insert Figure 5]

448 This knowledge can be valuable to identify potential differences in plant effects on
449 temperature of the surrounding environment. Genera/genotypes that normally heat up more
450 (i.e. with darker or thicker leaves) and/or that possess low typical g_s will inevitably re-radiate
451 more and release more heat by convection to the surrounding environment than others. In
452 highly urbanized areas, where temperatures can be considerably higher than in rural
453 environments (Oke 1987; Grimmond 2007), the increase of green space has been suggested
454 to be an effective way of reducing local air temperatures (Akbari et al., 2001; Gill et al.,
455 2007). Green roofs in particular have a potential to influence air temperatures as well as
456 building insulation, improving thermal comfort of residents (Saiz *et al.* 2006; Peng and Jim
457 2013). Based on the results discussed here we suggest that different genera and even
458 genotypes within the one genus may potentially have different cooling capacities, and thus
459 different benefits, when used on green roofs. Additionally, optimal substrate moisture is also

460 critical for keeping leaves cool. Consequently we suggest that maintaining transpirational
461 water loss by sustainable irrigation and selecting urban plants with advantageous
462 physiological/morphological traits are essential to maximize the thermal benefits (i.e.
463 increase latent heat loss, reduce convection and long wave emissions and reduce the heat
464 transferred into the buildings) provided by urban vegetation on green roofs and elsewhere.
465 Confirmatory findings to this effect will be presented in our follow-up papers.

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471 **References**

472 Akbari H, Pomerantz M, Taha H (2001) Cool surfaces and shade trees to reduce energy use
473 and improve air quality in urban areas. *Solar Energy* **70**, 295–310.

474 Ansari AQ, Loomis WE (1959) Leaf temperatures. *American Journal of Botany* **46**, 713–717.

475 Billings WD, Morris RJ (1951) Reflection of visible and infrared radiation from leaves of
476 different ecological groups. *American Journal of Botany* **38**, 327–331.

477 Blanusa T, Vaz Monteiro MM, Fantozzi F, Vysini E, Li Y, Cameron RWF (2013)
478 Alternatives to Sedum on green roofs: Can broad leaf perennial plants offer better
479 "cooling service"? *Building and Environment* **59**, 99–106.

480 Bowler DE, Buyung-Ali L, Knight TM, Pullin AS (2010) Urban greening to cool towns and
481 cities: A systematic review of the empirical evidence. *Landscape and Urban Planning*
482 **97**, 147–155.

483 Budescu D V (1993) Dominance analysis: A new approach to the problem of relative
484 importance of predictors in multiple regression. *Psychological Bulletin* **114**, 542–551.

485 Cameron R, Harrison-Murray R, Atkinson C, Judd H (2006) Regulated deficit irrigation: a
486 means to control growth in woody ornamentals. *Journal of Horticultural Science &*
487 *Biotechnology* **81**, 435–443.

488 Cameron RWF, Harrison-Murray RS, Fordham M, Wilkinson S, Davies WJ, Atkinson CJ,
489 Else MA (2008) Regulated deficit irrigation of woody ornamentals to improve plant
490 quality and precondition against drought stress. *Annals of Applied Biology* **153**, 49–61.

491 Campbell DR, Wu CA, Travers SE (2010) Photosynthetic and growth responses of reciprocal
492 hybrids to variation in water and nitrogen availability. *American Journal of Botany* **97**,
493 925–33.

494 Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osorio ML, Carvalho I,
495 Faria T, Pinheiro C (2002) How plants cope with water stress in the field?
496 Photosynthesis and growth. *Annals of Botany* **89**, 907–916.

497 Ehleringer J (1982) The influence of water stress and temperature on leaf pubescence
498 development in Encelia farinosa. *American Journal of Botany* **69**, 670–675.

499 Ehleringer JR, Mooney HA (1978) Leaf hairs: Effects on physiological activity and adaptive
500 value to a desert shrub. *Oecologia* **37**, 183–200.

