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Drought versus heat: what's the major constraint on Mediterranean green roof plants?

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ABSTRACT

Green roofs are gaining momentum in the arid and semi-arid regions due to their multiple benefits as compared with conventional roofs. One of the most critical steps in green roof installation is the selection of drought and heat tolerant species that can thrive under extreme microclimate conditions. We monitored the water status, growth and survival of 11 drought-adapted shrub species grown on shallow green roof modules (10 and 13 cm deep substrate) and analyzed traits enabling plants to cope with drought (symplastic and apoplastic resistance) and heat stress (root membrane stability). The physiological traits conferring efficiency/safety to the water transport system under severe drought influenced plant water status and represent good predictors of both plant water use and growth rates over green roofs. Moreover, our data suggest that high substrate temperature represents a stress factor affecting plant survival to a larger extent than drought *per se*. In fact, the major cause influencing seedling survival on shallow substrates was the species-specific root resistance to heat, a single and easy measurable trait that should be integrated into the methodological framework for screening and selection of suitable shrub species for roof greening in the Mediterranean.

Keywords: drought resistance, heat resistance, shallow substrate depths, shrub species, water status, mortality

INTRODUCTION

Green roofs are engineered ecosystems representing an effective strategy to address some of the most challenging environmental issues in urban areas (Castleton *et al.*, 2010; Berardi *et al.*, 2014). In particular, green roofs have the potential to mitigate the quantity and quality of storm-water runoff, provide thermal insulation to buildings with related energy savings, extend the roof lifespan, mitigate the ‘urban heat island’, and provide space and habitats for urban biodiversity (Castleton *et al.*, 2010; Madre *et al.*, 2014; Benvenuti and Bacci, 2010; Cao *et al.*, 2014; Vijayaraghavan and Raja, 2014). Extensive green roofs, characterized by shallow substrate, reduced weight and low maintenance costs, represent an innovative, energy-saving solution (Van Mechelen *et al.*, 2014; Price *et al.*, 2011). Over the last decades, the urban areas covered by green roofs has substantially increased in North and Central Europe and in temperate and sub-tropical regions worldwide (Castleton *et al.*, 2010; Madre *et al.*, 2014; Berardi *et al.*, 2014; Thuring and Grant, 2015). More recently, research has focused on the implementation of green roofs in Mediterranean regions, where high temperatures and prolonged drought significantly challenge plant survival in these artificial habitats (Olivieri *et al.*, 2013; Benvenuti and Bacci, 2010; Raimondo *et al.*, 2015; Rayner *et al.*, 2015).

A fundamental question addressed by Mediterranean green roof research is how to increase water retention capacity while keeping the substrate depth at a minimum. In fact, reducing substrate depth to limit installation costs apparently contrasts with the need to maximize the amount of water available to vegetation, and to minimize temperature extremes. In fact, another important aim of recent studies has been the selection of drought tolerant species that can survive the extreme green roof conditions in these hot and arid regions. There is evidence that targeted substrate amendments with hydrogel, peat, and biochar, or modifications to the layering design (substrate particle size, drainage panels etc.), have the potential to enhance the moisture retention properties of green roofs, thus increasing the volume of water available and improving plant water status and survival (Savi *et al.*, 2013; Cao *et al.*, 2014; Savi *et al.*, 2014; Vijayaraghavan and Raja, 2014; Raimondo *et al.*, 2015). Several criteria have been proposed to optimize species' selection for green roofs, but these are mainly based on ecological or morpho-anatomical approaches (Lundholm, 2006; Caneva *et al.*, 2015; Van Mechelen *et al.*, 2014; Rayner *et al.*, 2015). Moreover, most screening studies have been focused on succulents or herbaceous species (Benvenuti and Bacci, 2010; Price *et al.*, 2011; Van Mechelen *et al.*, 2014; Rayner *et al.*, 2015), while studies on shrubs as potential growth forms for green roof vegetation are still limited. Indeed, shrubs are generally characterized by a higher capacity in stomatal control of transpiration than herbaceous plants (Galmés *et al.*, 2007; Farrell *et al.*, 2013) and should be taken into serious consideration when selecting potential species assemblages for Mediterranean green roofs. Moreover, a selection process based on an ecophysiological approach might be more effective, at least when functional traits enabling plants to cope with stress factors, like drought and high temperature, are properly analyzed and quantified.

Plant tolerance to drought stress is commonly quantified in terms of symplastic and apoplastic vulnerability to dehydration. The former is generally correlated to the water potential inducing loss of cell turgor (Ψ_{tlp}, Bartlett *et al.*, 2012). Low Ψ_{tlp} values allow drought-adapted plants to maintain cell turgor, stomatal aperture, and positive carbon gain even under low soil water availability and/or high atmospheric evaporative demand. On the other hand, apoplastic vulnerability to water stress is generally quantified in terms of xylem vulnerability to embolism formation. In fact, intense or prolonged drought can affect the root-to-leaf water transport by causing the breakage of water columns in xylem conduits (Tyree and Sperry, 1989), potentially leading to plant desiccation and death (Nardini *et al.*, 2014b). Xylem hydraulic vulnerability is generally quantified in terms of P50 i.e., the xylem water potential inducing 50% loss of hydraulic conductivity (Choat *et al.*, 2012), with species displaying lower P50 generally performing better under drought stress (Nardini *et al.*, 2013) than species with relatively higher P50 values.

