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## XXVIII CICLO DEL CORSO DI DOTTORATO IN BIOLOGIA AMBIENTALE

### Optimization of green roof installations in the Mediterranean climate

Settore scientifico-disciplinare: **Fisiologia vegetale**

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# TABLE OF CONTENTS

<b>Ottimizzazione dei sistemi a verde pensile nel clima mediterraneo.....</b>	<b>1</b>
Riassunto.....	1
<b>1. General introduction.....</b>	<b>3</b>
1.1. A brief introduction to green roof technology.....	3
1.2. Thesis aims and structure.....	5
<b>2. Green roofs for a drier world: effects of hydrogel amendment on substrate and plant water status.....</b>	<b>12</b>
<b>3. Does shallow substrate improve water status of plants growing on green roofs? Testing the paradox in two sub-Mediterranean shrubs.....</b>	<b>30</b>
<b>4. Plant performance on Mediterranean green roofs: interaction of species-specific hydraulic strategies and substrate water relations.....</b>	<b>46</b>
<b>5. Leaf hydraulic vulnerability protects stem functionality under drought stress in <i>Salvia officinalis</i>.....</b>	<b>63</b>
<b>6. Composition and performance of succulent and herbaceous plant covers of green roofs in response to microclimatic factors.....</b>	<b>79</b>
<b>7. Drought versus heat: what's the major constraint to Mediterranean green roofs?.....</b>	<b>95</b>
<b>8. General conclusions.....</b>	<b>109</b>
<b>Publication list.....</b>	<b>111</b>

# OTTIMIZZAZIONE DEI SISTEMI A VERDE PENSILE NEL CLIMA MEDITERRANEO

## Riassunto

Le coperture a verde pensile sono impianti vegetali realizzati sui tetti degli edifici mediante l'uso di una serie di materiali specifici, in cui non vi è una continuità ecologica tra il verde e il suolo naturale. Le diverse stratificazioni (protezione antiradice, strato di accumulo idrico, strato drenante, strato filtrante, substrato e vegetazione) sono collocate sull'elemento di tenuta del tetto e formano, insieme con questo, un unico sistema in grado di mantenere nel tempo comunità vegetali e animali stabili. È stato largamente dimostrato che i tetti verdi forniscono numerosi benefici ecologici, economici e sociali e rappresentano degli efficaci strumenti di miglioramento della qualità della vita nei centri urbani. L'applicazione del verde pensile risulta essere ancora poco diffusa nelle regioni a clima mediterraneo caratterizzate da periodi siccitosi ed elevate temperature estive. Le attività di ricerca condotte nel corso della presente tesi di dottorato hanno permesso di sviluppare nuovi criteri per la realizzazione di coperture a verde pensile in area mediterranea, basati sulla conoscenza della risposta delle piante agli stress ambientali, nonché delle caratteristiche dei materiali e delle stratigrafie, con l'obiettivo di aumentare la quantità di acqua disponibile per la vegetazione pur contenendo spessori, pesi e costi del sistema.

La quantità di acqua garantita dal substrato è proporzionale allo spessore del substrato stesso, ma paradossalmente uno degli obiettivi principali della ricerca sul verde pensile punta al contenimento degli spessori utilizzati. Per aumentare le capacità di ritenzione idrica del sistema complessivo, mantenendo al tempo stesso spessori limitati, è stata valutata la possibilità di ricorrere a miscele di substrato e polimeri idrofili superassorbenti (SAP) in diverse proporzioni volumetriche. I SAP sono macromolecole sintetiche che hanno portato ad un significativo aumento della quantità di acqua disponibile per la vegetazione ottimizzando lo stato idrico delle piante di *Salvia officinalis* durante i periodi aridi. In particolare, il migliore stato idrico è stato riscontrato in piante cresciute su soli 8 cm di spessore di substrato, in quanto il ridotto volume limita l'accrescimento delle piante e, di conseguenza, promuove un uso più conservativo dell'acqua.

Essendo la riduzione degli spessori di substrato uno dei principali obiettivi della ricerca sul verde pensile, sono stati valutati lo stato idrico, i tassi di evapotraspirazione e di accrescimento di specie arbustive autoctone (*Cotinus coggygria* e *Prunus mahaleb*) cresciute in moduli sperimentali con spessori di substrato ridotti a soli 10 e 13 cm. Paradossalmente, i dati sperimentali hanno dimostrato come in condizioni di aridità ambientale lo stato idrico delle piante è risultato essere più favorevole nei sistemi caratterizzati da spessori ridotti (10 cm), in quanto essi promuovono un minore accumulo di biomassa vegetale e quindi un minor consumo di acqua, se paragonati a spessori superiori (13 cm). Inoltre, gli eventi piovosi garantiscono un più repentino ed efficiente recupero della ritenzione idrica del sistema stratigrafico complessivo quando vengono utilizzati spessori di substrato più limitati.

Con l'obiettivo di dimostrare l'importanza della selezione delle specie vegetali accoppiata a un'appropriata scelta del substrato, due specie arbustive (*Arbutus unedo* e *Salvia officinalis*) sono state fatte crescere in due substrati

per verde pensile che differivano leggermente in termini di caratteristiche di ritenzione idrica. Misure di parametri fisiologici effettuate in condizioni di elevata disponibilità idrica e in periodi di stress da aridità, hanno evidenziato come il tipo di substrato influenzi in maniera significativa lo stato idrico della vegetazione. Inoltre, le due specie oggetto di studio, pur essendosi dimostrate entrambe adatte per inverdimenti pensili in clima Mediterraneo, hanno mostrato una diversa strategia di risposta allo stress da aridità. Per approfondire le conoscenze sull'adattamento allo stress idrico della pianta modello *S. officinalis* è stato condotto un esteso studio ecofisiologico sulla specie, anche in ambiente naturale. I risultati hanno evidenziato come le foglie risultano essere più vulnerabili allo stress idrico in termini di perdita di efficienza di trasporto dell'acqua se paragonate ai fusti, ma dimostrano una sorprendente velocità nel recuperare il turgore cellulare non appena le condizioni di umidità del suolo lo permettono. Si può quindi concludere che la marcata tolleranza alla aridità di *S. officinalis* è, almeno in parte, conseguenza della segmentazione idraulica, in quanto la vulnerabilità delle foglie protegge la funzionalità del fusto.

Nelle regioni a clima mediterraneo, temperature elevate e deficit idrico impongono l'utilizzo nei sistemi a verde pensile di una vegetazione con buona tolleranza all'aridità e alle temperature estreme. Il presente lavoro, sulla base di uno studio che ha coinvolto 11 specie rappresentative della flora mediterranea, vuole contribuire alla ottimizzazione del processo di selezione delle piante arbustive più idonee per essere utilizzate nelle coperture pensili in climi aridi. Misure accurate dello stato idrico, test di sopravvivenza di specie diverse su spessori di substrato ridotti e lo studio di parametri fisiologici che conferiscono resistenza alla aridità, hanno evidenziato come i tratti che garantiscono efficienza/sicurezza al trasporto dell'acqua risultano essere buoni indicatori sia del tasso di accrescimento delle piante che del consumo delle risorse idriche. Nonostante le limitazioni imposte dallo stress idrico, le alte temperature raggiunte dal substrato nei mesi estivi risultano influenzare in maniera molto più significativa la capacità di sopravvivenza delle piante su un inverdimento pensile. La tolleranza specie-specifica dell'apparato radicale al calore, nonché la resistenza simpastica dell'apparato fogliare allo stress idrico, sono state evidenziate come caratteristiche funzionali essenziali per garantire un'adeguata copertura del verde pensile. La valutazione di tali tratti fisiologici, che risulta essere di facile e veloce misura, dovrebbe essere integrata nel processo metodologico per la selezione di specie idonee per l'inverdimento dei tetti in aree calde e tendenzialmente aride.

La tutela della biodiversità e la formazione di habitat per la flora e la fauna sono due dei benefici ecologici apportati dalle coperture a verde pensile. Nel corso della ricerca sono stati analizzati con regolarità lo sviluppo e la composizione floristica di coperture a piante erbacee e succulente sviluppate su volumi di substrato ridotti. L'utilizzo di una miscela di semi di specie erbacee ha permesso di ottenere in breve tempo una buona copertura del substrato e lo sviluppo di una comunità caratterizzata da elevata biodiversità. Complessivamente, sono state identificate più di 30 specie con spiccata tolleranza alla xericità, distribuite spazialmente e temporalmente in modo eterogeneo. La copertura a succulente ha subito una notevole regressione sia durante i periodi aridi estivi, che durante quelli freddi invernali, indicando come specie più resistenti e competitive *Sedum montanum* e *Sedum sexangulare*. Pertanto, in climi aridi si consiglia l'utilizzo di una miscela di piante erbacee e succulente che porterebbe a garantire una complementarietà nell'uso dell'acqua delle due tipologie vegetazionali ottimizzando la sopravvivenza delle piante durante i periodi aridi e la riduzione dei volumi di acque di deflusso durante gli eventi piovosi.

Il verde pensile rappresenta un sistema complesso dove molteplici fattori ne influenzano la stabilità nel tempo e la funzionalità. Le attività di ricerca descritte nella presente tesi hanno dimostrato la possibilità di realizzare coperture a verde pensile efficienti in climi aridi ricorrendo a soli 10 centimetri di spessore di substrato vegetati con specie accuratamente selezionate sulla base della loro resistenza alla aridità e tolleranza alle alte temperature.

# 1. GENERAL INTRODUCTION

## 1.1. A brief introduction to green roof technology

Green roofs, also known as ‘eco-roofs’ or ‘living roofs’, are engineered ecosystems covering the rooftops, in which specific materials and layerings support the growth of vegetation without physical or ecological continuity connecting plants with the natural ground. The structure of a green roof generally includes a waterproofing root-resistant barrier preventing root penetration and damage of the roof membrane, a water retention layer designed to store water, a drainage layer made up by grained porous media or plastic profiled elements which carry away the excess of water, a filter membrane preventing the washout of fine soil particles, a lightweight substrate, and vegetation (Getter & Rowe, 2006; Oberndorfer *et al.*, 2007; FLL, 2008).

Green roofs have often been indicated as complex systems requiring collaborative efforts by architects, engineers, urban planners, biologist, and horticulturists, with the result that related research is dispersed among many different journals in different fields (Theodosiou, 2009; Blackhurst *et al.*, 2010; Papafotiou *et al.*, 2013; Lamnatou & Chemisana, 2015; Lee *et al.*, 2015; Lundholm, 2015). It has been largely demonstrated that these bio-structures have great potential to bring about several benefits in different climatic conditions and building characteristics, and represent an effective strategy for the promotion of environmental sustainability of cities and, consequently, for the improvement of the human life quality in urban areas (Bowler *et al.*, 2010; Berardi *et al.*, 2014; Thuring & Grant, 2015). In fact, on a world-wide scale, and in particular in developing countries (United Nations, 2014), the level of urbanization is rising displacing natural areas with impervious surfaces, while severely modifying the energy and water balance of ecosystems (Cohen, 2003; Grimm *et al.*, 2008). The unsustainable use of natural resources, the continuous material demand, waste discharge, changes in urban hydrological cycles, and pollution coupled to ongoing climate changes have transformed cities in hotspots driving environmental changes at multiple scales (Grimm *et al.*, 2008). The consequent predicted high economic impacts and social costs are calling for the adoption of urgent mitigation strategies (Luber & McGeehin, 2008; Bowler *et al.*, 2010; Kan *et al.*, 2012).

Urban parks, trees, and green roofs represent effective tools to improve urban climate, as they effectively cool down air and surfaces through increasing albedo, evaporative processes, and shading effects (Bowler *et al.*, 2010; Mackey *et al.*, 2012), and remove large amounts of air pollutants (Nowak *et al.*, 2006; Yang *et al.*, 2008) with consequent positive effects on human health (Donovan *et al.*, 2013). In this light, it is undeniable the pressing need to increase the abundance and cover of vegetation in densely populated areas. On the other hand, the integration of new green areas into a well established urban context is a challenging task, as it would lead to the competition for space with human economic activities. Roof surfaces accounts for about 20-25% of the total urban surfaces and are widely unexploited areas (Akbari *et al.*, 2003), that can be potentially used for green roof installations.

Green roofs may bring direct and indirect benefits to either the building itself or to the urban environment on a wide scale. The technology represents a valid tool to replace the lost green spaces in towns, in that it recreates habitats for local flora (Van Mechelen *et al.*, 2015) and fauna (Madre *et al.*, 2013), while a spread network of installations enable higher connectivity between green spaces (Thuring & Grant, 2015). Reduction of storm-water runoff by means of water retention (Czemiel Berndtsson, 2010), and improvement of building thermal insulation with consequent

reduced energy consumption (Theodosiou, 2009; Nardini *et al.*, 2012), are among the most studied contributions of green roofs to environmental sustainability. Moreover, it has been largely demonstrated that living roofs improve the air (Yang *et al.*, 2008) and water (Czemiel Berndtsson, 2010) quality in cities, contribute to acoustic insulation of buildings (Veisten *et al.*, 2012), increase longevity of roof structures (Blackhurst *et al.*, 2010), and provide aesthetic appeal enhancing the quality of life of residents (Francis & Lorimer, 2011; Lee *et al.*, 2015). Vegetated roofs are often quoted to provide additional environmental/economic benefits, including increased photovoltaic efficiency through the reduction of temperature peaks (Lamnatou & Chemisana, 2015) and the possibility to produce bio-electricity exploiting plants and microbial fuel cells (Helder *et al.*, 2013). Moreover, cities that invest in green infrastructures increase the property values and create additional jobs (Veisten *et al.*, 2012).

