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Are succulence or trait combinations related to plant survival on hot and dry green roofs?

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ABSTRACT

The many ecosystem services that green roofs can provide rely on good plant coverage and plant survival, which is challenging in hot and dry climates. While true succulents like *Sedum* spp. have been shown to survive well on green roofs, there are limited studies relating individual traits or trait combinations to survival in other life-forms. Succulence is a rarely studied trait that describes plant water storage in leaves, stems and roots, regardless of life-form. This means that succulence can occur in plants which are not considered to be true succulents like *Sedum* or *Crassula* species. Improving water availability through succulence may improve survival on unirrigated green roofs, but succulence, as a trait, has rarely been investigated in plants which are not true succulents. We investigated whether succulence or trait combinations can relate well with survival and could be used to improve plant selection for different substrate depths on hot and dry green roofs. We conducted two experiments with the same 11 Mediterranean species (five herbs, three sub-shrubs and three shrubs); (1) a pot experiment to determine traits under well-watered conditions including: succulence, leaf lethal temperature, water use, root:shoot ratio and leaf area. These individual traits and the combinations of all these traits were used in the analyses of this experiment; and (2) a green-roof module experiment to determine survival in four substrate depths (10, 15, 20, and 25 cm). Survival was not related to succulence, indicating that increased internal water storage in non-succulent plants does not per se lead to greater survival in extreme conditions. Survival was also not related to individual traits relating to water use and leaf heat tolerance or trait combinations. Nevertheless, plants in the same functional groups had similar survival, which suggests plants with similar trait combinations can have similar survival on green roofs.

1. Introduction

Green roofs are recognised as an effective way to increase green space and relieve some of the environmental impacts of urbanisation such as the urban heat island effect, loss of biodiversity, increased stormwater runoff and reduced connection to nature (Vijayaraghavan, 2016; Shafique et al., 2018). However, plant survival on green roofs is challenging as green roofs often have shallow substrates (< 20 cm depth) to reduce weight-loading on buildings, which reduces plant water availability (Vijayaraghavan, 2016; Shafique et al., 2018). This is exacerbated in hot and dry climates where plants can experience long periods without water in summer, resulting in mortality of many species (Rayner et al., 2016; Savi et al., 2016). Plant survival on green roofs can be improved by increasing water availability through irrigation, improved substrate water retention properties, greater substrate depths or by selecting plants that have greater water availability through succulence (Farrell et al., 2012; Razzaghmanesh et al., 2014b).

Generally, plants growing in deeper green roof substrates are expected to have a greater survival and better growth, as deeper substrates store more water and provide more room for roots (Dunnett et al., 2008; Kazemi and Mohorko, 2017; Eksi and Rowe, 2019). Nevertheless, some studies have also demonstrated that plants can survive equally well or better in shallow substrates (Thuring et al., 2010; Zhang et al., 2014), which may be due to roots in shallow substrates accessing water in retention layers beneath substrates more quickly after planting (Savi et al., 2015). For example, Zhang et al. (2014) showed that the survival of some herbs (e.g. *Allium senescens* and *Leucanthemum vulgare*) in 10 cm was equal or greater than in 15 cm substrates. Substrate depths on green

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roofs cannot be increased indefinitely because of weight-loading limitations of roofs on existing buildings or on new buildings due to construction costs (Wong et al., 2005; Chow et al., 2016). Therefore, it is important to identify how important increased substrate depths are for plant survival on unirrigated green roofs in hot and dry climates, especially during summer months.

Plants that have greater water availability through internal water storage or 'succulence' should also have greater survival on green roofs. Succulence describes the 'storage of utilisable water in living tissues in one or several plant parts in such a way as to allow the plant to be temporarily independent from external water supply but to retain at least some physiological activity' (Eggli & Nyffeler, 2009). While succulence as a water storage trait occurs in all 'true succulents' like *Sedum* and *Crassula* species, other plants can also have succulence without being described as succulents. Succulent plants are a life-form group and are defined as 'having thick and juicy leaves, covered with a close membrane, through which the moisture cannot easily transpire, which makes them continue in dry places' (Eggli and Nyffeler, 2009).

The survival of true succulents, such as *Crassula* or *Sedum* species, on green roofs has been widely studied and their high survival has meant that these species are widely planted on green roofs worldwide (Thuring et al., 2010; Rowe et al., 2012). By contrast, the role of succulence as a plant trait in improving survival of other plant types, such as herbs and shrubs, has not been well explored. This may be because there has been no clear definition of 'succulence' as a trait until recently (Eggli and Nyffeler, 2009) and the focus for plant survival on green roofs has historically been on true succulents such as *Sedum* species (Shafique et al., 2018; Eksi and Rowe, 2019). As a trait, succulence is relatively easy to measure (Eggli and Nyffeler, 2009) and if it relates well with survival, it could be used to improve green roof plant selection. Expanding plant selection beyond true succulents is an important way of improving green roof plant diversity and functions such as stormwater retention and cooling (Farrell et al., 2013; Szota et al., 2017). There is evidence in a few studies that in both succulents and other life-forms not normally considered succulents, such as forbs and monocots, plants with greater *leaf* succulence (degree of leaf succulence; *Fresh leaf water content/Leaf area*, g cm^{-2}, Delf, 1912) have greater survival on green roofs in hot and dry climates (Farrell et al., 2013; Razzaghmanesh et al., 2014b; Rayner et al., 2016). However, there are also differences in survival among succulent plants with similar degrees of leaf succulence (Rayner et al., 2016). Therefore, it is important to investigate if herbs and shrubs with greater succulence have greater survival on green roofs; and whether succulence can be used as a trait to inform species selection for constructing more diverse and effective green roofs.

In addition to succulence, survival on green roofs will likely be linked with other plant traits or trait combinations that determine where a species is positioned on the plant economic spectrum (Males, 2017). For example, plant traits measured under well-watered conditions that are associated with resource acquisition include large specific leaf area (SLA), high leaf vein length per leaf area, high specific root length and fast growth rates are often related with low survival under water-deficit conditions (Reich, 2014). Conversely, plants with traits associated with resource conservation under well-watered conditions, such as high succulence, high wood density, small SLA, or small specific root length can have greater survival under water deficit (Eggli and Nyffeler, 2009; Reich, 2014). These traits or trait combinations may also reflect differences in survival across life-forms, although previous studies on green roofs have shown no difference in survival between shrubs and herbs (MacIvor and Lundholm, 2011; Nardini et al., 2012). Physiological traits such as evapotranspiration rates and leaf tolerance of high temperatures are also likely to influence plant survival. While evapotranspiration and heat tolerance have been assessed on woody plants and plants from dryland ecosystems in physiological studies (Gimeno et al., 2009), it is unknown whether plants with lower evapotranspiration or greater heat tolerance have greater survival on green roofs.