Water availability aside, high temperatures can also pose serious limitations to plant performance on green roofs. Heat stress can alter both membrane stability and enzymatic function and thus affects photosynthesis and respiration, altering carbon gain, growth, and secondary metabolism at the root and shoot levels (Wahid *et al.*, 2007; Huang *et al.*, 2012; Vile *et al.*, 2012). Most importantly, shallow green roof substrates potentially expose root systems to temperature extremes that largely surpass those experienced by plants in natural soils. In fact, the root system is generally more vulnerable to heat stress compared to the shoot (Kuroyanagi and Paulsen, 1988). The co-occurrence of both drought and heat stress over green roofs poses important challenges to plant life, frequently leading to foliage desiccation, plant die-back, and ultimately death (Allen *et al.*, 2010; Price *et al.*, 2011; Nardini *et al.*, 2013; Rayner *et al.*, 2015), and also complicates the identification of key physiological traits allowing to predict plant performance on green roofs installed in arid regions.

To the best of our knowledge, a comparative study of physiological traits conferring resistance to drought and heat stress has never been coupled to the monitoring of plant performance on extensive green roofs. In this study, we contribute to this literature gap, by analyzing the performance in terms of growth and survival of 11 Mediterranean shrub species, established on shallow green roof experimental modules, as related to several indicators of their physiological vulnerability to water stress and high temperatures. We monitored plant water status, leaf symplastic resistance to drought and stem vulnerability to xylem embolism, as well as root resistance to heat stress. We aimed at understanding which functional traits underlie plant performance and survival on Mediterranean green roofs. Our main hypothesis was that plant physiological traits conferring efficiency/safety to the water transport system under severe drought, as well as root resistance to heat stress, significantly influence the overall plant performance and survival. Moreover, on the basis of the results, we propose a methodological framework for screening and selection of suitable shrub species for roof greening in the Mediterranean.

2. MATERIALS AND METHODS

2.1. Study area and experimental set-up

The study was carried out between 2013 and 2015 on the experimental green roof installed on the rooftop of the Dept. of Life Sciences, University of Trieste (NE Italy; 45° 39’40’’N, 13°47’40’’E). Trieste lies on the upper Adriatic coast and it is characterized by a sub-Mediterranean climate, with mild winters and relatively warm, dry summers. Mean annual temperatures in the period 1994-2015 (www.osmer.fvg.it) averaged 15.7°C (highest 25.1°C in July, lowest 7.0°C in January). Maximum daily temperatures frequently exceed 30°C in summer, while in winter the minimum values drop under 0°C only occasionally. Mean annual rainfall is 869 mm, with relatively dry periods in July and January-February. Snow events are rare. The prevalent wind (Bora) blows from E-NE.

The experimental extensive green roof was composed of 10 modules, each covering an area of 2.5 m^2. Modules were built with a six-layer system by SEIC (Harpo Spa, Trieste, Italy), consisting of: a waterproof/root resistant membrane, a moisture retention layer, a drainage layer, a filter membrane, and substrate (dry bulk density=848 kg m^{-3}, organic matter=2.9%, pH=6.8, water content at saturation=0.44 g g^{-1}). The experimental modules were filled with 10 (D-10) or 13 (D-13) cm deep substrate (5 modules per depth). Each module had an independent discharge for excess water runoff, and was equipped with a temperature sensor (TT-500, Tecno.el srl, Roma, Italy) installed at the maximum substrate depth and a soil moisture content sensor (WC, EC-5, Decagon Devices, Pullman, WA, USA), both recording values at 1 h time intervals. In April 2013, the modules were vegetated with 11 woody species belonging to the Mediterranean and sub-Mediterranean flora (Pignatti, 2002). In particular, both evergreen (*Cistus salvifolius* L., *Ligustrum vulgare* L., *Phillyrea angustifolia* L., *Pistacia lentiscus* L., *Salvia officinalis* L.) and deciduous species (*Cotinus coggygria* Scop., *Emerus majus* Mill., *Paliurus spina-christi* Mill., *Prunus mahaleb* L., *Pyrus pyraster* Burgsd., *Spartium junceum* L., Conti *et al.*, 2008) were used (Table 1). The species were selected on the basis of the characteristics of their natural habitat (shallow soils with low water storage capacity, high temperatures) and their known tolerance to drought stress (Pignatti, 2002; Choat et al., 2012; Raimondo et al., 2015; Savi et al., 2015). The 2-3 year-old potted plants were provided by either a public (Regional Forestry Service, Tarcento, Italy) or a private nursery (Vita Verde, Bologna, Italy) in small pots of approximately 0.5 L. The substrate of the pots was carefully removed and the roots gently rinsed with water. Four individuals per species were randomly transplanted in each experimental module at a minimum distance of 20 cm between individuals, and abundantly irrigated. Moreover, 10 individuals per species were transplanted in 2 liters pots filled with the same green roof substrate (13 cm deep), and maintained nearby experimental modules for additional physiological measurements (see below). During the study period, both potted plants and those growing on green roof modules received natural rainfall and additional emergency irrigation only during severe drought (about 25 mm over the whole summer season).

Microclimatic parameters (i.e., wind, air temperature and humidity, irradiance, precipitations) during the study period were recorded by a weather station installed near the modules (Savi *et al.*, 2015; S1).

2.2. Plant water status

Plant water status was assessed in terms of pre-dawn (Ψ_{pd}) and minimum (Ψ_{min}) water potential, and leaf conductance to water vapor (g_L). Measurements were performed on two subsequent sunny days in June 2014 (high water availability) and August 2014 (dry period). During these days the substrate water potential ($\Psi_{substrate}$) recorded in D-10 modules was -0.04±0.02 MPa and -2.40±0.65 MPa, while in D-13 modules was -0.06±0.02 MPa and -

3.90±1.67 MPa for June and August, respectively. At 0500 h, at least three leaves per species (one leaf from each of three randomly selected individuals) and per substrate depth were detached, wrapped in cling-film, and inserted in plastic bags. Leaves were immediately transported in the laboratory and their Ψ_{pd} was measured with a pressure chamber (mod. 1505D, PMS Instruments, Albany, OR, USA). On the same days, g_L was measured at midday on at least three leaves per species and per substrate depth using a porometer (SC1, Decagon Devices, Pullman, WA, USA). After g_L measurements, leaves were sampled and transported to the laboratory for Ψ_{min} determination as described above. The water status measurements were performed on mature, fully expanded, healthy, and undamaged leaves.