On the basis of the required maintenance costs, modern green roofs are generally categorized as “intensive” or “extensive” systems. Intensive green roofs have the appearance of traditional gardens with considerable substrate layer depth (15-20 cm or more), which sustain a wide variety of plant species that may include trees and shrubs (Oberndorfer *et al.*, 2007; FLL, 2008). Intensive installations have the potential to increase the living and recreational spaces in densely populated areas (Francis & Lorimer, 2011). While intensive roofs require high investments in structure design and vegetation maintenance, green roofs termed “extensive” consist of a lightweight design, having shallower substrates (from 2 to 15-20 cm), and require little to no maintenance, as they are sowed with slow-growing and drought-tolerant plant communities comprising herbs, succulents, mosses, and creeping shrubs (Oberndorfer *et al.*, 2007; FLL, 2008; Berardi *et al.*, 2014). In addition, extensive green roofs can be accommodated upon a slope surface (Getter & Rowe, 2006; FLL, 2008). Due to the reduced weight loads, limited installation costs, low maintenance, and their self-regulating capacity extensive green roofs are widely applicable and represent the real sustainable solution for buildings in densely populated areas (Van Mechelen *et al.*, 2015).

While the green roof industry is booming in countries with temperate or sub-tropical climate (Oberndorfer *et al.*, 2007; Mackey *et al.*, 2012), a still low number of installations can be noted in arid-prone areas (Farrell *et al.*, 2012). In fact, in the Mediterranean-climate regions plants often face severe water stress and frequent high temperatures and irradiance, leading to scarce vegetation cover and poor green roof performance, therefore discouraging both industry and governments in the promotion of this technology (Razzaghmanesh *et al.*, 2014; Schweitzer & Erell, 2014; Van Mechelen *et al.*, 2015). Mediterranean cities, that would significantly benefit from a spread installation of green roofs, are often crammed around their old nucleus, which in many cases are characterized as a historical heritage. Here, the lack of areas that could be converted into conventional green spaces is particularly evident (Papafotiou *et al.*, 2013).

To significantly encourage installation of green roofs in water-scarce environments, current research is focused on the improvement of the amount of available water to vegetation ensured by the system, and on the selection of suitable drought-tolerant plant species. To match the first target, improving the water-holding capacity of substrates is essential. Indeed, Farrell *et al.* (2012) reported a correlation between the survival rate of plants under drought-stress and the water holding capacity of substrates, while several authors demonstrated that the substrate depth is the most significant factor affecting growth and survival of plants (Benvenuti & Bacci, 2010; Razzaghmanesh *et al.*, 2014; Van Mechelen *et al.*, 2015). Paradoxically, limiting the substrate depth and consequent weight load of the systems could greatly promote installation of green roofs in the Mediterranean, where most buildings are aged and with limited tolerance of additional weight loads (Papafotiou *et al.*, 2013). The development of new types of lightweight substrates, the study of different design of green roof elements, as well as the use of substrate amendments have been reported to effectively increase the water holding capacity of shallow substrate layers, while improving plant water status and survival under drought conditions (Young *et al.*, 2015; Papafotiou *et al.*, 2013; Savi *et al.*, 2013).

On Mediterranean extensive green roof, both summer and winter season extremes are intensified, while shallow substrates, prone to rapid desiccation, limit plant roots development and significantly reduce the number of suitable species (Young *et al.*, 2015). Taxa selected for roof greening must be able to tolerate prolonged drought conditions, extreme heat, high wind velocities, and sun exposure (Razzaghmanesh *et al.*, 2014; Van Mechelen *et al.*, 2015). The impressive plant biodiversity of the Mediterranean flora (Heywood, 1999) characterized by heterogeneity of adaptations to extreme environmental stresses and a variety of hydraulic strategies (Rotondi *et al.*, 2003; Galmés *et al.*, 2013; Nardini *et al.*, 2014), might represent an important resource for designing green roofs with specific technical features. A careful comparison of the ecology of plants growing in natural habitats with environmental conditions similar to those found on green roofs (extreme temperatures, shallow soils with high drainage, frequent drought, high wind speed etc.) may significantly improve the final performance of green roof structures. Knowledge of species requirements, the test of plant survival on experimental modules, as well as the study of their performance and physiological traits are crucial in this respect. Moreover, the use of mixtures of autochthonous species and different growth forms (succulents, herbs, and shrubs) would lead to better ecosystem functioning and resistance to environmental stresses, while increasing the green roof value in terms of local biodiversity conservation (Lundholm, 2015; Van Mechelen *et al.*, 2015).

## 1.2. Thesis aims and structure

As highlighted in the previous section, roof greening offers a multitude of benefits and is in many respects preferable to conventional roofs in urban areas. However, the application of the technique in water-scarce environments is relatively new and many questions still need to be answered.

The present research aims to contribute to the implementation of green roof technology in warm, drought-prone climates through the study of green roof design in terms of substrate type and depth, as well as through the monitoring of plant responses to environmental stresses. Activities carried out during the three-year long research project have been addressed at improving the amount of available water to vegetation on green roofs, while keeping the substrate depth at minimum, and at identifying criteria for the selection of plant species with high performance under heat and drought stress.

The main hypotheses addressed by the present PhD thesis can be summarized in three statements:

1. it is possible to install efficient extensive green roofs in arid-prone areas using extremely shallow substrate depths
2. the use of hydrogel amendment may increase the amount of water available to vegetation, thus improving the plant water status during drought
3. the selection of an appropriate set of plants for roof greening should be based on the study of species-specific resistance to drought stress.

The following six experimental chapters of this thesis are composed of self-contained units, presented in the style of scientific journal articles. Chapters 2 (Savi *et al.*, 2014), 3 (Savi *et al.*, 2015), 4 (Raimondo *et al.*, 2015), and 5 (Savi *et al.*, 2016) have been already published in international ISI journals, while Chapters 6 and 7 have been submitted to international ISI journals. A brief introduction to each chapter follows.

In Chapter 2, we assessed the effects of polymer hydrogel amendment on the water holding capacity of green roof substrate, as well as on the performance of the Mediterranean shrub *Salvia officinalis*. Plants were grown in green roof experimental modules containing shallow substrate (control) or blends of substrate and hydrogel at two different concentrations. We hypothesized that hydrogel amendment would increase the substrate's water content at saturation, as



well as the amount of water available to vegetation. As a consequence, we expected an enhanced water status and growth of sage plants established in modules containing the substrate-hydrogel blend.

Hydrogel amendment increased the substrate's moisture retention capability, as well as the volume of water available to plants. Our results provide experimental evidence that polymer amendments have the potential to significantly enhance water supply to vegetation on a green roof. In particular, the water status of plants was most effectively improved when reduced substrate depths were used, which also limited the biomass accumulation during early growing stages (Savi *et al.*, 2014).

Reducing the substrate depth of green roofs is essential to limit installation weight and costs, but this choice apparently contrasts with the need to maximize the amount of water available to plants. The second experiment (Chapter 3) was designed to monitor the performance of drought adapted shrubs (*Cotinus coggygia* and *Prunus mahaleb*) planted in experimental green roof modules filled with extremely shallow substrate (10 or 13 cm). In particular, the study aimed to identify the impact of substrate thickness on plant water status, survival, growth, and evapotranspiration, as a consequence of the available rooting volume coupled to the differences in terms of drainage and water accumulation capacity that characterize the two systems. In warm and dry climates, substrate depths of at least 15-20 cm are recommended for shrub-vegetated extensive green roofs. We hypothesised that efficient and fully functional extensive green roofs vegetated with drought-tolerant shrubs can be installed in arid-prone areas using extremely shallow substrate depths.

Experimental data provided evidence for the possibility to install fully functional green roofs using 10 cm deep substrate only. Indeed, the reduced depth translated into less severe water stress experienced by plants, because shallower substrate indirectly promoted lower water consumption as a consequence of reduced plant biomass. Moreover, we demonstrated that both large and small rainfalls induced better water content of the whole green roof system when shallow substrate was used (Savi *et al.*, 2015). Green roofs based on the combination of shallow substrate and drought-adapted vegetation may represent an optimal solution for solving urban ecological issues.

In Chapter 4 we describe an experiment performed to demonstrate the importance of an accurate selection of green roof substrate, which should be coupled to the study of the hydraulic strategies of the vegetation overly. Experiments were performed on two Mediterranean shrub species (*Arbutus unedo* and *Salvia officinalis*) grown in experimental modules filled with two green roof substrates slightly differing in their water retention properties. We expected that the differences in terms of substrates water retention capability will significantly affect the plant water status and the species-specific ability to cope with green roof environmental conditions.

Physiological measurements performed under high moisture availability, as well as under water deficit conditions showed that the substrate type significantly affect plant water status. The two studied species had a different hydraulic response to drought stress, with *Arbutus unedo* being substantially isohydric and *Salvia officinalis* more anisohydric. Despite the two shrubs adopted different hydraulic strategies to water limitations, both of them can be considered suitable species for roof greening in the Mediterranean (Raimondo *et al.*, 2015).

An extensive eco-physiological study was performed on the model species *Salvia officinalis* in order to highlight the strategy adopted by this species to survive under extreme environmental conditions characterizing its natural habitat, as well as green roof ecosystems, i.e. long-term decrease in soil water availability, high air temperatures and irradiance (Chapter 5). We expected to highlight high resistance to drought-induced dysfunction of the water transport system in both leaf and stem organ. Moreover, we hypothesized the existence of a functional coordination between leaf and stem hydraulics, which has been already proposed as a key trait of Mediterranean drought-tolerant plants.

The results highlighted that leaves of *S. officinalis* lose their water transport efficiency earlier than stems, although both plant organs showed surprisingly low apoplastic resistance, if compared to other drought-tolerant species. The fast recovery of leaf turgor upon restoration of soil moisture conditions suggests that the drought-induced reduction of leaf hydraulic conductance is not only a consequence of vein embolism, but cell shrinkage and consequent increase of resistance may play an important role. In this light we conclude, that the drought tolerance of *Salvia* arises, at least partly, as a consequence of vulnerability segmentation, since leaf hydraulic vulnerability seems to protect stem functionality (Savi *et al.*, 2016).

It is largely accepted that green roofs create habitats for local flora improving urban biodiversity. The Chapter 6 describes an experiment designed to study the early establishment and ecology of succulent and herbaceous vegetation grown on green roof modules filled with 8 or 10 cm deep substrate. In particular, we aimed to monitor the survival and development of the autochthonous crassulacean and herbaceous cover, as well as the efficiency in terms of evapotranspiration of both vegetation types over a two-year-long period. We hypothesized that the sowing of a local seed mixture can lead to the rapid development of a highly biodiverse herbaceous cover, while crassulacean species can ensure a satisfactory and continuous ground cover.

Our results highlighted that CAM metabolism ensures succulent species to thrive in the harsh habitat, although a significative regression of the vegetation ground cover was observed in both summer and winter season. In the highly biodiverse herbaceous modules, four different plant communities could be distinguished (for a total of 30 species) in four different times of the season (Boldrin *et al.*, Under review). Our data suggests that the association of succulent and herbaceous plants might ensure a trade-off between low water use for survival under drought conditions and high water use for storm-water runoff mitigation during rainfalls, but the use of a mix of the two growth forms deserves further studies.

In the last experiment (Chapter 7) the study of physiological traits conferring to woody species resistance to drought and heat stress was coupled to the monitoring of plant performance on green roof experimental modules filled with 10 and 13 cm deep substrate. In particular, the plant water status, mortality, leaf and stem resistance to drought, as well as the root resistance to heat stress of 11 drought-adapted shrubs belonging to the Mediterranean and sub-Mediterranean flora were addressed. We hypothesized that physiological parameters known to confer efficiency and safety to the water transport system under drought, significantly influence the overall plant performance and survival on green roofs with shallow depths. On the basis of the results, we aimed to propose a methodological framework for screening and selection of suitable shrub species for roof greening in the Mediterranean.

The results highlighted that several physiological traits can be used as indicators of plant's drought tolerance, low water needs/consumption, and reduced growth on a green roof. However, high substrate temperatures reached in shallow systems during summer season represented 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. Hence, both traits conferring drought tolerance, and in particular heat-stress resistance to plants should be included in the screening procedure of plant selection for green roof established in drought-prone climates (Savi *et al.*, Under review).

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## 2. Green roofs for a drier world: effects of hydrogel amendment on substrate and plant water status

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### HIGHLIGHTS

- Green roof technology is still under-represented in arid climates
- We assessed the potential advantages of polymer hydrogel amendment
- Hydrogel amendment significantly improved substrate and plant water status
- Reduced substrate depth sustained lower plant biomass independent of the amendment
- Hydrogel allowed to reduce substrate depth improving small sized plant water status

### ABSTRACT

Climate features of the Mediterranean area make plant survival over green roofs challenging, thus calling for research work to improve water holding capacities of green roof systems. We assessed the effects of polymer hydrogel amendment on the water holding capacity of a green roof substrate, as well as on water status and growth of *Salvia officinalis*. Plants were grown in green roof experimental modules containing 8 or 12 cm deep substrate (control) or substrate mixed with hydrogel at two different concentrations: 0.3 or 0.6%. Hydrogel significantly increased the substrate's water content at saturation, as well as water available to vegetation. Plants grown in 8 cm deep substrate mixed with 0.6% of hydrogel showed the best performance in terms of water status and membrane integrity under drought stress, associated to the lowest above-ground biomass. Our results provide experimental evidence that polymer hydrogel amendments enhance water supply to vegetation at the establishment phase of a green roof. In particular, the water status of plants is most effectively improved when reduced substrate depths are used to limit the biomass accumulation during early growth stages. A significant loss of water holding capacity of substrate-hydrogel blends was observed after 5 months from establishment of the experimental modules. We suggest that cross-optimization of physical-chemical characteristics of hydrogels and green roof substrates is needed to improve long term effectiveness of polymer-hydrogel blends.