501 Ferguson H, Eslick RF, Aase JK (1973) Canopy temperatures of Barley as influenced by
502 morphological characteristics. *Agronomy Journal* **65**, 425.

503 França M, Prados L, de Lemos-Filho J, Ranieri B, Vale F (2012) Morphophysiological
504 differences in leaves of Lavoisiera campos-portoana (Melastomataceae) enhance higher
505 drought tolerance in water shortage events. *Journal of Plant Research* **125**, 85–92.

506 Garnier E, Shipley B, Roumet C, Laurent G (2001) A standardized protocol for the
507 determination of specific leaf area and leaf dry matter content. *Functional Ecology* **15**,
508 688–695.

509 Gates DM, Alderfer R, Taylor E (1968) Leaf temperatures of desert plants. *Science* **159**, 994–
510 995.

511 Gausman HW, Cardenas R (1969) Effect of leaf pubescence of Gynura aurantiaca on light
512 reflectance. *Botanical Gazette* **130**, 158–162.

513 Getter K, Rowe D (2006) The role of extensive green roofs in sustainable development.
514 *HortScience* **41**, 1276–1285.

515 Gill S., Handley J., Ennos A., Pauleit S (2007) Adapting Cities for Climate Change: The Role
516 of the Green Infrastructure. *Built Environment (1978-)* **33**, 115–133.

517 Grant OM, Davies MJ, James CM, Johnson AW, Leinonen I, Simpson DW (2012) Thermal
518 imaging and carbon isotope composition indicate variation amongst strawberry

519 (Fragaria×ananassa) cultivars in stomatal conductance and water use efficiency.
520 *Environmental and Experimental Botany* **76**, 7–15.

521 Grant OM, Tronina L, Jones HG, Chaves MM (2007) Exploring thermal imaging variables
522 for the detection of stress responses in grapevine under different irrigation regimes.
523 *Journal of Experimental Botany* **58**, 815–825.

524 Grimmond S (2007) Urbanization and global environmental change: local effects of urban
525 warming. *The Geographical Journal* **173**, 83–88.

526 Hsiao TC (1973) Plant responses to water stress. *Annual Review of Plant Physiology* **24**, 519–
527 570.

528 Jones HG (1998) Stomatal control of photosynthesis and transpiration. *Journal of*
529 *Experimental Botany* **49**, 387–398.

530 Jones HG (1999) Use of thermography for quantitative studies of spatial and temporal
531 variation of stomatal conductance over leaf surfaces. *Plant, Cell & Environment* **22**,
532 1043–1055.

533 Kluge M, Ting IP (1978) "Crassulacean acid metabolism: Analysis of an ecological
534 adaptation." (Springer-Verlag: New York, USA)

535 Lewis DA, Nobel PS (1977) Thermal energy exchange model and water loss of a barrel
536 cactus, Ferocactus acanthodes. *Plant Physiology* **60**, 609–616.

537 López A, Molina-Aiz FD, Valera DL, Peña A (2012) Determining the emissivity of the
538 leaves of nine horticultural crops by means of infrared thermography. *Scientia*
539 *Horticulturae* **137**, 49–58.

540 Nagase A, Dunnett N (2010) Drought tolerance in different vegetation types for extensive
541 green roofs: effects of watering and diversity. *Landscape and Urban Planning* **97**, 318–
542 327.

543 Oberndorfer E, Lundholm J, Bass B, Coffman RR, Doshi H, Dunnett N, Gaffin S, Köhler M,
544 Liu KKY, Rowe B (2007) Green Roofs as urban ecosystems: Ecological structures,
545 functions, and services. *BioScience* **57**, 823.

546 Oke TR (1987) "Boundary layer climates." (Methuen & Co. Ltd)

547 Peng L, Jim C (2013) Green-roof effects on neighborhood microclimate and human thermal
548 sensation. *Energies* **6**, 598–618.

549 Rowe DB (2011) Green roofs as a means of pollution abatement. *Environmental pollution*
550 *(Barking, Essex : 1987)* **159**, 2100–10.