2.3. Physiological traits

Leaf water potential isotherms (PV-curves) were measured in July 2014 to evaluate the symplastic drought tolerance of the study species (Lenz *et al.*, 2006). At least three leaves per species were detached in the morning from different potted individuals and rehydrated for 30 min while wrapped in cling film. The initial leaf water potential (Ψ_{leaf}) was measured with the pressure chamber, followed by fresh weight measurements (FW). Leaves were left dehydrating on the bench and sequential measurements of Ψ_{leaf} and FW were performed until the relationship between $1/\Psi_{leaf}$ and cumulative water loss became linear. PV-curve elaboration (Tyree and Hammel, 1972) led to the extrapolation of the osmotic potential at full turgor (π_0) and the water potential at turgor loss point (Ψ_{tlp}).

To assess species-specific vulnerability to drought-induced xylem embolism, stem vulnerability curves (VCs) of the 11 study species were measured using the air injection method in summer 2015 (Ennajeh *et al.*, 2011; Cochard *et al.*, 2013). Potted plants were abundantly irrigated and after 24 h were cut under water at the root collar. The stem was re-cut under water several times at both ends to the final length, corresponding to 1.5 times the maximum vessel length, as estimated with the air-injection method (Jacobsen *et al.*, 2012), to avoid possible artefacts due to the presence of xylem conduits open at both sample ends (Ennajeh *et al.*, 2011). The basal end was connected to a tubing system and flushed with a perfusion solution (10 mM KCl) filtered at 0.2 μm for 30 min, under a pressure (P) of 0.18 MPa. The stem was then inserted through a 10 cm long double-ended pressure chamber and perfused with the reference solution at low pressure (5 kPa). The diameter of the tubing connected to the sample was large enough to allow the escape of air bubbles originating from the sample during pressurization. The flow (F) was measured by collecting effluent with pre-weighed vials filled with absorbent material over 1-min intervals (F_{max}, average of five measurements). The pressure in the chamber was progressively increased by 0.5 MPa intervals and F was measured after 5 min equilibration at each pressure level. The percentage loss of hydraulic conductivity (PLC) was calculated as $PLC=1-(F/F_{max})\times100$. At least three individuals per species were analyzed and PLC data corresponding to each applied pressure were averaged in a single VC. As a reference parameter indicating species-specific vulnerability to xylem embolism (Choat *et al.*, 2012), the value of xylem pressure inducing 50% loss of stem hydraulic conductivity (P50) was calculated from VCs.

2.4. Plant growth and mortality

In May 2013, the diameter at the root collar (D_i, calculated as the mean of two measurements taken at 90° angles), of all transplanted individuals was measured with a digital caliper (Absolute Coolant-Proof, Mitutoyo, Aurora, IL, USA). The diameter was re-measured in September 2014 (D_f) and the relative diameter increment was calculated as: $G=(D_f/D_i)-1\times100$.

The aim of these measurements was to estimate the species' growth rate after two years of establishment on the D-10 or D-13 modules.

Drought survival of the study species growing in the two substrate depths was estimated in September 2014 and 2015 on the basis of visual assessments. Desiccated plants without green foliage and vital buds were considered dead (D). Individuals with turgid and healthy leaves, even if limited in number, were classified as living plants (L). Species-specific mortality rates (M) for each category of substrate depth was calculated as the ratio between dead plants and the number of all planted individuals (D/D+L).

2.5. Root vulnerability to heat stress

On the basis of the significant differences found in substrate temperature and plant mortality between D-10 and D-13 modules (see Results), a laboratory experiment was performed in September 2015 to evaluate species-specific vulnerability of roots to heat stress. Root cell membrane stability at high temperatures was estimated with electrolyte leakage tests. Four potted plants per species were gently uprooted to collect about 200 mg (fresh weight) of fine roots (diameter<1 mm), which were rinsed with water and placed in two tubes (100 mg each) containing 1.5 ml of deionized water. The tubes were shaken for 1 h at laboratory temperature to eliminate remaining debris and ions entrapped in the root cortex apoplast (apparent free space, Bernstein and Nieman, 1960). The solution was afterward discarded and 1.5 ml of fresh deionized water was added to the samples. One tube per plant was incubated for 30 minutes in a bath containing water at 45°C (T, treatment), while the second tube was kept at lab temperature (C, control). After the heat stress treatments, all samples were allowed to reach room temperature, and the initial electrical conductivity (C_i) of the solution was measured (Twin Cond B-173, Horiba, Kyoto, Japan). Both T and C samples were then subjected to 3 freezing-thawing cycles (1 min in liquid N_2 followed by 30 min at room temperature) and the final electrical conductivity was measured (C_f). The relative leakage ratio was calculated as: $REL=(C_i/C_f)\times100$. The root cell membrane vulnerability to heat stress was estimated as: $\Delta REL=REL_T-REL_C$.

2.6. Statistical analysis

Statistical significance of differences and correlations was tested on the basis of unpaired Student's t-test and Pearson product-moment correlation. A multiple regression analysis was carried out to evaluate physiological traits as potential predictors of plant growth and mortality (R v. 3.2.2; www.r-project.org). All results were considered statistically significant at $P\leq0.05$. Means are reported ± standard error of the mean (SEM).