*Keywords* - polymer hydrogel, substrate depth, water availability, water status, drought stress, *Salvia officinalis*

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## 1. Introduction

Green roofs are an example of ecological engineering technology addressed at partially replacing vegetation that was removed to construct buildings. This green technology is largely accepted as a useful measure to address environmental impacts of urban areas while allowing sustainable development (Getter & Rowe, 2008). Recent studies have demonstrated that implementation of green roofs in urban areas can reduce the urban heat island effect (Kolokotsa *et al.*, 2013; Santamouris, 2014), reduce and delay storm-water runoff (Nagase & Dunnett, 2012; Speak *et al.*, 2013), improve air and water quality (Li *et al.*, 2010; Rowe, 2011; Vijayaraghavan *et al.*, 2012), improve noise reduction (Van Renterghem & Botteldooren, 2009), contribute to thermal insulation of buildings with consequent energy savings (Sailor, 2008; D’Orazio *et al.*, 2012), and favour habitat and biodiversity conservation (Baumann, 2006; Brenneisen, 2006; Bates *et al.*, 2013). Green roofs are often quoted to provide additional social (Francis & Lorimer, 2011) and environmental benefits, including the possibility to use or re-use recycled materials in their construction (Bates *et al.*, 2013; Farrell *et al.*, 2013; Mickovski *et al.*, 2013) and to produce bio-electricity exploiting living plants and microbial fuel cells (Helder *et al.*, 2013).

Modern green roofs generally include a waterproofing and root-resistant membrane which protects the rooftop against root penetration and damage, a water retention layer designed to store water, a drainage layer that allows excess water to flow away from the roof, a filter fabric preventing the loss of fine soil particles, and a lightweight mineral substrate and vegetation. Green roof installations can be categorized as intensive *versus* extensive. While intensive green roofs have thicker substrate depth (>15-20 cm) and can support shrubs and even small trees, extensive green roofs are characterized by thinner substrates (<15-20 cm), where only small sized vegetation can thrive successfully (Getter & Rowe,

2006; Oberndorfer *et al.*, 2007). Due to their lower costs as well as to widespread building mechanical limitations, extensive green roofs are much more common than intensive ones.

Green roof technology has become increasingly important in the last 20 years, and thousands of installations have been realized worldwide, especially in countries characterized by temperate and subtropical climates (Brenneisen, 2006; Li *et al.*, 2010; Smith & Roebber, 2011; Speak *et al.*, 2013). Germany is considered as one of the leading countries in green roof development, with over 14% of roofs artificially greened (Herman *et al.*, 2003). Chicago is one of the leading cities, with more than 50000 m<sup>2</sup> green roof installed only in 2008 (Smith & Roebber, 2011). In the Mediterranean climate, the interest in this technology is increasing, although research and installations efforts are still limited (D’Orazio *et al.*, 2012; Santamouris, 2014; Farrell *et al.*, 2013; Kolokotsa *et al.*, 2013; Olate *et al.*, 2013). This is likely due to the features of Mediterranean climate, characterized by high summer temperatures and prolonged seasonal drought, both making plant survival over green roofs quite challenging (Fioretti *et al.*, 2010; Nardini *et al.*, 2012; Savi *et al.*, 2013).

In order to promote the development of green roof technology in Mediterranean climate, research work should be mainly addressed to selecting native plant species capable to survive under harsh environmental conditions (MacIvor *et al.*, 2011; Olate *et al.*, 2013; Van Mechelen *et al.*, 2014), and to improving substrate water holding capacities to ensure larger amounts of available water while maintaining low substrate thickness, weight and related costs (Farrell *et al.*, 2013; Papafotiou *et al.*, 2013; Savi *et al.*, 2013). Suitable species can be found in local habitats characterized by micro-climatic conditions similar to those prevailing over green roofs. As an example, Van Mechelen *et al.* (2014) analyzed ten plant traits relevant for heat and water stress resistance of 372 Mediterranean open habitat species, and selected 28



species with estimated good ability to acclimate and survive on green roofs. On the other hand, Savi *et al.* (2013) have recently shown that slight modification of green roof layering can improve water availability to plants, and Papafotiou *et al.* (2013) found that the use of grape marc compost amendment ensured higher substrate water holding capacities, allowing reduction of substrate depth without causing restriction of plant growth and survival at the establishment phase and during drought events.

Over the last decade, several studies focusing on agriculture, nursery management and forestry practices have demonstrated the potential of different polymer hydrogel amendment to increase water holding capacity of potting mixtures and natural soils (Arbona *et al.*, 2005; Sojka *et al.*, 2007; Luo *et al.*, 2009). Hydrogels are synthetic superabsorbent polymers generally constituted by water-insoluble highly cross-linked polyacrylamides which can absorb water up to 400 times their own weight when saturated (Bouranis *et al.*, 1995; Oschmann *et al.*, 2009). Luo *et al.* (2009) recorded a 36% increase in water holding capacity when mixing the growing medium with 0.6% (w/w) of polymer hydrogel, while Akhter *et al.* (2004) reported a linear relationship between percentage of hydrogel amendment (0.1%, 0.2% and 0.3%) and increase of water content at field capacity for both sandy-loam (17%, 26% and 47%) and loam (23%, 36% and 50%) soils. Application of hydrogel to the rhizosphere of *Pinus sylvestris* seedlings improved the survival rate of plants by 19% during land reclamation (Sarvaš *et al.*, 2007). Apparently, when hydrogels are added to the substrate plant growth is improved, drought effects are delayed and the frequency of irrigations can be reduced (Akhter *et al.*, 2004; Arbona *et al.*, 2005; Shi *et al.*, 2010; Chirino *et al.*, 2011).

Recent studies have suggested that the use of hydrogel polymers can enhance the water holding capacity and plant available water of green roof substrates (Oschmann *et al.*, 2009; Olszewski *et al.*, 2010; Farrell *et al.*, 2013). As a consequence, the

timespan before permanent wilting of *Triticum aestivum* and *Lupinus albus* grown in green roof experimental modules, as well as their root and total dry mass, increased in response to hydrogel amendment (Farrell *et al.*, 2013). Oschmann *et al.* (2009) and Olszewski *et al.* (2010) found that hydrogels significantly increased coverage and regeneration of grasses and *Sedum* species over green roofs.

The aim of the present study was to specifically test the effectiveness of hydrogels added to green roof substrate in ameliorating plant water status, drought resistance and survival. We specifically tested: a) water relation properties and related variations over a short-time interval of substrate, polymer hydrogel and substrate-hydrogel blends; b) possible differences in water status of plants growing on substrate or substrate-hydrogel blends; c) minimum substrate thickness and suitable hydrogel concentrations assuring plant survival during intense drought episodes.

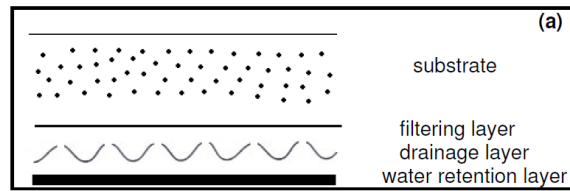
## 2. Materials and methods

### 2.1. Study area

The study was carried out over the roof of the Dept. of Life Sciences, University of Trieste (Trieste, 45°39'40" N, 13°47'40"E) between early April and late September 2013. Climate data for the area in the period 1995-2012 (<http://www.osmer.fvg.it>) report an average annual temperature of 15.7 °C, with a maximum of 25 °C and a minimum of 6.8 °C reached in July and January, respectively. Mean annual rainfall is 843 mm, with most precipitation occurring between September to November (290 mm) and relatively dry periods in January-February (105 mm) and July (55 mm).

### 2.2. Experimental modules and plant material

Wooden beams were used to construct three test beds (each measuring 2 m<sup>2</sup>) over a flat rooftop. Each test bed, lying on a 20 mm thick drainage element, was divided into ten experimental modules 40



Substrate depth 8 cm		Substrate depth 12 cm (b)	
control (substrate only)	<b>Sub8/Hyd0</b>	control (substrate only)	<b>Sub12/Hyd0</b>
substrate + 0.3% hydrogel	<b>Sub8/Hyd0.3</b>	substrate + 0.3% hydrogel	<b>Sub12/Hyd0.3</b>
substrate + 0.6% hydrogel	<b>Sub8/Hyd0.6</b>	substrate + 0.6% hydrogel	<b>Sub12/Hyd0.6</b>

**Fig. 1.** Schematic representation of green roof layering, and of the two main categories of substrate depth in which experimental modules were divided. Each category comprised control modules (substrate only) and modules filled with substrate-hydrogel 0.3 and 0.6% blends.

cm × 40 cm each (for a total of 30 modules) using wood dividers. The green roof layering was assembled using the following materials provided by Harpo Spa, Trieste, Italy: water retention tissue Idromant4 (thickness 4 mm, weight 400 g/m<sup>2</sup>), plastic profiled drainage panel Medidrain MD40 (thickness 4 cm, water retention 4 l/m<sup>2</sup>); geotextile filter membrane MediFilter MF1 and SEIC substrate for extensive green roof installation (dry bulk density 848 kg/m<sup>3</sup>, Fig.1a). The holes (2.5 mm) of Medidrain MD40 were widened to a diameter of 6 mm and increased in number (from 300 holes/m<sup>2</sup> to 600 holes/m<sup>2</sup>), according to Savi *et al.* (2013). The substrate is based on a mix of mineral material (lapillus, pomix and zeolite) enriched with 2.9% organic matter. Grain size ranged from 0.05 mm to 20 mm with a total porosity of 67.35%, pH = 6.8, drainage rate of 67.36 mm/min<sup>1</sup>, cation exchange capacity and electrical conductivity equaling about 23.8 meq/100 g and 9 mS/m, respectively.

Experimental modules were divided into two main categories on the basis of substrate depth: 8 cm and 12 cm. Within each category, 10 modules were filled with substrate mixed with a water-absorbent polymer hydrogel (cross-linked polyacrylic acid-potassium salt, STOCKSORB 660 medium, Evonik Industries) at two concentrations i.e. 0.3% w/w (5 modules) and 0.6% w/w (5 modules). Five modules per depth were used as controls (substrate only). Hence, six

different layering types were assembled, each replicated five times (Fig. 1b).

On April 17<sup>th</sup> 2013, one individual of *Salvia officinalis* L. (Common sage) was transplanted in each module. Potted plants were provided by a local nursery and were all of similar size at the time of planting. After planting, each module was irrigated three times within two weeks with a total of 34 mm of water. During the study period plants received natural precipitation, but additional irrigation (3-18 mm) was provided during extremely arid periods (Fig. 2), when leaves of at least 50% of plants appeared wilted and rolled up. *S. officinalis* is a perennial, evergreen subshrub with woody stems, grayish hairy leaves and purple flowers. It is native to the Mediterranean area but today is widely naturalized even outside the original habitat (Pignatti, 2002). Common sage was selected on the basis of its ability to survive green roof conditions (Savi *et al.*, 2013).

Air temperature and humidity (EE06-FT1A1-K300, E+E Elektronik), wind speed and direction (WindSonic 1, Gill Instruments), precipitation (ARG 100 Raingauge, Environmental Measurements Limited), and irradiance (MS-602, EKO Instruments) in the study site were recorded hourly by a weather station installed on the roof of the Dept. of Life Sciences.

### 2.3. Moisture release curves of substrate, polymer hydrogel and substrate-hydrogel blends

Relationships between water content and water potential (moisture release curves) of substrate, polymer hydrogel, and substrate-hydrogel 0.3% and 0.6% blends were measured at the beginning of experiments (April) and at the end of the vegetative period (September). Moisture release curves were elaborated to quantify the theoretical volume of water available to plants guaranteed by these substrate components (Savi *et al.*, 2013). A sample of substrate, polymer hydrogel or blend substrate-hydrogel was abundantly watered in a pot containing a piece of filter membrane to prevent the loss of fine particles. When saturation was reached, small sub-samples weighing a few grams each, were placed in sampling holders (diameter 40 mm; height 10 mm) and their initial water potential ( $\Psi$ ) was measured using a Dewpoint Hygrometer (WP4, Decagon Devices, Whalley *et al.*, 2013). Samples were then immediately weighted on a digital balance (fresh weight, FW) and then left to dehydrate on the bench before measuring again their  $\Psi$  and FW. Measurements were repeated until water potentials of -6/-7 MPa were reached. Finally, samples were oven-dried at 50° for 48 h in order to get their dry weight (DW). Water content (WC) of samples was calculated as follow: (FW-DW)/DW. The highest values of WC, measured immediately after saturation of the substrate sample were considered as water content at saturation (SWC). All water potential values recorded during sub-samples dehydration were plotted versus the corresponding WC values.

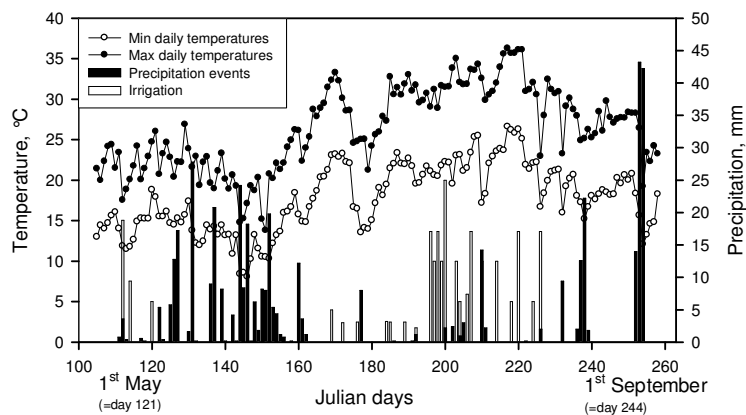
In September, samples for moisture release curves elaboration were collected by picking up approximately 1 liter of substrate from the whole depth of each experimental module. SWC was measured for all 30 modules, while one pressure-volume curve was elaborated for each green roof layering type.