The key aim of our study was to investigate whether succulence or trait combinations are related to plant survival in different substrate depths on hot and dry green roofs. Specifically, we investigated; 1) if plant survival is improved in deeper substrates; 2) if plants with greater succulence have greater survival; and, 3) if survival is related to other traits beyond succulence, or trait combinations.

2. Materials and methods

2.1. Plant selection and experimental approach

In order to maximise green roof biodiversity and broaden plant selection beyond true succulents, we selected a mix of evergreen herbs, sub-shrubs and shrubs for our green roof module experiment. Despite its classification as a temperate climate, Melbourne's summer can be very hot and dry, for example, Rayner et al. (2016) reported a mean maximum air temperature of 35 °C and total rainfall of 80.6 mm during a challenging summer for green roof plants. We therefore selected 11 species based primarily on their use as green roof plants in Mediterranean-type climates (Supplementary Table 1). We then narrowed down our selection to include a broad range of life-forms and a range of leaf succulence.

The eleven species included five herbs (*Ajuga reptans* L., *Armeria maritima* (Mill.) Willd., *Campanula poscharskyana* Degen, *Campanula portenschlagiana* Roem. & Schult., and *Origanum vulgare* L.), three sub-shrubs (*Convolvulus sabatius* Viv., *Lavandula angustifolia* Mill., *Senecio cineraria* DC.) and three shrubs (*Cistus salvifolius* L., *Rosmarinus officinalis* Spenn., *Teucrium fruticans* L.). Plants were purchased as standard sized tube stock (plugs) from three commercial nurseries (Larkman Nurseries Pty Ltd., Mansfield Propagation Nursery, and Renaissance Herbs).

This study consisted of two experiments with the same 11 species. The first was a glasshouse experiment to determine plant water use, succulence and other traits under well-watered conditions, and the second experiment determined plant survival in green roof modules with different substrate depths. We measured traits under identical well-watered conditions to avoid environmental impacts on trait expression and compare all plants on a level playing field, whereas survival had to be tested under stressed conditions to encompass all the mechanisms plants utilise to enable or prolong survival. There are also known linkages between well-watered condition traits and plant survival under stress in green roof studies (Lundholm et al., 2014; Van Mechelen et al., 2014; Du et al., 2019). Further, plants with traits associated with fast resource acquisition under well-watered conditions often have low survival under water-deficit conditions (Reich, 2014). Therefore, we investigated the relationship between plant survival in a green roof module experiment with the plant traits measured in the glasshouse experiment. Both experiments were undertaken at the Burnley Campus of the University of Melbourne, Australia (latitude 37°47'S; longitude 144°58'E).

2.2. Glasshouse experiment to determine plant water use, succulence and other morphological and physiological traits under well-watered conditions

A pot-based glasshouse experiment with a randomised block design was established in 6 June 2018 (winter). Five replicate plants of each species were planted into pots (14 cm diameter, 15 cm deep; one individual per pot) filled with 1400 g of a scoria-based green roof substrate (60 % aerolite black scoria 8 mm minus; 20 % 7 mm red scoria aggregate including fines and 20 % composted coir; air filled porosity = 13.8 %, bulk density at pot capacity =1.26 g cm^{-3}, water holding capacity = 45.9 %, and electrical conductivity =0.14 dS m^{-1}; Farrell et al., 2012). After planting, 0.77 g of slow-release fertiliser (Langley Macracote Coloniser Grey 8−9 M, NPK: 8−1−8) was applied to the surface of each pot. Glasshouse whole day temperatures ranged from 17 to 29 °C (average daily temperature 21.8 ± 0.1 °C) and the average daily humidity was 30.8 ± 0.8 %. All plants were watered twice a week to pot capacity to ensure well-watered conditions throughout the experiment.

2.2.1. Plant water use

Pots were weighed before and after each watering event to determine the *daily evapotranspiration* (*ET*) = (*weight after watering – weight before next watering event*) / *number of days between the two watering events* (g) from 7 July to 14 Sep. 2018. Evapotranspiration was also expressed on a shoot biomass basis (*daily ET/shoot dry weight*; ET.S).

2.2.2. Leaf lethal temperatures (T_{50})

The leaf lethal temperature (T_{50}) was measured on leaves of well-watered plants 30 days after potting up according to the protocol of Curtis et al. (2014). Leaves from each well-watered plant were collected at predawn (six hardboard leaf samples each individual plant, leaf number collected determined by leaf size), after which these samples were placed into 50 mL centrifuge tubes with deionised water. Considering the requirement of a linear relationship model, we chose to have five treatments, which were 45 °C, 47.5 °C, 50 °C, 52.5 °C and 55 °C, and a control (23 °C). These reflected the range of temperatures evaluated in Curtis et al. (2014). Leaves of each species were cut into the size that could fit into the fluorescence head and then stuck onto six hardboards with tape for each of the six temperature treatments. The leaves were then dark adapted for approximately 30 min at room temperature (23 °C) to prepare for the Fv/Fm measurement (Hansatech Plant Efficiency Analyser with fluorescence head). The hardboard strips, with leaves facing up, were then placed on a moistened paper towel in individual clear zip-lock bags and all bags were immersed at the control temperature (23 °C) for 15 min before they were placed into treatment temperatures for 15 min. Bags were then returned into the control water bath for another 90 min. After each water bath immersion, the leaves were dark-adapted for 30 min before measuring Fv/Fm. The bags were then placed in the dark overnight for extended recovery and Fv/Fm was measured the next day. The temperature which resulted in 50 % decline in Fv/Fm was determined from a linear relationship fitted between Fv/Fm and temperature.

2.2.3. Biomass and morphological traits

At the end of the experiment (100 days after planting, 14 Sep. 2018; spring), all plants were harvested to determine leaf area and fresh and dry weight of roots, stems, and leaves. Dry weights were measured after drying samples in an 80 °C oven for one week until reaching a constant weight. Total biomass and root:shoot ratio were determined from dry weights. Leaf areas were measured using a LI3100 area meter (Licor, Lincoln, NE, USA); specific leaf area (SLA) was calculated as *leaf area / dry weight of leaves* ($cm^2\ g^{-1}$); and leaf area ratio (LAR) was calculated as *total leaf area / total plant dry mass* ($cm^2\ g^{-1}$). Three expressions of succulence, including plant water content (PWC), leaf water content (LWC) and degree of leaf succulence (Dsucc) were determined. PWC and LWC were determined as (*fresh weight of total plants or leaves – dry weight of total plants or leaves*) / *dry weight of total plants or leaves* ($g\ g^{-1}$). Dsucc was calculated as (*leaf fresh weight – leaf dry weight*) / *leaf area* ($g\ cm^{-2}$) (Delf, 1912).