3. RESULTS

Table 2 reports the values of functional traits derived from PV-curves and stem VCs elaboration, as well as growth rates (G) assessed two years after planting. The overall mean Ψ_{tlp} and π_0 of the study species were -1.92±0.15 MPa and -1.42±0.12 MPa, respectively. The species with the lowest (more negative) values of Ψ_{tlp} and π_0 was *P. lentiscus*, while the highest values were recorded for *S. junceum.* P50 values ranged between -1.55 MPa in *P. lentiscus* (high vulnerability to drought-induced xylem dysfunction) and -5.00 MPa in *L. vulgare* (high resistance to embolism). Over two growing seasons, the diameter at the root collar increased by 60% and 84% in plants growing on 10 and 13 cm deep substrate, respectively. The G of *P. pyraster* individuals was not assessed due to high mortality in this species (see below). Interestingly, G was not correlated to P50, but a positive and significant correlation emerged with symplastic drought tolerance (Table 4, S2). Indeed the lowest G was recorded in *P. lentiscus* and the highest in *S. junceum*. A positive correlation was also observed between Ψ_{tlp} or π_0 and plant water status as recorded in June and August, in both D-10 and D-13 modules (Table 4). Overall, species characterized with lower Ψ_{tlp} and π_0 showed more negative Ψ_{pd} and Ψ_{min}, as well as lower g_L values. For example, in June *S. junceum* had the most favorable water status, while the lowest values of Ψ_{pd}, Ψ_{min}, and g_L were again found in *P. lentiscus*. Unfortunately, it was not possible to measure the g_L for *S. junceum* due to its small and drought-deciduous leaves (Pignatti, 2002). In August, *P. angustifolia* experienced the least favorable water status, reaching a Ψ_{min} of -4.2 MPa (Ψ_{tlp}=-2.49 MPa) and a g_L of about 110 mmol m^{-2} s^{-1} (the lowest after that of *P. lentiscus*).

Overall, the results point to a slightly more favorable water status in plants grown on 10 than on 13 cm deep substrate. In particular, the mean Ψ_{min} for all shrubs recorded in June was found to be -1.16±0.07 and 1.39±0.10 MPa for D-10 and D-13 plants, respectively ($P=0.08$). Moreover, the Ψ_{pd} in *P. mahaleb* and *P. spina-christi* was about 0.3 MPa more negative in plants grown on deeper substrate ($P<0.05$). Nevertheless, plants classified as on the basis of complete desiccation of their aerial portion were about 44% in D-10 modules and only 20% in D-13 ones ($P<0.05$), with notable differences among species (Fig. 1). Plants' mortality measured at the end of summer 2014 (data not shown) was not statistically different of data recorded in 2015. The lowest mortality rate was recorded for *P. angustifolia* (no dead plants in D-13), while the highest rates were found in *P. pyraster* (average M=71.1%) and *P. lentiscus* (average M=62.5%). No striking correlations were highlighted between M and plant water status, as well as Ψ_{tlp} and π_0. Surprisingly, a highly significant relationship ($P<0.01$) was observed between M and P50 in plants growing on 10 cm deep substrate but not in those growing on 13 cm (Table 4, S2).

Data on soil temperature at the maximum substrate depth revealed marked differences between the two categories of substrate depth. In particular, the temperatures recorded on a representative warm, summer day (mean air temperature=29.6°C) ranged between 26.5 and 43.6°C in 10 cm deep substrate, while the range was 29.3–39.2°C for the 13 cm deep substrate (Fig. 2A). The average daily thermal regime of the substrate in July (the hottest month) was about 15°C in D-10 and only 10°C in D-13 modules. Moreover, the maximum temperature peak was usually delayed by 2 hours in deeper modules (2000 h) if compared to the shallower ones (1800 h). A highly significant difference ($P<0.001$) was observed in terms of absolute daily maximum substrate temperatures reached during the study period between D-10 (43.8±0.49°C) and D-13 (39.4±0.68°C) modules (Fig. 2B).

Figure 3 summarizes the results of experiments designed to estimate the root vulnerability to heat stress. Cell membrane sensitivity to high temperatures, estimated as ΔREL, ranged from about 6% (low vulnerability to heat stress) to about 22% (high vulnerability to heat stress), as recorded in *C. coggygria* and *P. pyraster*, respectively. ΔREL was found to be significantly correlated with plant mortality in both 10 ($P=0.02$) and 13 ($P=0.001$) cm deep modules.

4. DISCUSSION

Our results provide experimental evidence that species-specific functional traits are useful and reliable proxies of plant performance on green roofs installed in Mediterranean-climate regions. In particular, our data suggest that traits conferring resistance to drought and high substrate temperatures represent the essential trademarks of plant species to be used for roof greening in warm and dry climates.

Our study was focused on the analysis of traits conferring symplastic and apoplastic drought tolerance, in terms of maintenance of positive turgor and efficient root-to-leaf pathway, both of which ensure maintenance of gas exchange rates and plant survival under drought conditions. The wide spectrum of Ψ_{tlp}, π_0, and P50 values recorded in the study species support the hypothesis that Mediterranean plants are flexible in their adaptation to drought and in fact display a range of different hydraulic strategies (Galmés *et al.*, 2007; Nardini *et al.*, 2014a).

Both Ψ_{tlp} and π_0 are considered reliable indicators of drought tolerance (Bartlett *et al.*, 2012). In fact, our data show that Ψ_{tlp} sets the limit that can be reached by Ψ_{pd} and Ψ_{min}. Progressively more negative Ψ_{tlp} allowed some species to reach and tolerate more negative Ψ_{pd} and Ψ_{min}, thus extending the time interval for maintenance of stomatal aperture, photosynthetic carbon gain, and growth (Sack and Holbrook, 2006; Lenz *et al.*, 2006). The highly significant positive correlation between Ψ_{tlp} or π_0 and g_L further points to symplastic drought resistance as a good predictor of plant water use over green roofs. In fact, low g_L values displayed by species with low Ψ_{tlp} translates into low evapotranspiration rates and a more conservative water use, which represents a desirable feature of plants selected for green roofs to be installed in drought-prone regions (Savi *et al.*, 2015). Similarly, low water use under drought conditions has been recently reported for granite outcrop shrubs capable to tolerate substantial Ψ_{leaf} drop under drought (Farrell *et al.*, 2013).