### 2.4. Monitoring plant water status, membrane integrity and biomass production

Water status of plants was monitored by periodic measurements of leaf water potential and leaf conductance to water vapor with the aim to highlight possible differences between plants growing in different experimental modules. At the beginning of the experiments, leaf water potential isotherms (pressure-volume curves) were also measured and elaborated.

Leaves for pressure-volume curve experiments (Tyree & Hammel, 1972) were collected early in the morning, wrapped in cling film and left rehydrating with the petiole immersed in water to a water potential ( $\Psi_{\text{leaf}} \geq -0.2$  MPa, as measured using a pressure chamber (mod. 1505D, PMS Instruments). Fully rehydrated leaves were immediately weighed (turgid weight, TW). Leaves were slowly dehydrated on the bench and sequential measurements of  $\Psi_{\text{leaf}}$  and fresh weight (FW) were performed until the relationship between  $1/\Psi_{\text{leaf}}$  and the cumulative water loss became strictly linear ( $r^2 > 0.98$ ). Pressure-volume curves were elaborated according to Salleo (1983) to calculate leaf osmotic potential at full turgor ( $\pi_0$ ) and water potential at the turgor loss point ( $\Psi_{\text{tlp}}$ ).

Leaf conductance to water vapor ( $g_L$ ) was measured on at least two leaves per experimental module (for a total of 8 measurements per layering type) using a portable porometer (SC1, Decagon Devices) calibrated at the beginning of each measurement session, according to manual specifications. Measurements were performed between 11.00 and 12.00 am (solar time) on two selected sunny days in spring (May 21<sup>st</sup>) and summer (July 12<sup>th</sup>). Air temperature ( $T_{\text{air}}$ ) and relative humidity (RH) data were recorded by the weather station (see 2.2.), while photosynthetic photon flux density (PPFD) was recorded with a portable quantum sensor (HD 9021, Delta Ohm). On the same dates when  $g_L$  was recorded, predawn water potential ( $\Psi_{\text{pd}}$ ) and minimum water potential ( $\Psi_{\text{min}}$ ) were measured on leaves collected at 5.00 am and 12.00 am (solar time), respectively. At least one leaf per individual, for a minimum total of



**Fig. 2.** Minimum (white circles) and maximum (black circles) daily temperatures and precipitation events (black columns) recorded over the rooftop between April 15<sup>th</sup> and September 15<sup>th</sup>. Additional irrigations are also reported (white columns).

four leaves per green roof layering type, were collected, immediately wrapped in cling film, inserted in plastic bags containing a piece of wet filter paper and placed in a cool bag. Leaves were transported in the lab where water potential was measured using a pressure chamber.

At 12.00 am (solar time), on the same dates of water status measurements, leaves for electrolyte leakage tests were also collected. The electrolyte test is a useful method to assess cell membrane stability and quantify the injury suffered by different plant tissue as caused by freezing, heating, drought and other environmental stresses (Prášil & Zámečník, 1998; Bajji *et al.*, 2001). Ten leaf disks (0.5 cm diameter) were punched from at least three leaves per module and immediately inserted in a test tube containing 7 ml of deionized water. Tubes were left for three hours on a stirrer at room temperature. Initial electrical conductivity ( $C_1$ ) of the solution were determined using a portable conductivity meter (Twin Cond B-173, Horiba). Then samples were subjected to three freezing (1 hour at  $-20^{\circ}\text{C}$ ) and thawing cycles (1 hour at lab temperature) in order to cause complete breakage of cell membranes. When the solution finally reached room temperature, its final electrical conductivity was assessed ( $C_2$ ). The relative electrolyte leakage (REL) was calculated as  $(C_1/C_2) \times 100$ , according to Prášil & Zámečník (1998).

At the beginning of the experiment (April), 10 potted plants of *S. officinalis* from the same stock used to vegetate experimental modules were sampled to determine initial aboveground biomass and calibrate a method for non-destructive biomass estimation during the study period. All leaves of each plant were counted ( $N_L$ ) and dry mass ( $DW_L$ ) of 10 representative leaves per plant were measured. The selected leaves were of heterogeneous sizes and reflected the structure of the plant canopy. Aboveground biomass was estimated as follows:  $N_L \times DW_{L\text{mean}}$ . Plants were then cut at the root-stem transition zone, the aboveground portions were oven-dried for 48 h at  $70^{\circ}\text{C}$  and their actual total dry mass ( $B_a$ ) recorded. An allometric relationship was fitted between estimated and actual plant biomass. At the end of June, biomass of plants growing in experimental modules were estimated by counting all leaves of each plant growing in the experimental modules, as well as measuring  $DW_L$  of 5 representative leaves per plant. Aboveground biomass of each plant was estimated as described above and the allometric relationship was used to extrapolate the plant actual total dry mass ( $B_a$ ).

## 2.5. Statistical analysis

Statistical analysis was performed using SigmaStat v. 2.03 (SPSS Inc.) and Statistica 7 (StatSoft Inc.). Significant differences between experimental groups

were assessed with unpaired Student's t-test, One-way-ANOVA, and Two-way-ANOVA. Effects of treatments on plant physiological parameters, as also potentially affected by plant biomass, were tested by General Linear Modelling (GLM). A GLM model was fitted for each dependent variable ( $\Psi_{pd}$ ,  $\Psi_{min}$ ,  $g_L$ , REL). Main and second-order interactive effects of substrate depth and hydrogel addition were tested, including above-ground biomass in the models as a covariate, treated as a continuous variable. Pairwise differences were tested using Tukey's HSD post hoc test. The significance of correlations was tested using Pearson product-moment correlation. All results were considered statistically significant at  $P \leq 0.05$ .

### 3. Results

#### 3.1. Climatic data

Figure 2 reports maximum and minimum daily temperatures and precipitation events recorded over the roof during the experimental period (April-September 2013), as well as supplementary irrigation supplied to modules. Mean daily temperature over the whole study period averaged  $21.6 \pm 4.5$  °C with an absolute minimum and maximum of 8.1 °C and 36.3 °C recorded on May 21<sup>st</sup> and August 5<sup>th</sup>, respectively. The average daily relative humidity over the rooftop ranged between 37% and 89%. During springtime, a total precipitation of 243 mm was recorded, while in summertime rain occurred only on rare occasions for a total of 185 mm, represented mainly by September rain events. As a consequence, during the summer dry period a total of 256 mm of supplementary irrigation was supplied (Fig. 2).

#### 3.2. Moisture release curves of substrate, polymer hydrogel and substrate-hydrogel blends

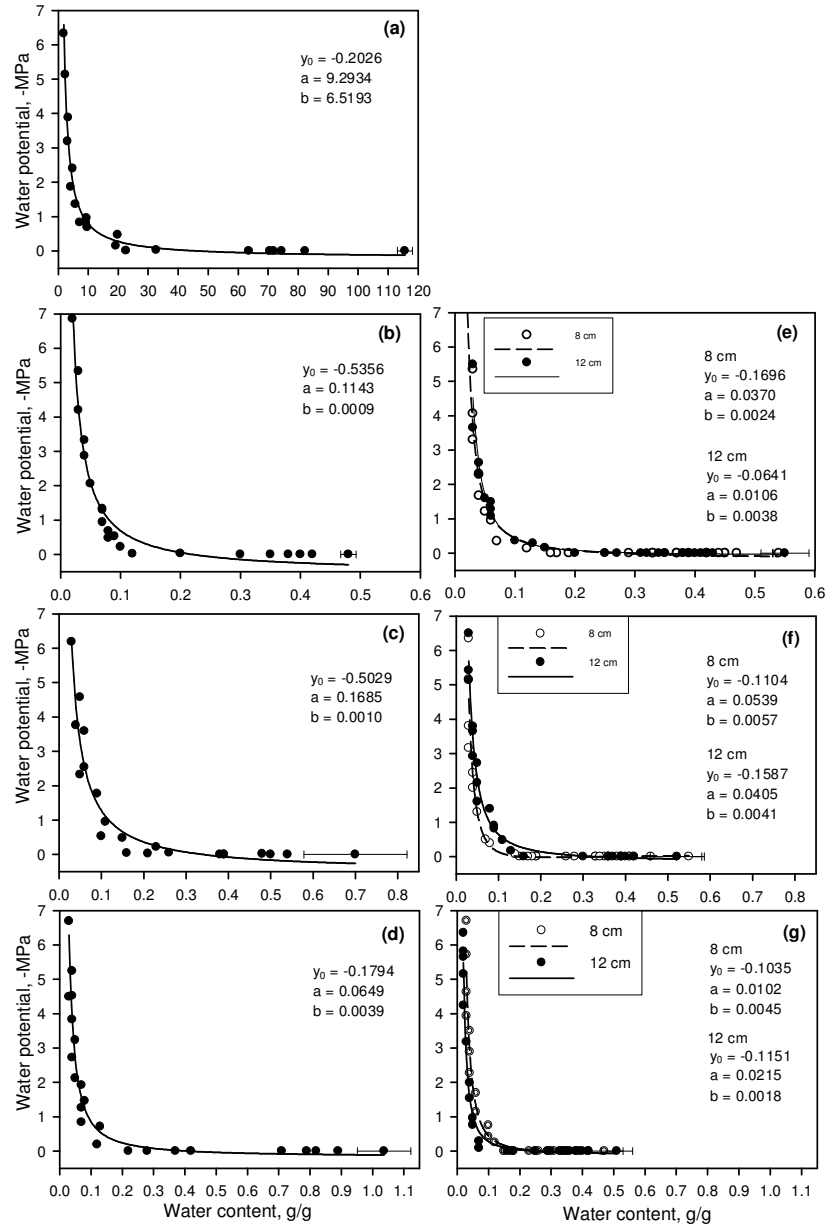
Figure 3 reports moisture release curves as obtained for polymer hydrogel (a), substrate (b, g), and substrate-hydrogel 0.3% (c, e) and 0.6% blends (d, f). Moisture release curves were measured in April (a-d) and in September (e-g) and each curve was based on at

least 21 measurements of  $\Psi$  (between 0 and -6.9 MPa) and corresponding sample water content. At the beginning of the experiment (April), water content at saturation (SWC) of substrate and substrate-hydrogel 0.3% and 0.6% blends were  $0.48 \pm 0.01$  g/g,  $0.70 \pm 0.12$  g/g and  $1.04 \pm 0.09$  g/g, respectively (Table 1a). SWC of the polymer hydrogel was  $115.6 \pm 2.46$  g/g. Hence, the addition of 0.3% and 0.6% hydrogel to the substrate led to an increase of water content at saturation by 45.8% and 116.7%, respectively. Regression curves, expressed by the function  $y = y_0 + (a/x) + (b/x^2)$ , were used to extrapolate water content at  $\Psi = -1.5$  MPa, that was considered as a reference permanent wilting point (Kramer & Boyer, 1995). The theoretical amount of water available to vegetation (AWC) was calculated as the difference between SWC and water content at  $\Psi = -1.5$  MPa. AWC of different substrate components are reported in Table 1a. About 88% of water stored by the substrate was actually available to plants, while in substrate-hydrogel 0.6% blend availability increased to 93%.

Table 1b reports SWC and theoretical AWC of substrate and substrate-hydrogel blends as recorded at the end of the experimental period (September). Water relations of substrate were similar to those recorded in April with an average water content at saturation of  $0.48 \pm 0.05$  g/g for samples collected from both 8 cm and 12 cm deep modules. SWC and AWC of substrate-hydrogel 0.3% and 0.6% blends decreased significantly (by about 27% and 25%, and 51% and 53%, respectively) with respect to values recorded in April ( $P < 0.001$ ). No significant differences in terms of SWC were found between samples collected from 8 and 12 cm modules ( $P = 0.55$ ), as well as between substrate and substrate-hydrogel blends ( $P = 0.08$ ).

#### 3.3. Plant water status, membrane integrity and biomass production

On the basis of leaf pressure-volume curves measured at the beginning of the experiment (April),



**Fig. 3.** Relationships between water potential ( $\Psi$ ) and water content (WC) as measured for polymer hydrogel (a), substrate (b, g) and substrate-hydrogel 0.3% (c, e) and 0.6% (d, f) blends. Moisture release curves were measured in April (left side, a-d) and in September (right side, e-g). Regression curves are expressed by the following function:  $y = y_0 + (a/x) + (b/x^2)$ . Coefficients  $y_0$ ,  $a$  and  $b$  are reported.  $r^2$  ranged between 0.92 and 0.98.