2.3. Green roof module experiment to determine survival in different substrate depths

The second experiment ran from June 2018 (winter) until May 2019 (autumn) (335 days in total). Climate data during the study period (16/6/2018 to 17/5/2019) were collected from Bureau of Meteorology Melbourne Olympic Park observation station, located 3 km west of the experimental site (Fig. 1). The 2018−19 summer was very hot and dry, and January 2019 was the hottest month on record for Australia to date of publication (Bureau of Meteorology, 2019).

There were four substrate depth treatments (10, 15, 20, and 25 cm) with five replicate modules planted with the above-mentioned 11 species. The experiment was a randomised block design with 20 modules. Each module was a 222 L black plastic crate with 15 evenly distributed holes (2 cm diameter) for drainage in the base and a geotextile filter fabric layer to prevent substrate loss. The trays were 116 cm long, 75 cm wide and 25.5 cm high and were placed on top of a black plastic grid pallet (20 cm height) to facilitate drainage, on a concrete pad outside in full sun. One of each of the 11 species was planted in each module randomly at fixed distances (about 25 cm), calculated based on the number of plants and module size. This distance reflected similar green roof module studies (Du et al., 2019). Plants were arranged apart in a quincunx pattern to ensure equal distances between adjacent plants and

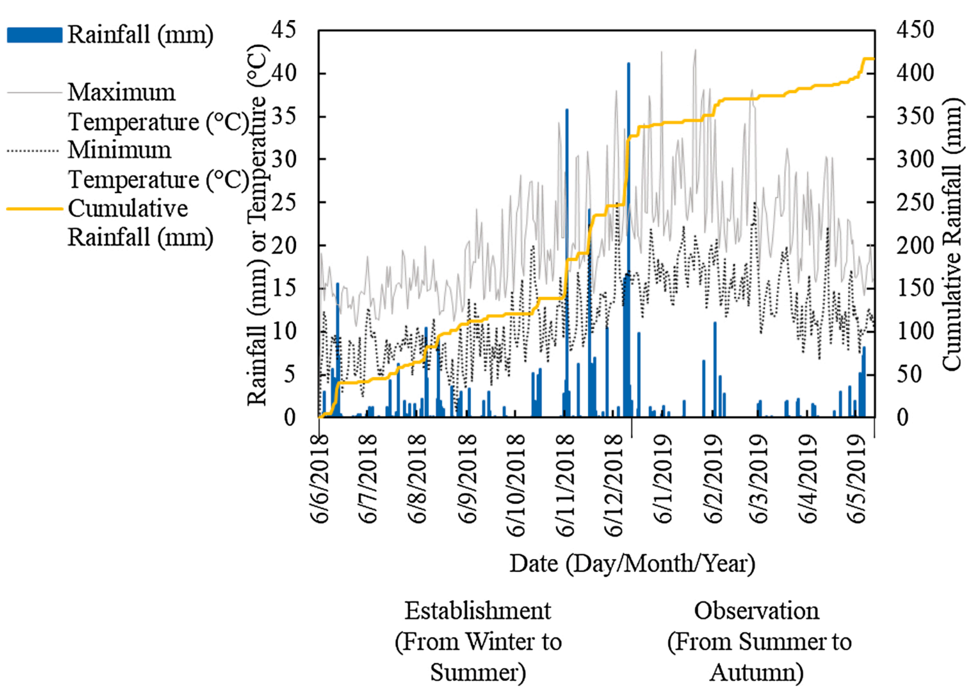

Fig. 1. Maximum and minimum temperature, daily rainfall and cumulative rainfall from 6/6/2018 – 17/5/2019 (Bureau of Metrology Melbourne Olympic Park observation station). Left y axis represents rainfall (mm), maximum and minimum temperatures (°C). Second y axis (right) represents cumulative rainfall (mm). Plant establishment was from 6/6/2018 to 17/12/2018 and experimental observations were from 17/12/2018 to 17/5/2019.

minimize plant competition. The substrates in the modules were same as used in the glasshouse experiment. Each module was fertilised with 43.5 g slow-release fertiliser (same as the pot experiment) on the substrate surface one month after planting. Modules were watered fortnightly in addition to rainfall for the first month after establishment, and after that, only received natural precipitation. Plant survival was initially recorded on 17/12/2018 (summer) and then was recorded weekly from 23/1/2019 (summer) to 10/4/2019 (autumn). Final survival was recorded on 17/5/2019 (autumn). Plants were considered to have died when all leaves and stems were dry and crispy.

2.4. Statistical analyses

Climate Wetness Index (CWI, *rainfall/evaporation*) from 6 Jun 2018 to 17 May 2019 was calculated based on the climate data collected from Bureau of Metrology Melbourne Olympic Park observation station. CWI can indicate the wetness of climate and when it is below 0.5, the environment is drier than a semi-arid climate (White et al., 2009). In order to answer the question of whether plant survival can be improved in deeper substrate depths, survival rates on each observation day were calculated; the number of days plants experienced a CWI < 0.5 from establishment to median survival was analysed; and survival analyses including Kaplan – Meier survival analysis, Log-rank tests and Cox proportional regressions were carried out using R (R studio team, 2016). Survival time used in the Kaplan - Meier survival analysis was from the module establishment date to the day on which a plant died or the observations ended (17/5/2019). The dataset of species name, module substrate depths, module number, survival time and whether the plant was dead on each specific date (died - 1; missing or observation stop - 0) were imported into R and analysed using survival and survminer packages. Log-rank tests and Cox proportional regressions were then conducted using the model built from Kaplan-Meier survival analysis to compare species survival over the whole experiment. To investigate the second and third questions of whether survival is associated with succulence, other traits, or combinations of traits, Pearson correlation was conducted between these traits and survival in Genstat 18.2 (VSN International, 2018). The survival parameter used for correlations was based on the survival rates on each observation day and the ranking method of Spearman (Spearman, 1904). The higher the mortality score for a species, the worse its overall survival in each depth. To determine the trait combinations, principal component analysis (PCA) was conducted in R to determine the variation of multiple traits among different species and the 11 species were then grouped through cluster analysis (hierarchical and k-means) based on the PCA results. The PCA included the traits T_{50}, Dsucc, ET.S, LAR, SLA, root:shoot ratio, LWC, and PWC for well-watered plants. The optimal number of clusters was determined by comparing the result of hierarchical and k-means cluster analyses as well as the 'elbow' method (Thorndike, 1953). Mean survival rates of each cluster on each observation day were compared among the four substrate depths (10, 15, 20 and 25 cm). Differences in water use, succulence and other morphological traits among species were analysed using one-way ANOVA (in randomised blocks; F-test) after using W-Tests to check normality and visually checking homogeneity of variance. Multiple comparisons were performed using Tukey's post hoc test ($P < 0.05$) to determine significant differences among means in Genstat. No data transformations were required.