551 Saiz S, Kennedy C, Bass B, Pressnail K (2006) Comparative life cycle assessment of
552 standard and green roofs. *Environmental Science & Technology* **40**, 4312–4316.

553 Schuepp PH (1993) Tansley Review No. 59. Leaf boundary layers. *New Phytologist* **125**,
554 477–507.

555 Skelton RP, Midgley JJ, Nyaga JM, Johnson SD, Cramer MD (2012) Is leaf pubescence of
556 Cape Proteaceae a xeromorphic or radiation-protective trait? *Australian Journal of*
557 *Botany* **60**, 104.

558 Taha H (1997) Urban climates and heat islands: Albedo, evapotranspiration, and
559 anthropogenic heat. *Energy and Buildings* **25**, 99–103.

560 Teeri JA, Turner M, Gurevitch J (1986) The response of leaf water potential and crassulacean
561 acid metabolism to prolonged drought in Sedum rubrotinctum. *Plant Physiology* **81**,
562 678–680.

563 Vile D, Garnier E, Shipley B, Laurent G, Navas M-L, Roumet C, Lavorel S, Díaz S, Hodgson
564 JG, Lloret F, Midgley GF, Poorter H, Rutherford MC, Wilson PJ, Wright IJ (2005)
565 Specific leaf area and dry matter content estimate thickness in laminar leaves. *Annals of*
566 *Botany* **96**, 1129–1136.

567 Voss DH (1992) Relating colorimeter measurement of plant color to the Royal Horticultural
568 Society colour chart. *HortScience* **27**, 1256–1260.

569 Wong NH, Chen Y, Ong CL, Sia A (2003) Investigation of thermal benefits of rooftop
570 garden in the tropical environment. *Building and Environment* **38**, 261–270.

571

572 **Tables**

573 **Table 1. Plant genotypes with key traits (colour, extent of pubescence and leaf**
574 **thickness) used in glasshouse experiments.**

Plant genus/species	**Plant genotype**	**Leaf colour** (visual perception)	**Leaf pubescence** (visual perception of length and density)	**Leaf thickness**	**Referred to as**
Heuchera	'Electra'	yellow	no	Thin	Yellow *Heuchera*
	'Café Olé'	dark green	no	Thin	Green *Heuchera*
	'Geisha´s Fan'	variegated purple/ white	no	Thin	Purple/ white *Heuchera*
	'Obsidian'	purple	no	Thin	Purple *Heuchera*
Salvia officinalis	Common form	green	yes (short and sparse)	Thin	Green *Salvia*
	'Berggarten'	green/grey	yes (long and sparse)	Thin	Grey *Salvia*
	'Purpurascens´	green/ purple	yes (short and sparse)	Thin	Purple *Salvia*
Sempervivum	'Reinhard'	green	no	thick/ succulent	Green *Sempervivum*
	'Red Shadows'	red	no	thick/ succulent	Red *Sempervivum*
	'Lively Bug'	green	yes (long and sparse)	thick/ succulent	Hairy *Sempervivum*

575

Table 2. *Heuchera*: The effect of genotype and irrigation regime ('WR' vs 'DR') on mean leaf lightness and leaf thickness on the last day of the experiment. Data are a mean of seven containers of each genotype per treatment; different letters correspond to statistically significant differences between means.

Measurements	Purple 'WR'	Purple 'DR'	Yellow 'WR'	Yellow 'DR'	Green 'WR'	Green 'DR'	Purple/ White 'WR'	Purple/ White 'DR'	LSD
Leaf lightness (%)	5.55 a	5.60 a	35.30 c	37.81 c	9.42 b	8.87 b	8.87 b	9.45 b	[A]
Leaf thickness (mm)	0.21 ab	0.20 a	0.20 a	0.21 ab	0.28 d	0.27 d	0.24 c	0.23 bc	0.022

[A] LSD not shown as it relates to transformed data.

Table 3. *Salvia*: The effect of genotype and irrigation regime ('WR' vs 'DR') on mean leaf lightness and leaf thickness on the last day of the experiment. Data are a mean of seven containers of each genotype per treatment; different letters correspond to statistically significant differences between means.