$\Psi_{\text{tip}}$  and  $\pi_0$  of potted plants of *S. officinalis* were found to be  $-1.02 \pm 0.09$  MPa and  $-0.73 \pm 0.04$  MPa, respectively. The water status of plants growing in experimental modules was assessed on two sunny days characterized by different substrate moisture conditions, as indicated by mean values of  $\Psi_{\text{pd}}$  (Fig. 4a and Fig. 5a). On May 21<sup>st</sup>,  $\Psi_{\text{pd}}$  was above the turgor loss point, and averaged  $-0.25$  MPa (Fig. 4a). Under this high substrate moisture conditions,  $\Psi_{\text{min}}$  dropped to about  $-0.65$  MPa and  $g_L$  ranged from an absolute

minimum of  $92 \text{ mmol m}^{-2} \text{ s}^{-1}$  to an absolute maximum of  $204 \text{ mmol m}^{-2} \text{ s}^{-1}$  (Fig. 4b). Values of  $g_L$  recorded in modules Sub8/Hyd0.6, Sub12/Hyd0 and Sub12/Hyd0.6 were slightly higher than those recorded in the other modules. The average REL measured on the same date was  $29.9 \pm 2.1\%$  (Fig. 4c). For all physiological parameters no significant effects of substrate depth and hydrogel amendment were found (Two-ways-ANOVA,  $P > 0.05$  in all cases).

On July 12<sup>th</sup>,  $\Psi_{pd}$  values were below the turgor loss point and ranged between  $-1.6 \pm 0.35$  MPa and  $-3.13 \pm 0.80$  MPa, in plants growing in Sub8/Hyd0.6 and Sub12/Hyd0 modules, respectively (Fig. 5a). Intermediate values were recorded in the other modules. On the same date  $\Psi_{min}$  ranged between a maximum of  $-2.55 \pm 0.42$  MPa (Sub8/Hyd0.6 modules) and a minimum of  $-4.20 \pm 0.89$  MPa (Sub12/Hyd0 modules). No statistically significant first-order effects of treatments (substrate depth and hydrogel addition) were highlighted on  $\Psi_{pd}$  as well as  $\Psi_{min}$  (GLM,  $P > 0.05$ ). The statistically significant differences between Sub8/Hyd0.6 and Sub12/Hyd0 ( $\Psi_{pd}$ ,  $P = 0.02$ ;  $\Psi_{min}$ ,  $P = 0.002$ ) were due to direct or interactive effects of biomass with treatments (see Supplementary data, Table S1). Under low substrate moisture conditions,  $g_L$  averaged  $200 \text{ mmol m}^{-2} \text{ s}^{-1}$  with a maximum of  $385.2 \pm 42.5 \text{ mmol m}^{-2} \text{ s}^{-1}$  recorded in Sub8/Hyd0.6 modules (Fig. 5b). It is worth noting that  $g_L$  of plants growing in Sub8/Hyd0.6 modules was approximately 220% higher than that recorded in modules with 12 cm deep substrate ( $P = 0.01$ ). Figure 5c reports the REL values recorded on July 12<sup>th</sup>. The average REL of all experimental groups was  $25.0 \pm 4.5\%$ . Minimum values were recorded in plants growing in Sub8/Hyd0.6 modules ( $20.3 \pm 2.9\%$ ), while maximum values were recorded in Sub8/Hyd0.3 modules ( $32.7 \pm 4.2\%$ ), with intermediate values recorded for the other

modules. It is worth noting that plants growing in modules with 12 cm deep substrate showed an overall 21% higher REL if compared to values recorded for plants growing in Sub8/Hyd0.6 modules. Pairwise significant differences were observed among several treatment combinations (see Supplementary data, Table S1). A significant effect of hydrogel addition (GLM,  $F = 6.89$ ,  $P = 0.01$ ) as well as of its interaction with biomass (GLM,  $F = 6.04$ ,  $P = 0.02$ ) was found.

A significant correlation ( $r = 0.99$ ,  $P < 0.01$ ) was observed between initial estimated above-ground biomass of plants and the actual values ( $B_a$ ) recorded in April (Fig. 6a). The initial  $B_a$  of potted plants of *S. officinalis* averaged  $8.0 \pm 1.4$  g. The correlation function was used as a non-destructive method to estimate plant biomass at the end of June (Fig. 6b). A general increase of  $B_a$  was recorded in all experimental groups. Plants growing in modules with 8 cm deep substrate increased their biomass by about 190%, while plants growing in 12 cm deep substrate increased biomass by about 320%. The substrate depth influenced significantly the biomass accumulation (Two-way-ANOVA,  $F = 9.09$ ,  $P = 0.01$ ). The lowest value of  $B_a$  was found in Sub8/Hyd0.6 modules ( $20.3 \pm 5.6$  g) and the highest one in Sub12/Hyd0.6 ( $37.4 \pm 9.3$  g), with intermediate values recorded in the other groups.

(a)	Substrate	Polymer hydrogel	Sub/Hyd 0.3	Sub/Hyd 0.6
SWC, g/g	$0.48 \pm 0.01^a$	$115.6 \pm 2.46$	$0.70 \pm 0.12^b$	$1.04 \pm 0.09^c$
AWC, g/g	0.42	109.5	0.61	0.97

(b)	Sub8/Hyd0	Sub 8/Hyd0.3	Sub 8/Hyd0.6	Sub12/Hyd0	Sub12/Hyd0.3	Sub12/Hyd0.6
SWC, g/g	$0.47 \pm 0.06^a$	$0.52 \pm 0.07^a$	$0.52 \pm 0.07^a$	$0.50 \pm 0.05^a$	$0.50 \pm 0.06^a$	$0.50 \pm 0.06^a$
	(-2.1%)	(-25.7%)	(-50.0%)	(+4.2%)	(-28.6%)	(-51.9%)
AWC, g/g	0.41	0.47	0.47	0.46	0.45	0.44
	(-2.4% n.s.)	(-24.0%*)	(-51.6%*)	(+9.5% n.s.)	(-26.2%*)	(-54.6%*)

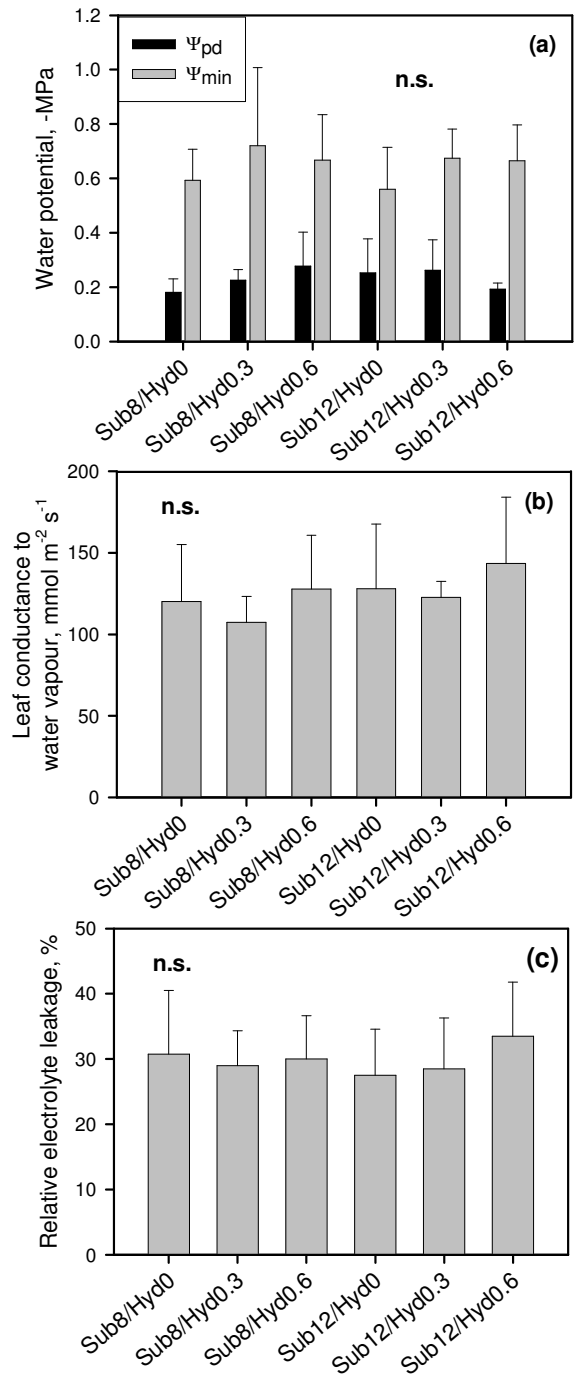
**Table 1.** Water content at saturation (SWC) and theoretical water available to vegetation (AWC) of substrate, polymer hydrogel and substrate-hydrogel 0.3 and 0.6% blends, as recorded in April (a) and in September (b) collecting samples from both 8 and 12 cm deep modules. AWC was calculated as the difference between SWC and water content at  $\Psi = -1.5$  MPa. Different letters indicate significant differences between groups (a), while same letters indicate lack of significant differences (b) in SWC measured in experimental groups, as tested using One-way ANOVA followed by a post hoc Tukey's pairwise comparison. Percentage variation of SWC and AWC as recorded at the end of experimental period with respect to data measured in April, are also reported (b, in brackets). n.s. indicates lack of significant differences, \* indicates significant differences between SWC recorded in April and in September, as tested using unpaired Student's t-tests.

#### 4. Discussion

Our data provide experimental evidence for a positive effect of polymer hydrogel amendment on water status of plants growing on extensive green roof, while also highlighting some possible limitations that need to be addressed by future research in order to assure long-term improvement of green roof water relations.

The substrate used in our experiments showed a water holding capacity of  $0.48 \pm 0.01$  g/g (Table 1a), with a consequent saturated weight below  $1300$  kg/m<sup>3</sup>. Generally, natural soils are characterized by significantly higher saturated weights, even up to about  $2300$  kg/m<sup>3</sup> (Olate *et al.*, 2013). Indeed, over the last decades several lightweight substrates with low organic matter content and high water holding capacity have been specifically developed for green roof technology, thus improving water available to plants even under the harsh conditions of these semi-natural ecosystems (Oberndorfer *et al.*, 2007; Fioretti *et al.*, 2010). On the basis of substrate PV analysis, it was calculated that the theoretical amount of available water to plants ensured by the substrate used in this study was approximately 28% in volume (Table 1a). In the recent scientific literature, the saturated water content of substrates specifically designed for green roof installations is often reported (Nardini *et al.*, 2012; Vijayaraghavan *et al.*, 2012; Olate *et al.*, 2013), but information about the actual amount of water available to plants as guaranteed by these substrates is generally lacking. In a recent study by some of us (Savi *et al.*, 2013) the amount of water available to plants by an intensive green roof substrate was reported to average 34%, a value in substantial agreement with our current results.

The polymer hydrogel used in this study absorbed water up to 115 times its weight (Table 1a), thus proving its potential as an effective soil conditioner. Similar SWCs ranging between 97 to 122 g/g were reported by Bai *et al.* (2010) for four different hydrogels. In the present study, the addition of 0.3% and 0.6% (w/w) hydrogel significantly increased



**Fig. 4.** Values of pre-dawn ( $\Psi_{pd}$ , black columns) and minimum water potential ( $\Psi_{min}$ , grey columns, a), leaf conductance to water vapour ( $g_L$ , b), and relative electrolyte leakage (REL, c) recorded in plants growing in experimental modules on May 21<sup>st</sup>. Means are reported  $\pm$  standard deviation. n.s. indicates lack of significant differences between experimental groups.

( $P < 0.001$ ) the substrate water content at saturation by 46% and 117%, respectively. This also translated into an increase of water available to plants by +45% and +131% for the 0.3 and 0.6% blend, respectively (Table



1a). Our results are consistent with those reported by Farrell *et al.* (2013), where the addition of only 1 g/l of hydrogel to a green roof scoria-based substrate increased SWC and AWC by about 12% and 18%, respectively. Similar magnitudes of SWC increase have been reported for several other green roof substrates (Olszewski *et al.*, 2010) and potting mixtures (Arbona *et al.*, 2005; Apostol *et al.*, 2009). As a consequence, hydrogels have been widely adopted in agriculture, nursery management, and forestry practices (Akhter *et al.*, 2004; Sarvaš *et al.*, 2007; Sojka *et al.*, 2007; Chirino *et al.*, 2011), but little is known about the persistence of their effects on physiochemical properties of soils over the medium-term (Bai *et al.*, 2010). The PV-curves measured at the end of our experimental period (September) i.e. about 5 months after field release of the hydrogel, revealed a significant reduction of water holding capacities for both 0.3% and 0.6% substrate-hydrogel blends with respect to data recorded in April ( $P < 0.001$ ). In fact, SWC as measured in September was not statistically different between substrate and substrate-hydrogel blends collected from both 8 and 12 cm modules ( $P > 0.05$ ). These changes in the water retention properties of substrate-hydrogel blends might suggest limited stability of substrate-hydrogel blends over time. Akhter *et al.* (2004) reported that hydrogels have high water absorption during the first wetting, but decreased efficacy during subsequent wetting cycles. High temperatures, UV exposure, wetting/drying cycles, and microbial activity can cause degradation of polymer chains, resulting in the release of monomers and a consequent decrease of substrate water holding capacity (Holliman *et al.*, 2005; Sojka *et al.*, 2007).

However, such an abiotic-biotic hydrogel degradation is thought to be a relatively slow process that can take several years to be completed (Sojka *et al.*, 2007; Wilske *et al.*, 2014). Therefore, we hypothesize that the reduction of substrate-hydrogel blends' water holding capacity observed in our study, might result from a washout process. Polymer

hydrogels are generally anionic molecules characterized by carboxylate hydrophilic groups which can determine an electrostatic repulsion with negative charges on the surface of substrate particles (Sojka *et al.*, 2007). These anion-anion repulsive forces might reduce absorption of polymer hydrogel molecules to the substrate. As a result, the hydrogel could be easily lost when the substrate is leached by water during intense precipitation or frequent irrigation, with a consequent decrease of the water holding capacity of the blend within some months. In April 2014, one year after field release of hydrogel, experimental modules were disassembled and small amounts of hydrogel aggregates were still observed in both 8 and 12 cm deep substrate originally mixed with 0.6% hydrogel. This observation might suggest that adding higher hydrogel concentration in green roof substrate at the establishment phase might ensure higher amount of available water over longer time intervals. Clearly, further research is needed to improve the long-term effectiveness of hydrogels/substrate blends for their use in green roof installations in drought-prone areas.