3. Results

3.1. Climatic conditions during the green roof module experiment

The summer of 2018–2019 was the hottest summer on record for most places in Australia, including Melbourne, and January 2019 was the hottest month on Australian record to the date of publication, with three days maximum temperature over 40 °C in Melbourne (Bureau of Meteorology, 2019, 2020). From January to April, rainfall was very low (Fig. 1), with a mean monthly precipitation of 0.4 mm for these four months, and evaporation exceeded rainfall for most of this time, with 141 days out of 151 days (from 17/12/2018 to 17/2/2019) where CWI < 1, and 133 days where CWI < 0.5 (Fig. 2A).

3.2. Plant survival and substrate depth

Plant survival differed significantly among both substrate depths and species. When all 11 species were considered together in the survival analysis, plant survival increased significantly with each increase in substrate depths ($P < 0.001$; Fig. 2B). Generally, survival of individual species also increased in deeper substrates, although the magnitude of differences among depths varied with species (Supplementary Table 2). At the last observation day in autumn, all plants in 10 cm deep substrates were dead and only two species survived in 15 cm (*C. sabatius*; sub-shrub and *T. fruticans*; shrub) and 20 cm deep substrates (*C. sabatius*; sub-shrub and *C. salvifolius*; shrub) (Supplementary Table 2). In the deepest substrate depth (25 cm) woody plants (shrubs and sub-shrubs) had greater survival than herbaceous plants, with only one herb species (*A. maritima*) alive on the last observation day. Likewise, when we analysed survival by comparing the number of days plants experienced a CWI < 0.5 between establishment and median survival, generally plants in deeper substrate depths took much longer until median survival, which indicated greater survival (Fig. 3). Woody plants generally took longer until median survival compared to herbaceous plants (Fig. 3). Interestingly, not all plants that took longer until median survival were the plants with greater survival rates on a specific observation day (Fig. 3; Supplementary Table 2). For example, in 25 cm deep substrates, *A. maritima* experienced longer CWI < 0.5 days than *L. angustifolia*, but their survival rates on 17/5/2019 were the same.

When we compared mortality scores of different species in each

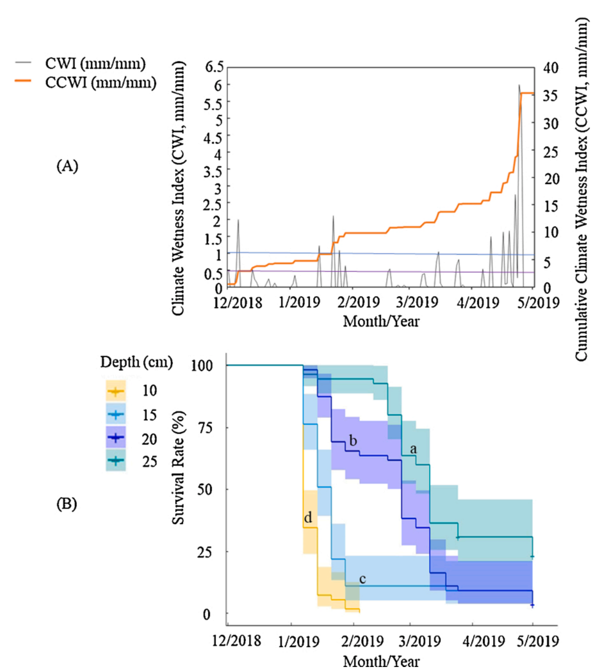

Fig. 2. (A) Climate Wetness Index (CWI) and cumulative CWI during 17/12/2018 – 17/5/2019, calculated as *rainfall/evapotranspiration* based on Fig. 1. Y axis (left) represents CWI and y axis (right) represents cumulative CWI. Above blue line, CWI > 1, means the evaporation is less than rainfall, while below the purple line (CWI < 0.5) represents when rainfall is less than half the evaporation. (B) Survival curve for all species in different substrate depths of 10, 15, 20 and 25 cm. Survival possibilities are on y axis. The ribbon around the survival curves indicates their confidence intervals. The alphabetic symbols represent the Log-rank test results, indicating significant differences in survival curves for each depth.

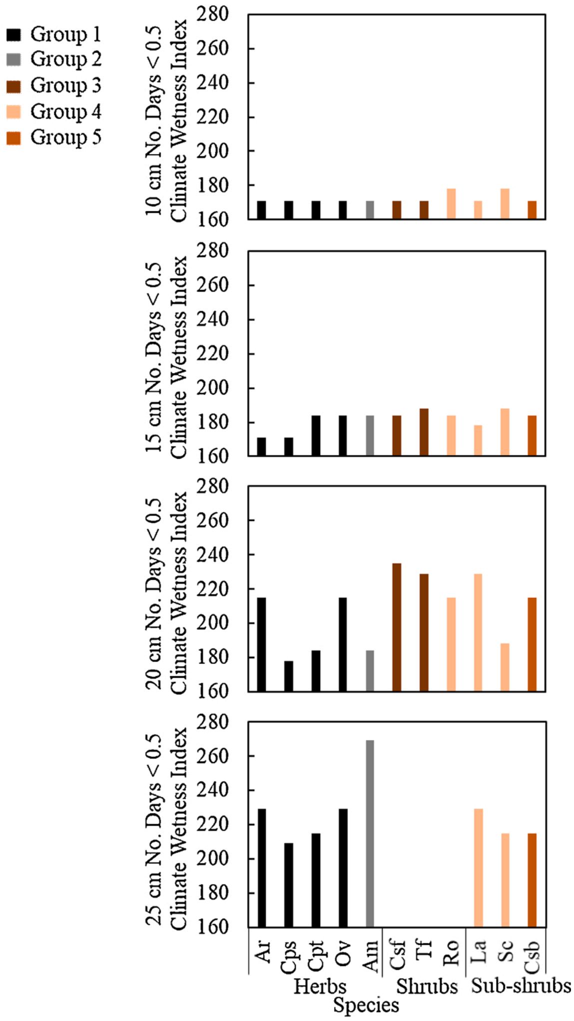

Fig. 3. Number of days plants experienced a CWI < 0.5 from establishment to median survival in four substrate depths (10, 15, 20 and 25 cm). Am = *Armeria maritima*, Ar = *Ajuga reptans*, Cps = *Campanula poscharskyana*, Cpt = *Campanula portenschlagiana*, Csb = *Convolvulus sabatius*, Csf = *Cistus salvifolius*, La = *Lavandula angustifolia*, Ov = *Origanum vulgare*, Ro = *Rosmarinus officinalis*, Sc = *Senecio cineraria*, Tf = *Teucrium fruticans*. Species sharing the same colour are in the same group determined using PCA and cluster analysis (Fig. 6); Group 1 = black, group 2 = grey, group 3 = brown, group 4 = yellow, group 5 = orange. Csf, Tf and Ro in 25 cm had not reached median survival at the final observation day, so they are omitted from this graph.