Plants with more negative π_0 or Ψ_{tlp} also displayed significantly lower growth rates in both 10 and 13 cm deep modules (Table 4, S2). Low growth rates in these species might arise as a consequence of both limited g_L and reduced carbon gain, and osmoregulation processes involving substantial carbon investment. The reduction of π_0, driven by active accumulation of compatible solutes in cells, protects membranes during stress and preserves metabolic functionality, but requires high energetic costs (Lenz *et al.*, 2006; Dichio *et al.*, 2009; Bartlett *et al.*, 2012) at the expense of plant growth. In any case, low growth rates translate into the development of small-sized vegetation, representing a desirable characteristic for extensive green roofs due to associated reduction of installation load and maintenance costs (Caneva *et al.*, 2015; Berardi *et al.*, 2014; Savi *et al.*, 2014).

An overall more favorable water status (albeit only marginally significant, P=0.12) was recorded in plants growing on D-10 than on D-13 modules. As an example, Ψ_{pd} measured for *P. spina-christi* in both June and August was significantly higher in D-10 than in D-13 modules. In a recent experiment by some of us, it was shown that reduced substrate depth may translate into less severe plant water stress, as a likely consequence of reduced plant biomass, coupled to faster recovery of hydration of substrate and water retention layer during rainfalls (Savi *et al.*, 2015). The results of the present experiment support these conclusions, as shrubs growing on 13 cm deep substrate showed an overall tendency to grow faster when compared to the individuals growing on 10 cm, and also displayed lower water potentials.

Even if the water status of plants grown on D-10 modules was more favorable, the recorded mortality rate exceeded 40% in these modules, while it was less than 20% in D-13 modules. In fact, for *E. majus* 73% of the plants established on shallow substrate died, while a 100% survival rate of the same species was observed in deeper substrate. Moreover, an overall high M (62.5 %) was observed for *P. lentiscus*, despite the high symplastic resistance to drought of this species (low Ψ_{tlp} and π_0). This result suggests that a difference of only 3 cm in substrate depth can produce significant effects on plant performance. Our data are consistent with recent studies, reporting improved plant survival in green roof installations with deep substrates than in shallower ones (Dunnett *et al.*, 2008; Razzaghmanesh *et al.*, 2014; Zhang *et al.*, 2014). However, our mortality data, coupled to measurements of plant water status and analysis of functional traits related to species-specific drought resistance, suggest that water stress is not the only, and probably not the major cause of plant failure on Mediterranean green roofs.

Xylem hydraulic vulnerability as estimated in terms of P50 was correlated with Ψ_{pd} and g_L measured in June in the shallow modules (D-10). This result indeed suggests that high resistance to stem hydraulic dysfunction (more negative P50) may allow plants to tolerate lower Ψ_{leaf} while maintaining positive safety margins (calculated as P50–seasonal minimum Ψ_{leaf}) towards massive embolism formation (Choat *et al.*, 2012; Nardini *et al.*, 2014a). The reduced Ψ_{leaf} enhances the driving force for the water movement in the root-to-leaf pathway, enabling the plant to absorb water at lower $\Psi_{substrate}$. A very interesting result was the lack of correlation between P50 and M in D-13 modules, while such relationship was highly significant in shallow modules ($P<0.01$). In particular, the highest mortality was observed for species characterized by low P50 values, i.e. *P. lentiscus* (P50=-1.55 MPa) and *P. pyraster* (P50=-1.70 MPa). This is in accordance with recent studies reporting correlations between tree die-back and species-specific P50 in natural habitats characterized by extremely shallow limestone soils (Nardini *et al.*, 2012). On the other hand, the lowest M was recorded for *C. coggygria* (P50=-3.88 MPa), known to be a drought resistant species colonizing limestone cliffs and degraded areas (Pignatti, 2002). More than 50% of the tested species showed almost complete survival on D-13 modules, suggesting that just 3 cm of deeper substrate might significantly enhance the chances of plant survival. Aside from P50, however, no significant correlations were found between M and other physiological traits related to drought resistance. The trend towards improved plant growth/survival on deeper substrates has been related to the higher volume of available water to vegetation, or to the mitigation of temperature extremes ensured by deep substrates compared to shallow ones (Dunnett *et al.*, 2008; Price *et al.*, 2011; Razzaghmanesh *et al.*, 2014). Surprisingly enough, to the best of our knowledge, a clear demonstration of the relative importance of drought versus heat stress in driving plant mortality over green roofs is still lacking.

In our study, the 3 cm difference in substrate depth translated into an increase of saturated water content by 30% in D-13 versus D-10. However, as discussed above, plant water status was overall more favorable in D-10 than in D-13. On the other hand, we observed that both minimum and maximum temperatures, as well as daily thermal regime recorded at the maximum substrate depths, were significantly different in D-10 and D-13 modules. The 25% deeper substrate led to a 4.4°C difference in the absolute temperature peak reached during summer. In particular, the temperature in D-10 modules frequently exceeded 42°C, while it was constantly below such critical threshold in modules that were just 3 cm deeper. The temperatures recorded in our study are in accordance with those reported for a 15 cm deep green roof established in Mediterranean climate (Olivieri *et al.*, 2013) and slightly higher (by about 3°C) of those measured under 10 cm deep substrate layer under subtropical climate conditions (Simmons *et al.*, 2008). On the basis of the maximum temperature peak reached in D-10 modules, the species-specific root vulnerability to heat stress (ΔREL) was estimated after a 45°C treatment. Interestingly ΔREL was correlated to plant mortality in both D-10 and D-13 modules, thus suggesting that high substrate temperature represents a stress factor affecting plant survival on green roofs to a larger extent than drought *per se* (Fig. 3; S2). In fact, several authors have reported that both chronic and abrupt heat stress can reduce root growth and limit nutrient and water uptake, since roots are often more sensitive to heat stress than shoots, Huang *et al.*, 2012). High temperatures at the root level may adversely affect respiration and cell membrane stability, as well as modulate levels of hormones and primary and secondary metabolites, with a consequent effect on root-to-shoot signaling (Kuroyanagi and Paulsen, 1988; Wahid *et al.*, 2007; Huang *et al.*, 2012). Moreover, the effects of high temperature and water deficit stress, both of which characterize green roof ecosystems, are globally additive (Vile *et al.*, 2012) and their combined effect is known to be even more deleterious for plant life in both natural and semi-natural ecosystems (Allen *et al.*, 2010; Price *et al.*, 2011; Nardini *et al.*, 2013).