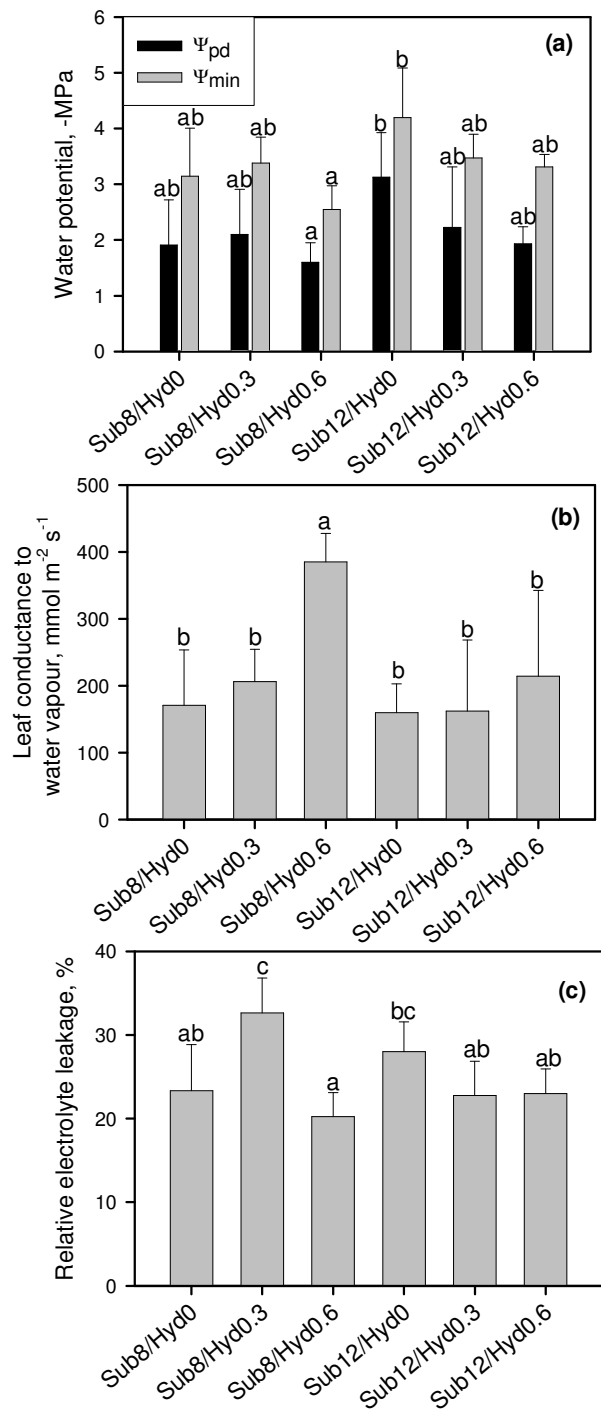
All plants of *S. officinalis* were successfully established in experimental modules due to the rainy 2013 spring (Fig. 2). Physiological parameters of potted plants ( $\Psi_{\text{tip}} = -1.02 \pm 0.09$  MPa and  $\pi_0 = -0.73 \pm 0.04$  MPa) as derived from PV-curves were comparable to those recorded by Savi *et al.* (2013) over the whole vegetative period. On May 21<sup>st</sup>, under high substrate moisture conditions (Fig. 4a),  $\Psi_{\text{pd}}$  and  $\Psi_{\text{min}}$  did not fall below the turgor loss point of the species. Values of  $g_L$  averaged  $125 \text{ mmol m}^{-2} \text{ s}^{-1}$ , while REL averaged 30% mainly due to electrolytes leaking out from the punching area of leaf discs. Indeed, in well-watered and unstressed plants the amount of leakage from controls depends on the species and tissue type, and sometimes it can reach relatively high values (Prášil & Zámečník, 1998). Under low substrate moisture conditions (Fig. 5a), both  $\Psi_{\text{pd}}$  and  $\Psi_{\text{min}}$  dropped below the turgor loss point, highlighting interesting differences between plants growing in the

six experimental groups. Differences were observed also in terms of gas exchange rates and membrane stability (Fig. 5b-c) suggesting that different substrate depths and polymer hydrogel amendments guaranteed different volumes of water available to plants. Notably, the best water status was maintained by plants growing in the Sub8-/Hyd0.6 modules, comprising the shallowest substrate depth but the highest hydrogel concentration.  $\Psi_{pd}$ ,  $\Psi_{min}$  and  $g_L$  recorded in Sub8/Hyd0 and Sub8/Hyd0.3 modules were sharply lower than those recorded in Sub8/Hyd0.6 modules, suggesting again that 0.3% hydrogel amendment provides less advantages to vegetation performance with respect to the 0.6% amendment. Akhter *et al.* (2004) demonstrated that increasing hydrogel addition in the soil improves water status and growth of seedlings of barley, wheat and chickpea, while Olszewski *et al.* (2010) recorded a progressive increment of shoot dry weight and coverage of *Sedum* species growing in green roof substrate amended with increasing hydrogel concentrations. In our study, plants growing in Sub12/Hyd0 modules, containing only 12 cm deep substrate, showed the lowest water potentials as well as leaf conductance to water vapor. It is worth noting that the addition of 0.3% and 0.6% hydrogel led to slightly higher values of  $\Psi_{pd}$ ,  $\Psi_{min}$  and  $g_L$  in 12 cm modules, but water status of plants was always less favorable compared to that estimated for plants growing in modules with 8 cm deep substrate.

The lowest value of REL recorded in Sub8/Hyd0.6 modules (Fig. 5c), indicating the highest level of cell membrane integrity, confirmed that plants growing on substrate mixed with 0.6% hydrogel were favored with respect to plants growing in other modules. A recent study showed that hydrogel treatment can reduce root membrane leakiness of *Quercus rubra* seedlings by 31% after a single desiccation exposure (Apostol *et al.*, 2009). Notably, the highest REL were recorded in Sub12/Hyd0 modules, highlighting the highest leaf tissue injury

caused by drought, high temperatures and other environmental stresses under these conditions.

The recorded trends of  $\Psi_{pd}$ ,  $\Psi_{min}$ ,  $g_L$  and REL

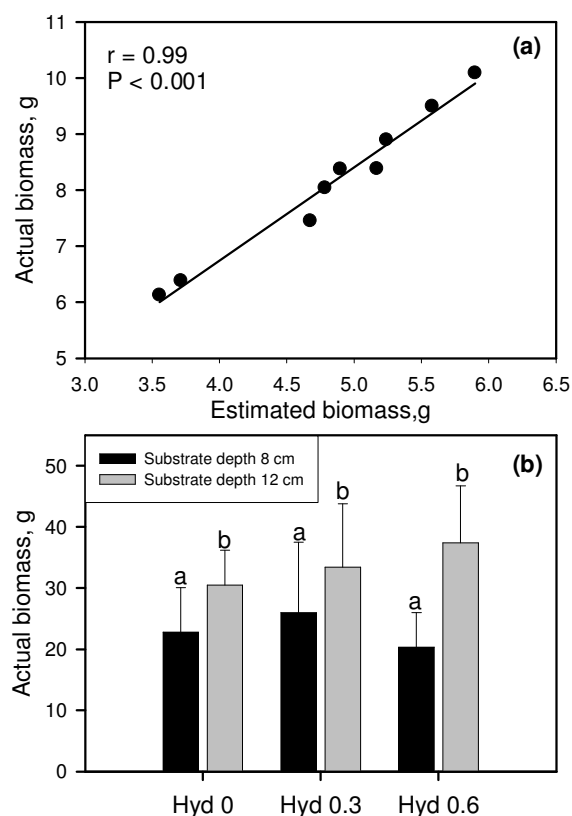


**Fig. 5.** Values of pre-dawn ( $\Psi_{pd}$ , black columns) and minimum water potential ( $\Psi_{min}$ , grey columns, a), leaf conductance to water vapour ( $g_L$ , b), and relative electrolyte leakage (REL, c) recorded in plants growing in experimental modules on July 12<sup>th</sup>. Means are reported  $\pm$  standard deviation. Different letters indicate significant differences between experimental groups (post hoc Tukey's test for the interactive effect of substrate depth and hydrogel addition, from GLM models in Supplementary data, Table S1).

in different modules could be explained on the basis of different plant aboveground biomass as estimated at the beginning of the drought period (Fig. 6b). The best performance in terms of  $g_L$  recorded in Sub8/Hyd0.6 modules was associated to a lower biomass of these plants (Two-way-ANOVA,  $F=9.09$ ,  $P=0.01$ , see Supplementary data, Table S2), also with a significant interactive effects of biomass with substrate depth (GLM,  $F=20.35$ ,  $P=0.001$ , see Supplementary data, Table S1) and with hydrogel amendment (GLM,  $F=9.51$ ,  $P=0.004$ , see Supplementary data, Table S1). In fact, aboveground biomass of plants growing in 12 cm deep substrate was approximately 50% higher than that recorded in 8 cm deep modules. In agricultural studies (Semchenko *et al.*, 2007; Pires *et al.*, 2011), as well as in green roof research (Dunnnett *et al.*, 2008; Papafotiou *et al.*, 2013), it has been largely demonstrated that restricted substrate volume affects plant growth, possibly through chemical and/or mechanical self-inhibition of root growth (Semchenko *et al.*, 2007). Plants can sense the available soil volume and consequently, the developed root mass, as well as total biomass, is a function of available rooting volume (Hess & De Kroon, 2007; Markham & Halwas, 2011). Dunnnett *et al.* (2008) tested the performance of fifteen perennial grass and herb species established into experimental green roof modules containing either 100 or 200 mm depth substrate. Greatest size, survival and flowering performance of planted species were recorded at 200 mm depth. McConnaughay & Bazzaz (1991) grew several colonizing annual species over a wide range of pot volume highlighting that all species had greater vegetative growth in larger pot volumes. In particular, some species nearly doubled their root and shoot mass with doubling of the rooting volume, which is consistent with our results where a 50% higher substrate volume available in 12 cm deep modules with respect to 8 cm deep ones translated in a 50% higher biomass accumulation. The positive correlation between deeper substrates and plant growth has been mainly attributed to the increased water holding

capacity of substrates and to the evidence that shallower substrates lose their moisture content faster during a drought period. In our study, treatments that included the use of hydrogel (higher SWC) promoted a slight increase of plant dry mass, with respect to treatments without the hydrogel, but such effects were not statistically significant (Two-way-ANOVA,  $F=0.27$ ,  $P=0.77$ ).

Our results, based on a five month study, apparently confirm that larger substrate volumes available for root system development favour biomass accumulation, which in turn leads to a faster depletion of water reserves during drought periods. Shallow



**Fig. 6.** Relationship between initial estimated above-ground biomass of potted plants of *S. officinalis* and the actual values ( $B_a$ ), as measured at the beginning of the experiment (a). The correlation coefficient  $r$  and  $P$  value (Pearson Product Moment Correlation) are reported. Actual above-ground biomass of plants growing in different green roof systems, as estimated at the end of June (b) using the correlation function in a. Means are reported  $\pm$  standard deviation. Within each level of hydrogel addition, different letters indicate significant differences between substrate depths (Tukey's post-hoc test from Two-way-ANOVA in Supplementary data, Table S2).

substrate depth resulted in reduced plants' growth, that translated into a more conservative use of available water and better water status of vegetation at the establishment phase. The use of shallow substrate added with a hydrogel in extensive green roof settings could lead to improved performance under drought, reduction of the weight load on infrastructure, as well as of the installation costs of the system. The resulting small sized vegetation would also assure low maintenance costs, representing an appreciated characteristic for extensive green roof.

## 5. Conclusion

Our data show that even small amounts of hydrogels mixed to green roof substrates have the potential to significantly improve the amount of available water to plants. Polymer hydrogel amendment enhanced water supply to plants and improved their performance in green roof systems under drought. In particular, the functional advantage of hydrogels is higher when reduced substrate depths are involved. This experimental evidence suggests that the use of hydrogels can improve water status of plants and could help to avoid water stress in substrates with low water storage due to open texture or reduced depth. Reduced weight load on infrastructure and limited installation as well as maintenance costs would be also achieved. However, the recorded loss of improved water holding capacity of substrate-hydrogel blends over a relatively short-time interval raises questions about how to improve hydrogels long-term effectiveness. More efforts should be invested in the study of interactions between different polymer hydrogels and potential green roof substrates. Future research should be based on comparison and evaluation of physical-chemical characteristics of hydrogels and their effects on substrate and plant water status over long lifespans.