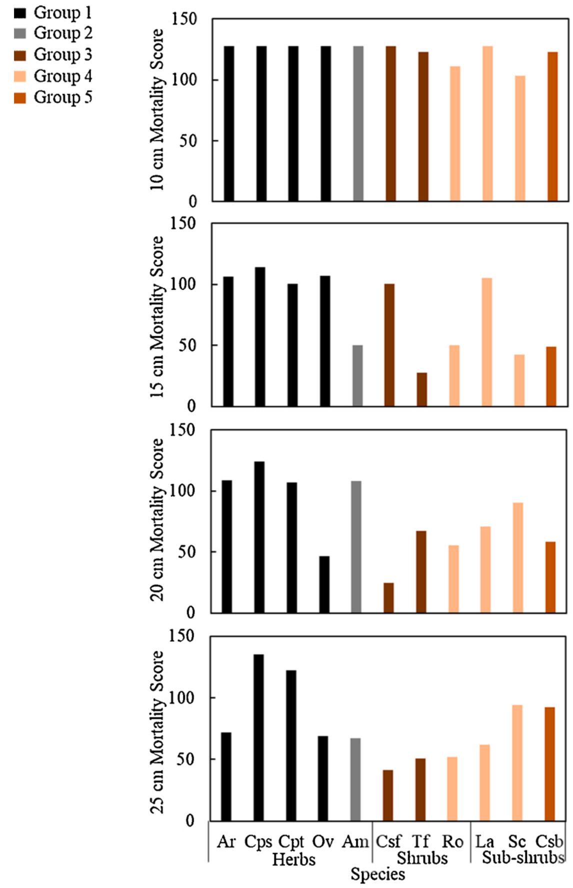

Fig. 4. Total mortality scores by species within each substrate depth. Mortality scores were determined using the Spearman method within each depth and cannot be compared across depths. Note that higher mortality scores indicate worse survival. Species and group codes are the same as in Fig. 3.

substrate depth and ranked them from the greatest mortality score (worst survival) to the lowest mortality score (greatest survival), the ranking of species was not consistent across substrate depths, even for species of the same life-form (Fig. 4). While *C. salvifolius* and *T. fruticans* (both shrubs) had higher survival than other species in all substrate depths, *S. cineraria* (sub-shrub) was among the best surviving species in 10 and 15 cm, but among the worst surviving species in 20 and 25 cm deep substrates (Fig. 4). Conversely, *A. maritima* (herb) had the lowest survival in 10 and 20 cm deep substrates, but its survival performance ranked middle in 15 and 25 cm deep substrates. *C. poscharskyana* (herb) had the worst survival in all substrate depths (Fig. 4).

3.3. *Traits variation, trait combinations and functional groups*

The species with the highest mortality score in all substrate depths (*C. poscharskyana*) had greater T_{50}, leaf water content, total plant water content and root:shoot ratio than many other species (Fig. 5). By contrast, the two shrubs with the highest survival (*C. salvifolius* and *T. fruticans*) had lower water use, T_{50}, root:shoot ratio and larger succulence (degree of leaf succulence, leaf water content and total plant water content) than many other species (Fig. 5). Trait combinations were characterised using PCA and the first three PCA axes together explained 86.7 % of the total variation. The first PCA axis (PC1, Fig. 6) explained 44.6 % of the total variance and represented morphological traits including SLA (loading vector 0.48), LAR (0.46) and root:shoot ratio (0.39). Axis two (PC2) explained 24.3 % of the variance and represented leaf succulence traits (LWC, -0.56 and Dsucc, -0.50). The remaining axis (PC3) accounted for 17.8 % of the total variance and represented ET.S (-0.57), PWC (0.40) and T_{50} (0.50), although these traits were also partly explained by PC1 and PC2. The PCA and cluster analysis showed that herbs, sub-shrub and shrubs clustered into five different functional groups; Group 1, a group of five herbs (Group 1–5 Herbs); Group 2, a group with *A. maritima* (Group 2 - Am); Group 3, a group consists of two shrubs (Group 3−2 Shrubs); Group 4, a group with one shrub and two sub-shrubs (Group 4 - Shrub and Subshrubs); and Group 5, a group with *C. sabatius* (Group 5 - Csb) (Fig. 6). Despite significant differences in individual traits among species, including within species of the same life-form (Fig. 5), the five functional groups largely had life-form uniformity, except for Group 4 - Shrubs and Subshrubs, which contained two sub-shrubs (*L. angustifolia* and *S. cineraria*) and one shrub (*R. officinalis*).

3.4. *Relationships between succulence, other traits and trait combinations and survival in different substrate depths*