5. CONCLUSION

Our data highlight the importance of plant physiological traits conferring resistance against both drought and high substrate temperatures as proxies to be taken into account when selecting species for roof greening in the Mediterranean-climate regions. In fact, drought-tolerant species had also lower water needs and growth rates, while the ability to survive in harsh microclimate conditions was significantly correlated to the resistance of the root system to heat stress. In has been demonstrated that reducing soil temperature while maintaining air temperature relatively high improve the growth and the functional status of both roots and shoots, ensuring plant survival (Kuroyanagi and Paulsen, 1988; Price *et al.*, 2011; Huang *et al.*, 2012). One of the main targets in green roof research is reducing substrate depth, to limit installation weight and costs (Cao *et al.*, 2014). However, our results show that such a strategy might contrast with the need to minimize temperature extremes in the substrate and assure plant survival. Future experiments should test possible solutions to increase albedo on green roof systems with shallow substrates. In this light, the optimal design for green roofs in arid-prone areas should include a carefully selected drought resistant vegetation, able to save water and tolerate extreme below-ground temperatures.

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CONFLICT OF INTEREST

We declare that there are no conflicts of interest including any financial, personal or other relationships with other people or organizations that could influence this work.

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LEGENDS

Table 1. List of the 11 study species and relative family, growth form (Ch, camaephyte, partially woody shrubs; NP, nano-phanerophyte, low woody shrubs; P, phanerophyte, small

trees with shrub-like growth habit), leaf habit (E, evergreen; D, deciduous), habitat and altitude range. *Maquis*, evergreen sclerophyllous shrubland; *garigue*, low, soft-leaved scrubland.

Table 2. Leaf water potential at turgor loss point (Ψ_{tlp}, MPa), osmotic potential at full turgor (π_0, MPa), and water potential inducing 50% loss of stem hydraulic conductivity (P50, MPa) of the 11 Mediterranean and sub-Mediterranean woody species. The relative diameter increment (G, %) as estimated 2 years after planting in 10 cm (D-10) and 13 cm (D-13) thick experimental modules is also reported.

Table 3. Pre-dawn (Ψ_{pd}) and minimum (Ψ_{min}) leaf water potential (MPa), and leaf conductance to water vapor (g_L, mmol m^{-2} s^{-1}) as recorded for the 11 study species in 10 cm (D-10) and 13 cm (D-13) experimental modules in June (high water availability) and in August (limited water availability) 2014.

Table 4. Correlation matrices reporting the coefficient r and P value (as asterisks, Pearson product moment correlation) for correlations between pairs of traits: water potential at turgor loss point (Ψ_{tlp}), osmotic potential at full turgor (π_0), water potential inducing 50% loss of stem hydraulic conductivity (P50), plant mortality (M), relative diameter increment (G), pre-dawn and minimum water potentials (Ψ_{pd}, Ψ_{min}), and leaf conductance to water vapor (g_L), as measured in 10 and 13 cm deep green roof modules. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Fig. 1. Plant mortality (M, %) of the 11 study species growing in 10 cm (D-10, black columns) and 13 cm (D-13, gray columns) deep green roof modules. The average plant mortality calculated for 10 or 13 cm thick substrate (n=11) is also reported. * indicates statistically significant difference between experimental categories (Student's t-test, $P<0.05$).

Fig. 2. A) Temperature course (°C) recorded at the maximum substrate depth in 10 cm (D-10, closed circles) and 13 cm (D-13, open circles) on a representative warm summer day. The average thermal regime of the substrate in July (the hottest month) is also reported. B) The absolute maximum substrate temperature reached during the study period in D-10 (black columns) and D-13 (gray columns). * indicates statistically significant difference between experimental categories (Student's t-test, $P<0.05$).

Fig. 3. Relationship between root vulnerability to heat stress (ΔREL, %) and plant mortality (M, %) as measured in September 2015 in 10 cm (D-10, closed circles) and 13 cm (D-13, open circles) experimental modules. The correlation coefficient r and P value (Pearson product moment correlation) are reported.