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## Supplementary material

<b>Predawn water potential</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Factor I-Substrate depth	0.04	1	0.04	0.12	0.7379
Factor II-Hydrogel addition	0.17	2	0.08	0.26	0.7731
Biomass	2.49	1	2.49	7.80	0.0175
Factor I-Substrate depth*Factor II-Hydrogel addition	0.10	2	0.05	0.15	0.8634
Factor I-Substrate depth*Biomass	0.18	1	0.18	0.56	0.4715
Factor II-Hydrogel addition*Biomass	0.60	2	0.30	0.94	0.4205
<b>Minimum water potential</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Factor I-Substrate depth	0.52	1	0.52	2.85	0.1172
Factor II-Hydrogel addition	1.10	2	0.55	3.01	0.0873
Biomass	2.17	1	2.17	11.86	0.0049
Factor I-Substrate depth*Factor II-Hydrogel addition	0.41	2	0.20	1.11	0.3614
Factor I-Substrate depth*Biomass	0.52	1	0.52	2.84	0.1176
Factor II-Hydrogel addition*Biomass	1.79	2	0.89	4.90	0.0278
<b>Leaf conductance to water vapour</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Factor I-Substrate depth	46813.78	1	46813.78	29.92	0.0002
Factor II-Hydrogel addition	16053.26	2	8026.63	5.13	0.0267
Biomass	768.97	1	768.97	0.49	0.4978
Factor I-Substrate depth*Factor II-Hydrogel addition	72401.45	2	36200.72	23.14	0.0001
Factor I-Substrate depth*Biomass	31837.58	1	31837.58	20.35	0.0009
Factor II-Hydrogel addition*Biomass	29754.70	2	14877.35	9.51	0.0040
<b>Relative electrolyte leakage</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Factor I-Substrate depth	0.02	1	0.02	0.00	0.9604
Factor II-Hydrogel addition	117.80	2	58.90	6.89	0.0115
Biomass	8.30	1	8.30	0.97	0.3453
Factor I-Substrate depth*Factor II-Hydrogel addition	39.57	2	19.79	2.32	0.1447
Factor I-Substrate depth*Biomass	1.32	1	1.32	0.15	0.7021
Factor II-Hydrogel addition*Biomass	103.15	2	51.57	6.04	0.0170

**Table S1.** Summary of the generalized linear models (GLM) testing the effects of substrate depth, hydrogel addition, and biomass, as well as their interactions, on physiological parameters ( $\Psi_{pd}$ ,  $\Psi_{min}$ ,  $g_L$ , REL) recorded in experimental modules on July 12<sup>th</sup>.

<b>Biomass</b>	<b>SS</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Factor I-Substrate depth	652.85	1	652.85	9.09	0.008
Factor II-Hydrogel addition	38.66	2	19.33	0.27	0.767
Factor I-Substrate depth*Factor II-Hydrogel addition	116.86	2	58.43	0.81	0.46

**Table S2.** Summary of the Two-Way-ANOVA testing the effects of substrate depth, hydrogel addition and their interaction on above-ground biomass of plants growing in different green roof systems, as estimated at the end of June.



# 3. Does shallow substrate improve water status of plants growing on green roofs? Testing the paradox in two sub-Mediterranean shrubs

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## HIGHLIGHTS

- Green roof technology is under-represented in warm sub-Mediterranean areas
- Substrate depth reduction is mandatory in order to limit installation weight
- Water status of drought-adapted shrubs was monitored in 10 or 13 cm deep substrate
- Reduced substrate depth translates into less severe water stress suffered by plants
- Rainfalls lead to faster water availability recovery if shallow substrates are used

## ABSTRACT

Green roofs are artificial ecosystems providing ecological, economic, and social benefits to urban areas. Recently, the interest in roof greening has increased even in Mediterranean and sub-Mediterranean areas, despite the climate features and reduced substrate depth expose plants to extreme stress. To limit installation weight and costs, recent green roof research aims to reduce substrate depth, which apparently contrasts with the need to maximize the amount of water available to vegetation. We monitored water status, growth, and evapotranspiration of drought-adapted shrubs (*Cotinus coggygria*, *Prunus mahaleb*) growing in experimental green roof modules filled with 10 or 13 cm deep substrate. Experimental data showed that: a) reduced substrate depth translated into less severe water stress experienced by plants; b) shallower substrate indirectly promoted lower water consumption by vegetation as a likely consequence of reduced plant biomass; c) both large and small rainfalls induced better recovery of water content of substrate, drainage, and water retention layers when shallow substrate was used. Evidence was provided for the possibility to install extensive green roofs vegetated with stress-tolerant shrubs in sub-Mediterranean areas using 10 cm deep substrate. Green roofs based on shallow substrate and drought-tolerant plants may be an optimal solution for solving urban ecological issues.

*Keywords* - substrate depth, water availability, drought stress, evapotranspiration, *Cotinus coggygria*, *Prunus mahaleb*

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## 1. Introduction

The negative environmental impacts of urbanization are partially driven by the replacement of natural vegetation with hard, impervious surfaces such as concrete and asphalt (Grimm *et al.*, 2008). Urban trees and green areas (Armson *et al.*, 2012), as well as green roofs (Berardi *et al.*, 2014; Susca *et al.*, 2011; Thuring & Dunnett, 2014) represent effective mitigation strategies that can partially offset the negative consequences of expanding urban areas. Several recent studies have highlighted the potential of green roofs to provide environmental, economic, and social benefits to towns, including reduction and delay of water run-off (Qin *et al.*, 2013; Voyde *et al.*, 2010), mitigation of heat island effects (Susca *et al.*, 2011), thermal (MacIvor *et al.*, 2011; Olivieri *et al.*, 2013) and acoustic (Connelly & Hodgson, 2013) insulation of buildings with related energy savings (Zinzi & Agnoli, 2012), increased photovoltaic efficiency (Chemisana & Lamnatou, 2014), pollution abatement (Göbel *et al.*, 2007; Whittinghill *et al.*, 2014), habitat and biodiversity conservation (Benvenuti, 2014; Cook-Patton & Bauerle, 2012; Madre *et al.*, 2014), and creation of pleasant recreational spaces (Lee *et al.*, 2014; White & Gatersleben, 2011).

A green roof is generally composed of several functional layers, i.e. a waterproofing and root resistant membrane, a drainage layer, a filter membrane, a lightweight mineral substrate, and vegetation. A water retention tissue is often placed under the drainage layer. Extensive green roofs are characterized by a thin substrate layer (< 20 cm), supporting the growth of small sized plants (less than 50 cm tall) like succulents, stress tolerant herbs, and woody creeping shrubs, generally requiring low maintenance costs (Berardi *et al.*, 2014; Schweitzer & Erell, 2014). An irrigation system is often not necessary (Bernardi *et al.*, 2014), but an increasing number of authors have suggested that irrigation may be essential for the establishment of extensive green roofs in arid and semi-arid regions (Benvenuti, 2014; Kotsiris *et al.*, 2012; Ntoulas *et al.*,

2013; Schweitzer & Erell, 2014). Indeed, green roofs represent challenging environments for plant survival due to high temperatures and dramatic fluctuations in water availability (Nagase & Dunnett, 2010). In regions with a temperate climate, the roof surfaces covered by vegetation are increasing year after year (Berardi *et al.*, 2014; Connelly & Hodgson, 2013; Thuring & Dunnett, 2014). In Mediterranean regions high summer temperatures and prolonged seasonal drought make the installation of efficient and fully functional green roofs more difficult. However, research efforts and public interest for the development of this technology are increasing (Benvenuti & Bacci, 2010; Kotsiris *et al.*, 2012; Razzaghmanesh *et al.*, 2014; Schweitzer & Erell, 2014).

In order to promote the adoption of green roof technology in drought-prone areas, the plant selection process as well as the improvement of the amount of water available to vegetation are key research targets (Berardi *et al.*, 2014; Savi *et al.*, 2014). The selection of suitable plant species should be based on an ecophysiological approach, starting from identification of autochthonous plants adapted to dry shallow soils, coupled with sound analysis of physiological traits related to drought resistance (Caneva *et al.*, 2013; Razzaghmanesh *et al.*, 2014; Savi *et al.*, 2013). The survival of plants over green roofs has been reported to be positively correlated with the substrate depth (Kotsiris *et al.*, 2012; Madre *et al.*, 2014; Papafotiou *et al.*, 2013). This trend has been mainly related to the higher water-holding capacity of deep substrates compared to shallow ones (Getter & Rowe, 2009; Ntoulas *et al.*, 2013), and to the mitigation of temperature extremes (Boivin *et al.*, 2001). However, green roof installations have to be reconciled with buildings' structural features, and deep substrates lead unavoidably to larger structural loads. The densely populated Mediterranean cities are mostly occupied by aged buildings with limited tolerance of additional weight loads and in this case extensive green roofs with a shallow substrate depth are often the only option

available (Ntoulas *et al.*, 2013; Papafotiou *et al.*, 2013). Hence, a key target of green roof research is to increase the amount of water available to plants, while maintaining reduced substrate depth (Farrell *et al.*, 2013; Papafotiou *et al.*, 2013; Savi *et al.*, 2013; Savi *et al.*, 2014). To this aim, Papafotiou *et al.* (2013) investigated the combined effect of the type/depth of the substrate, as well as of irrigation frequency on the growth performance of six Mediterranean xerophytic species. The use of grape marc compost as an organic component of the green roof substrate, instead of peat, helped to reduce the water needs of plants, as well as the substrate depth, while not affecting plant growth. Recent studies by some of us provided experimental evidence that slight modifications in the geometrical features of drainage elements can improve plant survival during prolonged drought events (Savi *et al.*, 2013). It was also suggested that the use of polymer-hydrogel amendment might lead to a marked increase of the amount of water available to vegetation, improving the plant water status, particularly when reduced substrate depths are used (Savi *et al.*, 2014).

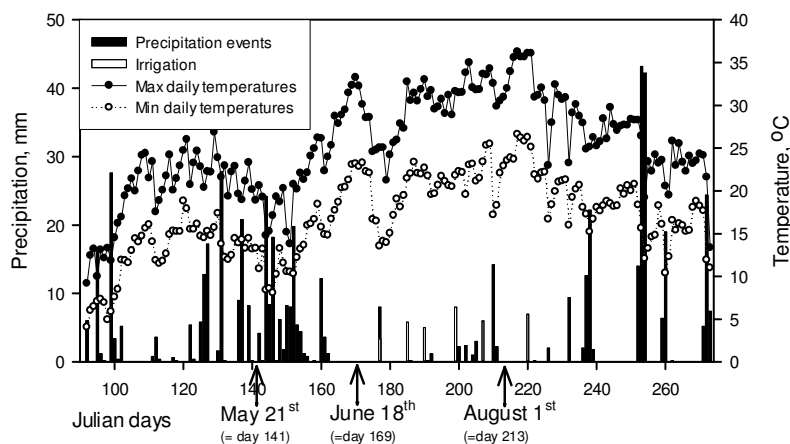
The present study aims to: 1) investigate the performance of two sub-Mediterranean shrubs grown over green roofs with extremely shallow substrate depths; 2) identify the impact of substrate thickness on

shrubs water status, survival, and growth in a sub-Mediterranean climate; 3) verify implications of two different substrate depths in terms of evapotranspiration rates; 4) quantify eventual differences in drainage and water accumulation capacity of green roof systems characterized by different substrate depths.

## 2. Materials and methods

### 2.1 The study area

The study was carried out between early April and late October 2013, over the flat rooftop of a building of the University of Trieste (45°39'40" N, 13°47'40" E; altitude 125 m a.s.l.). The area is characterized by a sub-Mediterranean climate with a relatively hot and dry summer. Mean annual temperature in the period 1994-2013 (<http://www.osmer.fvg.it>) averaged 15.7 °C, with maxima and minima monthly averages of 25 °C and 6.8 °C recorded in July and January, respectively. Mean annual rainfall is 869 mm, with a peak of precipitation in November (106 mm) and monthly minima of 55 mm (July) and 51 mm (January). The dry and cold Bora (ENE) is the predominant wind that blows in the study area for approximately 3000 h/year (Martini, 2009).



**Fig. 1** Precipitation events (black columns), supplied irrigation (white columns), and maximum and minimum daily temperatures (black and white circles, respectively) recorded between 1 April and 30 September 2013 on the rooftop near the experimental modules. The tree sampling days (21 May, 18 June, and 1 August) are marked.

## 2.2 Experimental modules and plant material

In April 2012 wooden beams were used to construct six experimental modules with an overall surface of 2.5 m<sup>2</sup> each. The modules were laying on a 30 cm high polystyrene panel platform to allow drainage of rainwater from each module. A 6-layered green roof was installed using the SEIC extensive system (Harpo Spa, Trieste, Italy) which includes a waterproof and root resistant PVC membrane (Harpoplan ZDUV 1.5), a moisture retention layer with water holding capacity up to 14 L/m<sup>2</sup> (Idromant 4), a drainage layer of plastic profiled elements (MediDrain MD 40, water retention 4 L/m<sup>2</sup>), a filter membrane (MediFilter MF1) and SEIC substrate for extensive green roof installations (dry bulk density = 848 kg/m<sup>3</sup>). The cavities of the Medidrain MD40 were modified with holes of 4 mm diameter (340 holes/m<sup>2</sup>) to promote the coupling between retention layer and substrate (Savi *et al.*, 2013). The substrate was a blend of lapillus, pomix (light highly porous rock of volcanic origin) and zeolite enriched with 2.9% organic matter (peat), with grain size ranging between 0.05 mm and 20 mm. The substrate had pH = 6.8, total porosity = 67.35%, drainage rate = 67.36 mm min<sup>-1</sup>, water content at saturation = 0.44 g g<sup>-1</sup>, cation exchange capacity = 23.8 meq 100 g<sup>-1</sup>, electrical conductivity = 9 mS m<sup>-1</sup>.

The experimental modules were divided into two categories on the basis of substrate depth: 10 cm (D-10, 3 modules) and 13 cm (D-13, 3 modules). Each experimental module was equipped with a soil moisture content sensor (WC, EC-5, Decagon Devices Inc., USA) installed in the middle of the soil profile. The WC data were recorded at 60 min intervals. At the beginning of the experiments, the relationships between water content and water potential (moisture release curve) of the substrate was measured according to Savi *et al.* (2013) and the regression curve function was used to convert values of WC recorded by the soil moisture content sensors in values of substrate water potential ( $\Psi_{\text{sub}}$ , MPa).