While there were differences in traits and survival among the 11

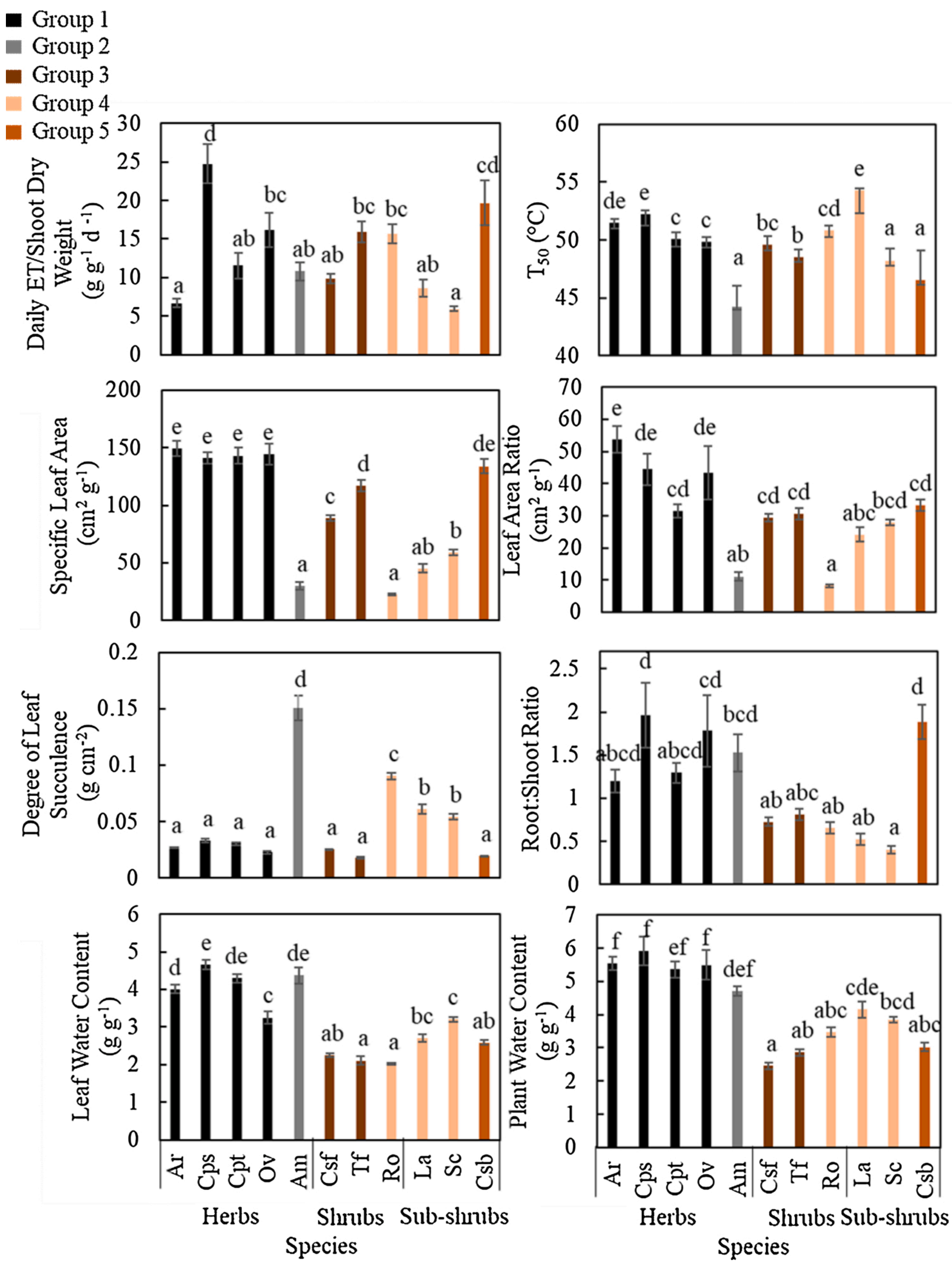

Fig. 5. Trait variation among species. Species and group codes are the same as in Fig. 3. Error bars represent the standard error in all graphs except T_{50}. Error bars in T_{50} graph represent confidence intervals. Species sharing common letters have no significant difference in that trait.

species, strong relationships between individual traits and survival were only observed for three traits (Table 1). Across all species, greater succulence (leaf and plant water content) was associated with greater mortality in 15, 20, 25 cm deep substrates (i.e. succulence was positively correlated with mortality scores) and greater leaf thermal tolerance (T_{50}) was associated with higher mortality in 15 cm deep substrates. The combination of greater SLA, LAR and root:shoot ratio represented by PC1 was associated with greater mortality in 25 cm deep substrates. There were no other significant relationships between traits and survival.

Species clustered in the same functional group had similar survival in each depth except for two herb species (*A. reptans* and *C. poscharskyana*) in 25 cm deep substrates (Supplementary Table 2). In 10 cm deep substrates, survival was greater for Group 4 - Shrub and Subshrubs, which included two sub-shrubs (*L. angustifolia*, and *S. cineraria*) and one shrub (*R. officinalis*), than the other groups (Fig. 7; Supplementary Table 2). In 15 cm deep substrates, survival was similar among the five functional groups except Group 1 - 5 Herbs (including *A. reptans*, *C. poscharskyana*, *C. portenschlagiana* and *O. vulgare*; herbs), who had the worst survival, whereas in 20 cm deep substrates, the best and worst performers in terms of survival changed over time (Fig. 7; Supplementary Table 2). Group 1 - 5 Herbs had the lowest average survival rates during January to February 2019 and Group 2 - Am (*A. maritima*; herbs) had the lowest survival rates during February to May 2019 in 20 cm deep substrates (Fig. 7). Meanwhile, Group 3 - 2 Shrubs (*C. salvifolius* and *T. fruticans*; shrubs) and Group 5 - Csb (*C. sabatius*; sub-shrubs) had the greatest mean survival rates in 20 cm deep substrates (Fig. 7). Likewise, the groups with worst survival in 25 cm deep substrates changed from Group 1 - 5 Herbs to Group 5 - Csb in February 2019, but Group 3 - 2 Shrubs had the greatest mean survival rates in 25 cm deep substrates throughout the whole regular observation phase (Fig. 7). Overall, groups with woody plants generally had greater mean survival rates than

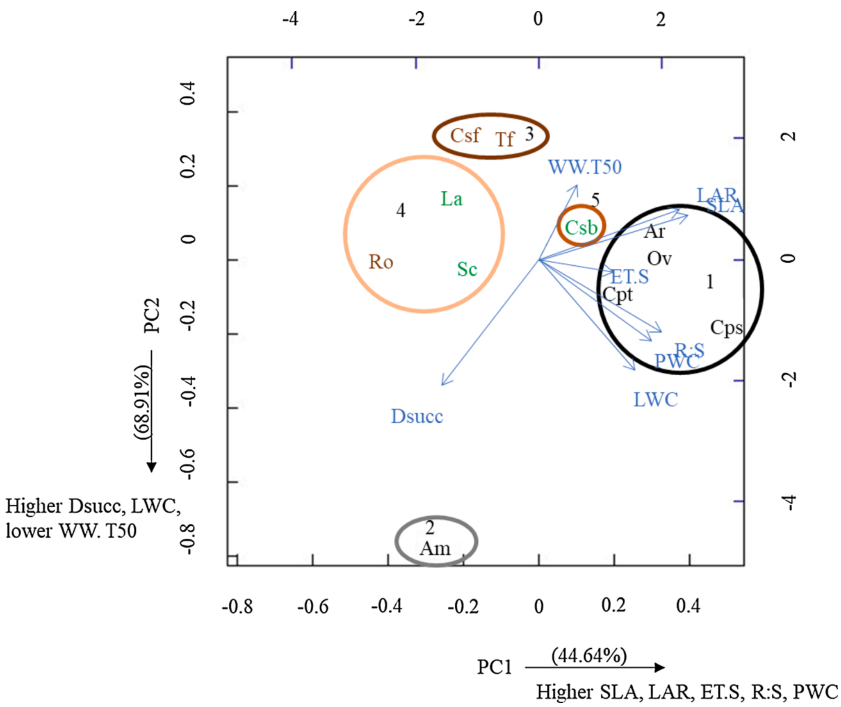

Fig. 6. Principal component analysis (PCA) of plant traits showing five functional groups determined using cluster analysis. Species groups (within circles) are numbered, and the colour of species abbreviations indicates different life-forms; black = herbs, green = sub-shrubs, brown = shrubs. Percent variation explained by each axis is shown. Traits used: WW. T50 = well-watered plants T_{50}, Dsucc = degree of leaf succulence, ET. S = ET/shoot dry weight, LAR = leaf area ratio, SLA = specific leaf area, R:S = root:shoot ratio, LWC = leaf water content, PWC = plant water content. Species codes are the same as in Fig. 3.