Species	Family	Growth form	Leaf habit	Habitat and altitude range
Cistus salviifolius L.	Cistaceae	NP	E	*Maquis* and oak forests, on acid or limestone soil; 0-1200 m
Cotinus coggygria Scop.	Anacardiaceae	NP	D	Brush and cliffs, on limestone soil; 0-900 m
Emerus majus Mill.	Fabaceae	NP	D	Brush and woodlands; 0-1650 m
Ligustrum vulgare L.	Oleaceae	NP	E	Termophilic deciduous forest margins and brush; 0-1300 m
Paliurus spina-christi Mill.	Rhamnaceae	P	D	Woodland and bush, on steep slopes; 0-500 m
Phillyrea angustifolia L.	Oleaceae	P	E	Hot and very dry *maquis* and *garigues*; 0-600 m
Pistacia lentiscus L.	Anacardiaceae	P	E	*Maquis* developed on the coast; 0-700 m
Prunus mahaleb L.	Rosaceae	P	D	Thermophilous submediterranean forests; 0-1900 m
Prunus spinosa L.	Rosaceae	P	D	Deciduous forests, brush, hedges; 0-1500 m
Pyrus pyraster Burgsd.	Rosaceae	P	D	Deciduous forests, on nutrient-rich soil; 0-1400 m
Salvia officinalis L.	Lamiaceae	Ch	E	*Garigue*, rocky meadows arid cliffs; on limestone soil; 0-300 m
Spartium junceum L.	Fabaceae	P	D	Sun exposed brush; 0-2000 m

Table 1

Species	**Ψtlp**		**π0**		**P50**	**Growth, %**	
	-MPa		-MPa		-MPa	**D-10**	**D-13**
C. salviifolius	1.64	± 0.14	1.28	± 0.05	4.40	59.3	128.5
C. coggygria	1.89	± 0.22	1.32	± 0.18	3.9	81.1	87.0
E. majus	1.90	± 0.17	1.44	± 0.17	2.76	47.8	103.4
L. vulgare	1.75	± 0.12	1.15	± 0.09	5.00	74.6	106.1
P. spina-christi	2.02	± 0.1	1.51	± 0.03	2.13	30.4	34.9
P. angustifolia	2.49	± 0.02	1.78	± 0.16	2.7	41.3	25.0
P. lentiscus	2.69	± 0.15	2.23	± 0.08	1.6	0.0	15.9
P. mahaleb	2.15	± 0.12	1.55	± 0.14	5.0	34.4	48.5
P. pyraster	2.32	± 0.29	1.68	± 0.28	1.7	x	x
S. officinalis	1.26	± 0.04	1.03	± 0.02	2.51	122.2	72.9
S. junceum	1.02	± 0.16	0.69	± 0.14	3.66	202.6	219.1

Table 2

Species	**Ψpd, -MPa**								**Ψmin, -MPa**								**gL, mmol m-2 s-1**							
	June				**August**				**June**				**August**				**June**				**August**			
	D-10		D-13		D-10		D-13		D-10		D-13		D-10		D-13		D-10		D-13		D-10		D-13	
C. salviifolius	0.57	±0.11	0.56	±0.24	1.19	±0.39	1.33	±0.08	1.20	±0.11	1.35	±0.09	2.03	±0.33	2.43	±0.12	527.9	±155.2	493.0	±58.1	151.6	±28.4	210.4	±76.1
C. coggygria	0.20	±0.02	0.15	±0.02	1.29	±0.12	1.06	±0.03	1.10	±0.07	1.13	±0.06	2.17	±0.11	2.24	±0.05	425.6	±16.6	466.4	±21.9	203.5	±22.5	216.7	±38.0
E. majus	0.80	±0.14	0.61	±0.09	0.59	±0.04	1.43	±0.53	1.30	±0.02	1.55	±0.18	1.25	±0.15	2.57	±0.39	81.3	±9.5	339.9	±75.8	157.3	±42.9	182.2	±120.4
L. vulgare	0.56	±0.05	0.78	±0.22	0.65	±0.05	1.84	±0.64	1.32	±0.09	1.28	±0.21	1.83	±0.53	2.76	±0.28	338.7	±110.3	226.8	±32.8	325.7	±82.7	168.1	±132.7
P. spina-chris	0.88	±0.05	1.14	±0.1	1.34	±0.07	1.84	±0.02	1.30	±0.12	1.42	±0.12	2.57	±0.29	2.99	±0.34	189.2	±25.7	340.3	±107.3	242.0	±104.4	228.9	±102.2
P. angustifoli	0.88	±0.31	1.05	±0.05	2.80	±0.8	2.12	±1.2	1.13	±0.3	2.03	±0.37	4.20	±0.75	3.62	±1.53	164.7	±41.3	111.7	±12.8	108.8	±41.4	176.8	±25.0
P. lentiscus	1.30	±0.02	1.44	±0.07	1.98	±0.08	1.75	±0.65	2.20	±0.02	2.34	±0.29	3.71	±0.36	3.37	±0.31	95.5	±15.6	231.5	±54.8	66.4	±26.8	154.5	±60.0
P. mahaleb	0.54	±0.1	0.58	±0.12	0.97	±0.05	1.25	±0.03	1.20	±0.2	1.34	±0.25	2.06	±0.11	2.29	±0.07	435.8	±10.5	435.9	±24.9	212.8	±40.8	212.8	±49.8
S. officinalis	0.73	±0.05	0.64	±0.06	0.74	±0.04	0.80	±0.02	1.06	±0.14	0.86	±0.05	1.68	±0.12	1.85	±0.7	468.5	±183.2	475.9	±133.5	389.9	±68.4	468.0	±151.6
S. junceum	0.27	±0.09	0.25	±0.03	0.71	±0.21	0.59	±0.24	0.54	±0.07	0.60	±0.03	1.23	±0.26	2.36	±0.19	x	x	x	x	x	x	x	x

Table 3

(A)	Ψpd				Ψmin				gL			
	June		August		June		August		June		August	
	D-10	D-13	D-10	D-13	D-10	D-13	D-10	D-13	D-10	D-13	D-10	D-13
Ψtlp	**0.67 ***	**0.71 ***	**0.73 ***	-0.15	**0.76 ****	**0.94 *****	**0.82 ****	**0.76 ****	**0.67 ***	**0.67 ***	**0.8 ****	**0.7 ***
π0	**0.78 ****	**0.77 ****	**0.7 ***	-0.067	**0.86 ****	**0.96 *****	**0.81 ****	**0.72 ***	**0.67 ***	0.56	**0.82 ****	0.56
P50	**0.73 ***	0.61	0.43	-0.12	0.42	0.45	0.45	0.43	**0.67 ***	0.31	0.28	-0.17
M-10	**-0.7 ***	-0.57	0.14	-0.47	-0.57	-0.33	0.013	-0.24	-0.64	-0.21	-0.11	0
M-13	-0.2	-0.06	0.08	-0.15	-0.24	0.076	-0.009	0.4	0.33	0.4	0.22	0.64
G-10	**0.67 ***	x	0.48	x	**0.81 ****	x	0.61	x	0.64	x	**0.79 ****	x
G-13	x	**0.73 ***	x	-0.11	x	**0.74 ****	x	0.51	x	0.5	x	0.08