Measurements	Green 'WR'	Green 'DR'	Purple 'WR'	Purple 'DR'	Grey 'WR'	Grey 'DR'	LSD
Leaf lightness (%)	12.93 b	12.69 b	9.61 a	10.06 a	14.16 b	13.89 b	1.669
Leaf thickness (mm)	0.29 a	0.30 a	0.28 a	0.30 a	0.30 a	0.29 a	0.023

587 **Table 4. *Sempervivum*: The effect of genotype and irrigation regime ('WR' vs 'DR') on**
588 **mean leaf lightness on the middle of the experiment and leaf thickness on the last day of**
589 **the experiment. Data are a mean of seven containers of each genotype per treatment;**
590 **different letters correspond to statistically significant differences between means.**

Measurements	Red 'WR'	Red 'DR'	Green 'WR'	Green 'DR'	Hairy 'WR'	Hairy 'DR'	LSD
Leaf lightness (%)	7.52 a	7.52 a	17.57 b	17.20 b	16.67 b	16.11 b	1.826
Leaf thickness (mm)	2.17 ab	2.10 a	2.46 c	2.49 c	2.45 c	2.40 bc	0.271

591

592 **Table 5. Leaf temperature variation accounted for by the multiple regressions for four**
593 **different days of each experiment (DOE) representing different stages of drying. The**
594 **regression relates leaf temperature to all significant predictors (with $P < 0.05$) from leaf**
595 **stomatal conductance (g_s)/daily water loss, leaf lightness, hair length and leaf thickness.**
596 **Individual contributions of significant plant factors were determined by dominance**
597 **analysis and are reported on the right side of the table.**

Plant types	DOE	Variation accounted for by the multiple regression (%)	Individual contributions of significant plant factors (%)			
			g_s/ daily water loss	Leaf lightness	Hair length	leaf thickness
A. *Heuchera*	0	57.6	24.5	33.1		
	7	53.5	31.0	22.5		
	12	38.7	21.5	17.2		
	16	56.5	38.0	18.5		
B. *Salvia*	0	34.6		34.6		
	6	86.3	64.7	11.0	10.7	
	13	77.5	71.6		6.0	
	17	58.4	32.0		26.4	
C. *Sempervivum*	0	24.5				24.5
	7	14.1				14.1
	11	23.0	16.6			6.4
	15	30.3	30.3			

598

Figure legends

Figure 1. Images of all plant genotypes used for the experiments.

Figure 2. *Heuchera*: A. air temperature profile within the glasshouse over the full extent of the experiment and B. substrate moisture content (SMC) C. leaf stomatal conductance (g_s) and D. leaf temperature of different genotype/irrigation treatments on four days of the experiment (DOE). Data for SMC, g_s and leaf temperature are a mean of seven containers of each genotype per treatment. LSD values (5%) were calculated for each day separately and are shown at the top of the figures; different letters on top of bars correspond to statistically significant temperature differences between means.

Figure 3. *Salvia*: A. air temperature profile within the glasshouse and B. substrate moisture content (SMC). C. leaf stomatal conductance (g_s) and D leaf temperature of different genotype/irrigation treatments on four days of the experiment (DOE). Data for SMC, g_s and leaf temperature are a mean of seven containers of each genotype per treatment. LSD values (5%) were calculated for each day separately and are shown at the top of the figures; different letters on top of bars correspond to statistically significant temperature differences between means.

Figure 4. *Sempervivum*: A. air temperature profile within the glasshouse and B. substrate moisture content (SMC). C. daily plant water loss and D. leaf temperature of different genotype/irrigation treatments on four days of the experiment (DOE). Data for SMC, plant water loss and leaf temperature are a mean of eight containers of each genotype per treatment. LSD values (5%) were calculated for each day separately and are shown at the top of the

figures; different letters on top of bars correspond to statistically significant water loss and temperature differences between means.

Figure 5. Factors influencing leaf temperature in various leaf types in our experiments when substrate moisture content is optimal (dark blue) or low (light blue).