Table 1
Pearson correlation between mortality scores in four substrate depths (10, 15, 20, and 25 cm) and individual plant traits and trait combinations represented by the first 3 axes from the PCA.

Substrate Depths	Specific Leaf Area	Leaf Area Ratio	Root:Shoot Ratio	Daily ET/Shoot Dry Weight	Degree of Leaf Succulence	Leaf Water Content	Plant Water Content	T_{50}	PC1	PC2	PC3
10 cm	0.468	0.386	0.532	0.201	−0.173	0.355	0.347	0.147	0.513	−0.110	−0.008
15 cm	0.429	0.565	0.271	0.005	−0.322	0.437	0.606*	0.663*	0.591	0.070	0.505
20 cm	0.162	0.216	0.292	0.016	0.247	0.856**	0.708*	0.022	0.392	−0.590	0.383
25 cm	0.467	0.372	0.521	0.381	−0.197	0.715*	0.609*	0.089	0.639*	−0.318	0.056

** represents $P < 0.001$.
* represents $P < 0.05$. Note that higher mortality scores indicate worse survival.

herbaceous plants, but exceptions existed, and group rankings did change over time.

4. Discussion

4.1. *Plant survival is improved in deeper substrates*

The survival of the 11 Mediterranean plants was greater in deeper substrate depths, which is consistent with most green roof studies (Dunnett et al., 2008; Kazemi and Mohorko, 2017). The results of our experiment in terms of final survival rates after an extremely hot and dry summer suggests that irrigation over summer will be essential in extreme conditions to promote growth, maintain plant cover and diversity of these Mediterranean green roof species. Nevertheless, while greater substrate depths improved plant survival overall, the survival ranking of the 11 species differed among depths. This meant that not all species with greater survival in shallower substrates had greater survival in deeper substrates. This has also been demonstrated for non-succulent life-forms in green roof studies in other climates, including oceanic (Dunnett et al., 2008) and humid continental (Zhang et al., 2014) climates. The fact that some species surviving better in shallower substrates did not have greater survival in deeper substrates may be because roots in shallow substrates can access water in retention layers beneath substrates (Savi et al., 2015). The external utilisable water for these plants in shallower substrates can be the same or greater than in deeper substrates due to their root lengths. When we ranked the survival of the 11 species as well as the 5 functional groups in each substrate depth at different time points (from low to high), the survival ranking changed over time. This meant that not all species or groups with greater survival at one time point had greater survival than other species or groups at other time points. This indicates that analysing survival rate at one point in time and using this to make conclusions of the best survivor can be misleading.

Our result that woody life-forms generally had greater survival than herbs is in contrast with the results reported by MacIvor and Lundholm (2011), who showed no difference in survival among life-forms on an extensive green roof. However, MacIvor and Lundholm (2011) evaluated green roof survival in a maritime climate where species did not suffer extreme drought and they used 6 cm deep substrates. Generally, shrubs are thought to be more vulnerable to drought stress on green roofs due to their inability to maintain plant water status in shallow substrates, where roots cannot access deep stores of water (Farrell et al., 2013). In contrast, herbs have been reported to survive better on green roofs as they can maintain their water status and avoid drought stress through early stomatal closure (Farrell et al., 2013). The extended hot and dry conditions in our study, however, were likely too extreme for differences in drought resistance to improve plant survival (Farrell et al., 2017). Therefore, the improved survival of some shrubs in our study is

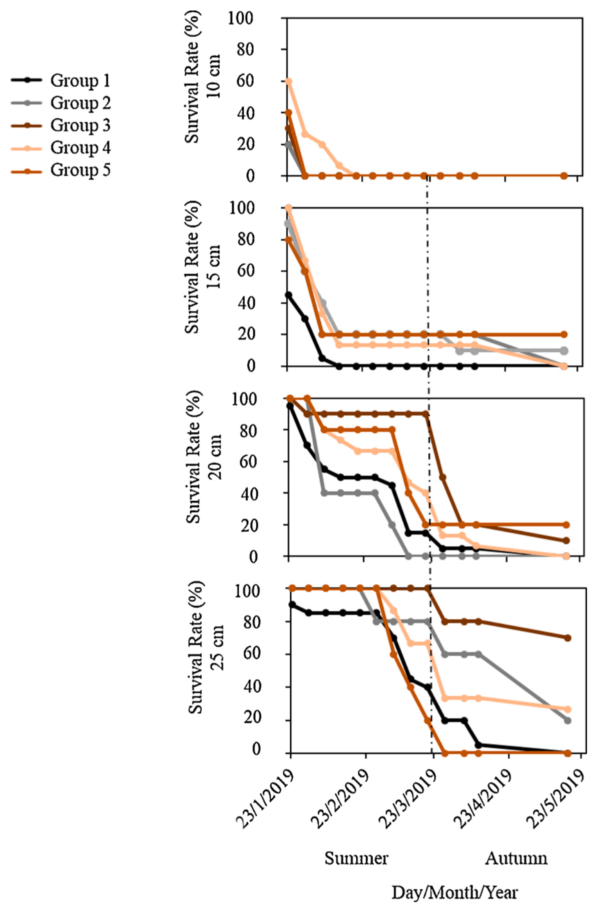

Fig. 7. Mean survival rates of five functional groups during regular observation phase (23/1/2019 – 17/5/2019) in 10, 15, 20 and 25 cm substrate depths. Group codes are the same as in Fig. 3. The dashed line indicates the end of summer and the start of autumn.

not likely due to their physiological drought strategies. It may reflect other characteristics like xeromorphic characteristics such as waxy leaves, thick cuticles and leaf shedding (Aroca, 2012).