(B)	Growth		Mortality	
	D-10	**D-13**	**D-10**	**D-13**
Ψtlp	**0.89 *****	**0.83 ****	-0.22	-0.12
π0	**0.89 *****	**0.84 ****	-0.35	-0.2
P50	0.24	0.43	**-0.73 ****	-0.42

Table 4

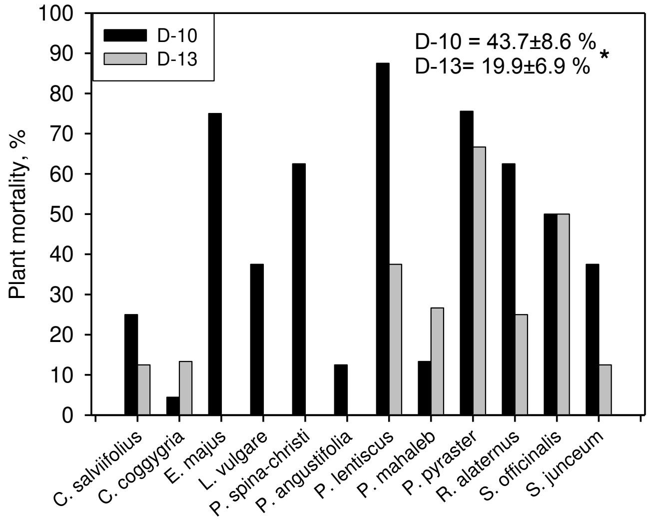
100
90
80
70
60
50
40
30
20
10
0
Plant mortality, %
D-10
D-13
D-10 = 43.7±8.6 %
D-13= 19.9±6.9 %
*
C. salviifolius
C. coggygria
E. majus
L. vulgare
P. spina-christi
P. angustifolia
P. lentiscus
P. mahaleb
P. pyraster
R. alaternus
S. officinalis
S. junceum

Fig. 1

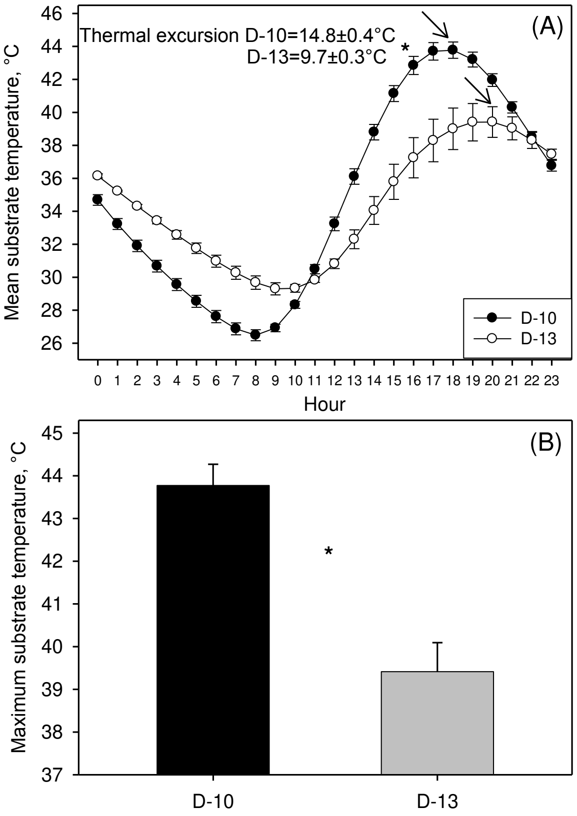
(A)
Thermal excursion D-10=14.8±0.4°C
D-13=9.7±0.3°C
*
Mean substrate temperature, °C
46
44
42
40
38
36
34
32
30
28
26
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hour
D-10
D-13
(B)
Maximum substrate temperature, °C
45
44
43
42
41
40
39
38
37
*
D-10
D-13

Fig. 2

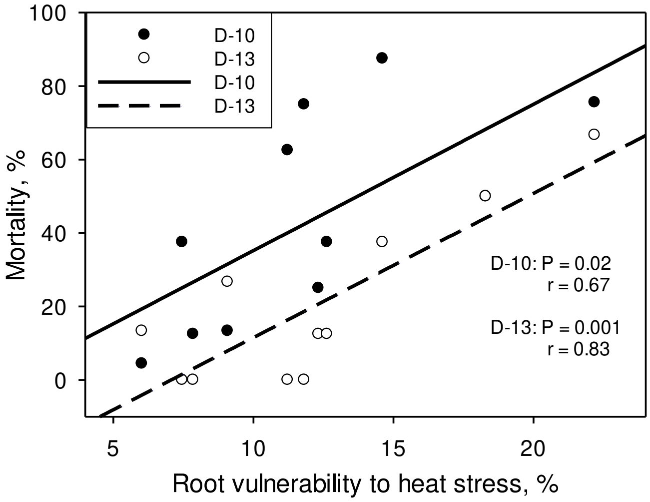
100
80
60
40
20
0
Mortality, %
5
10
15
20
Root vulnerability to heat stress, %
● D-10
○ D-13
D-10
D-13
D-10: P = 0.02
r = 0.67
D-13: P = 0.001
r = 0.83

Fig. 3