4.2. Plants with greater succulence do not have greater survival

Surprisingly, plants with greater succulence (plant water content, leaf water content and degree of leaf succulence) did not have greater survival in our study. This may because the plant types we evaluated in our experiments were shrubs and herbs, unlike other studies which have shown that a greater degree of leaf succulence was related to greater survival for plants with true succulence (Farrell et al., 2013; Razzaghmanesh et al., 2014b; Rayner et al., 2016). Further, while other studies have tended to show a positive relationship between degree of leaf succulence and survival, survival of plants with the same degree of succulence can vary (Farrell et al., 2013; Razzaghmanesh et al., 2014a, b) and suggests that plants use stored water differently during periods of water limitation. For example, species with greater succulence can have lower survival during drought if they also have greater water use (Farrell et al., 2012). It is important to note, however, that while succulence is a trait that characterises stored water in different plant parts, this stored water may not be available for plants to use to maintain physiological function under drought stress (Von Willert et al., 1992; Eggli and Nyffeler, 2009). The water stored in plants can be free water, loosely bound water or tightly bound water and tightly bound water is not available for physiological function, although this can change under stress (Rascio et al., 1992; Lamont and Lamont, 2000). Thus, differences in internal water availability and water use strategies may explain why not all plants with greater succulence had greater survival in our study and this deserves further investigation.

4.3. Survival was not related to other individual traits or combinations of traits, but functional groups had similar survival

Survival was also not related to any of the other morphological or physiological traits measured in our experiment including water use, specific leaf area, leaf area ratio, root:shoot ratio and T_{50}. Theoretically, plants with smaller specific leaf area and leaf area ratio have greater survival under drought stress, and this has been shown in a few forest studies (Lusk and Pozo, 2002; Poorter and Bongers, 2006). In contrast, Sterck et al. (2006) did not observe significant relationships between specific leaf area and survival in rainforest trees and suggested that plants with smaller specific leaf area had longer leaf life span and therefore had greater survival rates. Therefore, although smaller specific leaf area and leaf area ratio can theoretically indicate greater survival, the relationships can be inconsistent depending on their relationships with other plant traits. In terms of root:shoot ratio, Padilla and Pugnaire (2007) reported similar results to ours in that root:shoot ratio was not related with survival, however they found that maximum rooting depth was positively related to survival in five transplanted Mediterranean woody species. Lopez-Iglesias et al. (2014) also showed that survival of Mediterranean woody species in a pot-based drought experiment was positively related to rooting depth per leaf area. This may mean that actual rooting depth better reflects the ability to acquire water during drought stress. However, in green roofs, the depth of green roof substrate is limited due to weight loading constraints, which may mean that roots are restricted and species rooting depth in natural environments may not reflect survival under green roof conditions.

Plants with greater rates of water use (ET under non-limiting conditions) did not die faster than plants with more conservative water use. Our finding is consistent with Du et al. (2019) who also did not observe a relationship between survival and water use for shrubs from different climates grown under green roof conditions. They suggested that the reason for the lack of a relationship was that the regulation of water use also affects mortality in addition to the rates of water use (Du et al., 2019).

Likewise, plants with greater leaf thermal tolerance also did not have improved survival in our study. The greatest daily maximum air temperature was 42.8 °C during our study; in extremely hot and dry places like high deserts, air temperatures of 40 °C can lead to leaf temperatures over 50 °C (Blonder and Michaletz, 2018). Green roof substrates can also have much greater temperatures than ambient air due to their low albedo. For example, Rayner et al. (2016) reported substrate temperatures up to 65 °C (when air temperature was about 35 °C) on a green roof in Melbourne. We observed inconsistent survival among species with similar thermal tolerance. For example, among the species with the greatest leaf thermal tolerance, *C. poscharskyana* (T_{50} = 52.2 °C) was the worst survivor and *L. angustifolia* (T_{50} = 54.3 °C) was among the best survivors. Further, two of the best survivors (*A. maritima* and *C. sabatius*) had the lowest thermal tolerance with T_{50} approximately 45 °C. One reason for the lack of a relationship between T_{50} and survival in our study could be that factors including albedo, leaf hairs, spines, wax layers, leaf orientation and architecture can improve plant drought and thermal tolerance, but these traits are not reflected in the measure of T_{50} (Willey, 2015; Kumar et al., 2019). Other reasons may be that root vulnerability to heat stress may be more critical for plant survival than leaf thermotolerance, or that heat stress did not influence survival in these plants and that plant mortality was more related to water deficit (Huang and Xu, 2000).

While survival was not related to trait combinations, species within functional groups had similar survival. When multiple traits were considered together, the 11 species separated out on the basis of three trait combinations which were made up of both fast traits (traits related to resource acquisition; specific leaf area, leaf area ratio, daily ET/shoot dry weight, and root:shoot ratio) and slow traits (traits associated with resource conservation; degree of leaf succulence, leaf water content and T_{50}). For example, species with a high specific leaf area (149.5 $cm^2\ g^{-1}$), and leaf area ratio (53.8 $cm^2\ g^{-1}$) had low water use (6.7 $g\ g^{-1}\ d^{-1}$) and degree of leaf succulence (0.03 g cm^{-2}) and greater allocation to roots (root:shoot ratio 1.2). Therefore, it is likely that the combination of contrasting fast and slow traits in each functional group resulted in there being no significant relationships between trait combinations and survival in our study (Reich, 2014). However, our results showed that species in the same functional group had similar survival, and that species in the same functional group had similar trait combinations. Therefore, plants with similar trait combinations can have similar survival, but what the best combination of traits is to predict survival needs further investigation.

5. Conclusion

Our research showed that after an extremely hot and dry summer, survival of plants was greater in deeper substrates and shrubs and subshrubs survived better than herbs. Our study also demonstrated that for the life-forms we evaluated, plants with greater succulence do not have greater survival. Survival was also not related to any other individual trait or trait combination. This suggests that in this study, the stored water was not utilisable for maintaining physiological function and improving survival. It may also be that under these extreme conditions, traits relating to succulence, water use, T_{50} or trait combinations do not relate well with survival on green roofs, as water limitations on green roofs occur swiftly and may mask relationships which are evident in natural ecosystems. Nevertheless, plants in the same functional groups had similar survival, which suggests plants with similar trait combinations can have similar survival on green roofs. Nevertheless, in our study we only assessed eight plant traits associated with succulence, water use and leaf thermal tolerance. While these traits are associated with drought tolerance on green roofs, other traits such as rooting depth, leaf loss and root thermal tolerance should be investigated in further studies as these may relate better with survival on hot and dry green roofs.

CRediT authorship contribution statement

Bihan Guo: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Stefan Arndt:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Rebecca Miller:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Nuonan Lu:** Conceptualization, Investigation. **Claire Farrell:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ufug.2021.127248.

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