In mid April 2012, 15 individuals of *Cotinus coggygria* Scop. and 15 individuals of *Prunus mahaleb* L. were randomly planted in each experimental module, for a total of 30 plants per module (distance between plants = 27 cm). Shrubs were selected because woody plants show generally an isohydric response (Nardini *et al.*, 2003) and have, hence, higher probability to survive in the harsh environmental conditions of green roofs. Two-year old potted plants were provided by the Pascual Regional Forest Service Nursery (Tarcento, Udine, Italy). After planting, each individual was irrigated with 2 L of water. During the 2012 and 2013 vegetative seasons, modules received natural precipitation. In order to avoid severe water deficit stress to plant material, additional irrigation (3-12 mm) was supplied during severe drought (for a total of 7 events between May and August 2013), i.e. when the substrate water potential of D-10 modules dropped below -3 MPa. The pre-set value was based on the water potential at the turgor loss point ( $\Psi_{\text{tlp}}$ ) data of *C. coggygria* and *P. mahaleb* (around -3 MPa) as recorded in July-August in the natural habitat of the species (Nardini *et al.*, 2003). All modules were watered at the same time. The supplied water did not fully saturate the substrate profile, but allowed the  $\Psi_{\text{sub}}$  to increase by about 0.5 MPa.

*C. coggygria* is a deciduous shrub native to southern Europe and central Asia (Pignatti, 2002). *P. mahaleb* is a large shrub or small tree native to SE Europe and NE Turkey (Pignatti, 2002). The two species were selected on the basis of their high resistance to drought stress (Nardini *et al.*, 2003; Nardini *et al.*, 2012) and relative abundance in the surrounding local vegetation growing on shallow limestone soils with low water storage capacity (Poldini, 2009), and their previously reported capability to survive green roof conditions (Nardini *et al.*, 2012).

Air temperature and humidity (EE06-FT1A1-K300, E+E Elektronik, USA), precipitation (ARG 100 Raingauge, Environmental Measurements Limited,

UK), wind speed and direction (WindSonic 1, Gill Instruments, UK), and irradiance (MS-602, EKO Instruments, Japan) on the rooftop were recorded, at 5 min time intervals, during the entire study period by a weather station installed a few meters from the experimental modules.

### 2.3 Monitoring plant water status and membrane integrity

Leaf water potential isotherms (P-V curves) of *C. coggygria* and *P. mahaleb* were measured at the end of May and at the end of August 2013, i.e. one year after planting. The water potential at the turgor loss point ( $\Psi_{\text{tlp}}$ ) and osmotic potential at full turgor ( $\pi_0$ ) were derived from PV curves, according to Tyree & Hammel (1972).

Leaves for P-V curves were collected before 0900 h (solar time) from both D-10 and D-13 modules. Mature leaves were wrapped in cling film and left rehydrating with the petiole dipped in distilled water for approximately 1 hour. Measurements of water potential ( $\Psi_{\text{leaf}}$ ) were made with a pressure chamber (mod. 1505D, PMS Instruments, USA, Scholander *et al.*, 1965), and the experiment continued only for fully hydrated leaves ( $\Psi_{\text{leaf}} > -0.2$  MPa). After  $\Psi_{\text{leaf}}$  measurement, the turgid weight (TW) of leaves was immediately measured. Leaves were then left dehydrating on the bench and sequential measurements of  $\Psi_{\text{leaf}}$  and fresh weight (FW) were performed. The cumulative water loss of leaves ( $Wl = TW - FW$ ) was plotted versus  $1/\Psi_{\text{leaf}}$ , and experiments were concluded when this relationship became linear ( $r > 0.98$ ). The  $\pi_0$  was calculated by extrapolating the linear part of the P-V curve to  $Wl = 0$ , while  $\Psi_{\text{tlp}}$  was estimated as the flex point transition between the curvilinear and linear parts of the relationship (Bartlett *et al.*, 2012; Tyree & Hammel, 1972).

In order to assess possible differences in terms of plant water status among species and experimental modules, pre-dawn ( $\Psi_{\text{pd}}$ ) and minimum ( $\Psi_{\text{min}}$ ) leaf water potential, and leaf conductance to water vapor

( $g_L$ ) were monitored on a monthly basis. Measurements were performed on the following selected sunny days: 21 May, 18 June, and 1 August 2013.

$\Psi_{\text{pd}}$  and  $\Psi_{\text{min}}$  were measured on leaves sampled before 0500 h and between 1200 and 1300 h (solar time), respectively. At least 3 leaves per species and per module were randomly collected and immediately wrapped in cling film, inserted in plastic bags, and transported to the laboratory using a refrigerated bag. The water potential was measured with a pressure chamber as described above. The  $g_L$  was measured on at least one leaf of three different individuals per experimental module (for a total of 9 measurement per species per substrate depth), between 1200 and 1300 h (solar time), using a steady-state porometer (SC1, Decagon Devices, WA, USA). Before each measurement session, the porometer was left equilibrating for 30 min nearby the experimental modules and then calibrated, according to manual specifications. In each sampling day, different individuals randomly selected among 15 plants of *C. coggygria* and *P. mahaleb* were measured in each experimental module. Climatic data (air temperature and humidity) were provided by the weather station (see above), while photosynthetic photon flux density was measured with a portable quantum sensor (HD 9021, Delta Ohm, Italy).

On 1 August, after  $g_L$  and  $\Psi_{\text{min}}$  measurements, leaves were collected for an electrolyte leakage test in order to assess eventual differences in cell membrane integrity (Bajji *et al.*, 2001; Vasquez-Tello *et al.*, 1990) among species and modules. For each experimental module, ten leaf disks (area = 0.2 cm<sup>2</sup>) were punched from at least 4 leaves per species and immediately inserted in a test bottle containing 7 ml of deionized water. The bottles were left on a stirrer at room temperature. After about three hours, the initial electrical conductivity ( $C_i$ ) of the solution was measured, using a conductivity meter (Twin Cond B-173, Horiba, Japan). Samples were then subjected to three freezing (1 h at - 20 °C) and thawing (1 h at lab

(a) $\Psi_{pd}$ , 21st May	SS	df	MS	F	P
Substrate depth	0.075	1	0.075	20.465	0.002
Species	0.508	1	0.508	138.342	<0.001
Factor I*Factor II	0.029	1	0.029	7.893	0.023
Residual	0.029	8	0.004		
(b) $\Psi_{pd}$ , 1st August	SS	df	MS	F	P
Substrate depth	0.306	1	0.306	9.191	0.016
Species	0.758	1	0.759	22.765	0.001
Factor I*Factor II	0.012	1	0.012	0.367	0.562
Residual	0.267	8	0.033		
(c) $\Psi_{min}$ , 1st August	SS	df	MS	F	P
Substrate depth	1.211	1	1.211	11.695	0.003
Species	0.065	1	0.065	0.624	0.439
Factor I*Factor II	0.031	1	0.031	0.294	0.594
Residual	1.967	19	0.104		
(d) $g_L$ , 1st August	SS	df	MS	F	P
Substrate depth	55670.1	1	55670.1	4.356	0.05
Species	32907.1	1	32907.1	2.575	0.124
Factor I*Factor II	5054.4	1	12778.9	0.396	0.537
Residual	255578.7	20	12778.9		
(e) G, 1 year after planting	SS	df	MS	F	P
Substrate depth	322.7	1	322.7	3.601	0.094
Species	5522.5	1	5522.5	61.617	<0.001
Factor I*Factor II	45	1	45	0.502	0.499
Residual	6607.2	11	600.7		

**Table 1.** Summary of the Two-way-ANOVA testing the effects of substrate depth (D-10 and D-13, Factor I), plant species (*C. coggygia* = CC and *P. mahaleb* = PM, Factor II), and their interaction on pre-dawn water potential ( $\Psi_{pd}$ , a-b), minimum water potential ( $\Psi_{min}$ , c), leaf conductance to water vapor ( $g_L$ , d), and relative growth (G) as estimated on 21 May 2013 (a), 1 August 2014 (b-d) 2013, and one year after planting (e) in experimental green roof modules.

temperature) cycles in order to cause complete membrane disruption and electrolyte release from leaf tissue, and the final electrical conductivity ( $C_f$ ) was measured. The relative electrolyte leakage (REL) was calculated as:  $REL = (C_i / C_f) \times 100$ .

#### 2.4 Estimation of plant growth and evapotranspiration rates

In April 2012, the diameter at the root collar ( $S_{d_i}$ ) of all planted individuals of *C. coggygia* and *P. mahaleb* was measured using a digital caliper (Absolute Coolant-Proof, Mitutoyo, USA). In order to estimate eventual differences in growth of plants growing on D-10 or D-13 modules, the diameter was measured again at the beginning of June 2013 ( $S_{d_f}$ ).

The relative diameter increment (G) was expressed as follows:  $(S_{d_f} - S_{d_i}) / S_{d_i} \times 100$ .

The soil moisture content sensors (see above) allowed a regular monitoring of substrate water content (WC) in D-10 and D-13 modules. The dry mass of the substrate ( $M_s$ ) contained in D-10 and D-13 modules was calculated multiplying the substrate volume with substrate dry bulk density. The WC data (g of water per g of substrate) recorded by soil moisture content sensors every day at midnight, were used to calculate the total amount of water contained in the substrate of each module as follows:  $WCI = WC \times M_s$ . Changes in WCI were used to estimate daily evapotranspiration rates with the following equation:  $ET = (WCI - WCI_{+24h}) / A$ , where  $WCI_{+24h}$  is the substrate water content measured 24 hours after the previous WCI measurement, and A is the area of the experimental modules (2.5 m<sup>2</sup>). For evaluation of ET only data recorded on days without rain events or supplied irrigation were used.

#### 2.5 Testing water content recovery of green roof layers

On the basis of collected data, highlighting significant differences in water status of plants growing in green roof modules, supplementary laboratory experiments were carried out in September-October 2013 to evaluate eventual differences in terms of water drainage and substrate water content/potential recovery after rainfall in 10 and 13 cm deep modules. Small-scale models of D-10 and D-13 modules were reconstructed using plastic tube segments (diameter 12 cm; height 14 cm). The segments' bottom was covered with filter membrane fixed with a plastic band. The small module was placed on a square plastic profiled element and moisture retention layer (30×30 cm) previously weighed (DW). Modules were filled with 10 or 13 cm deep dry substrate. The substrate was gently air-dried at laboratory temperature for at least 5 days and then placed in an oven for 8 hours at 30 °C. A spray bottle was used to simulate small (5 and 10 mm) or large (30 and 40 mm) rain events in 15 min time

intervals. Modules were then covered with cling film for at least 15 min in order to allow water drainage, favored by the drainage rate of the substrate used (= 67.36 mm min<sup>-1</sup>). Finally, modules were disassembled and plastic profiled element and moisture retention layer were re-weighed (FW). The amount of water drained and accumulated by the two layering elements (AW) was calculated as FW–DW. Simulation of small rain events did not result in any water drainage. Hence, the substrate from modules subjected to 5 and 10 mm rain events simulation was carefully mixed and small samples were collected to measure substrate water potential ( $\Psi_{\text{sub}}$ ) with a dewpoint hygrometer (WP4, Decagon Devices, USA, Whalley *et al.*, 2013). After  $\Psi_{\text{sub}}$  measurement, fresh weight (FW) of samples was immediately recorded. Samples were oven-dried for 24 h in order to obtain their dry weight (DW). Water content (WC) was calculated as (FW–DW) / DW.

## 2.6 Statistics

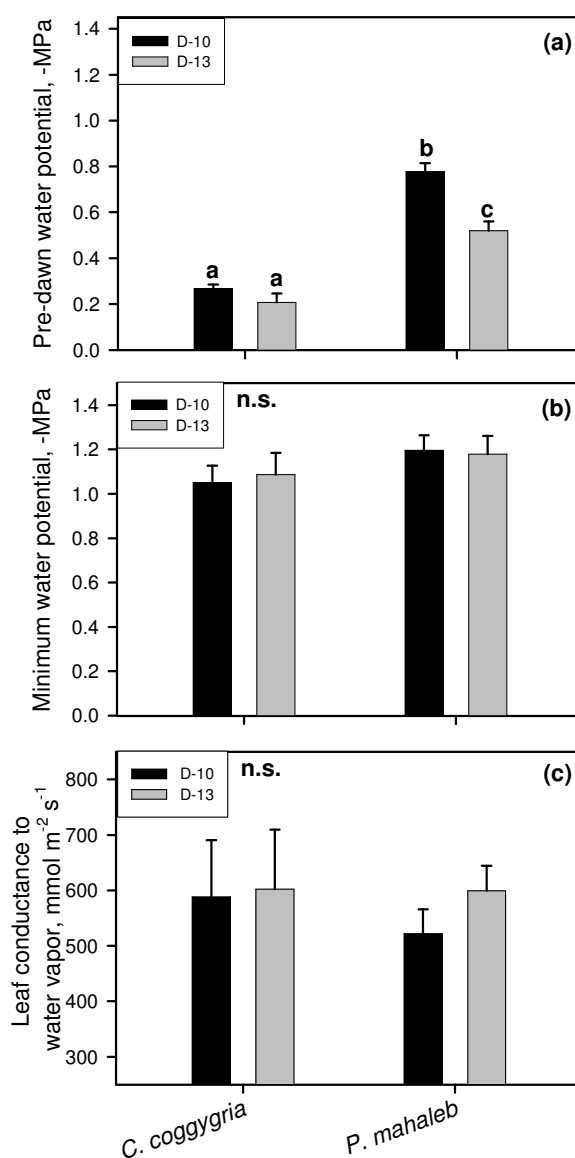
Data were analyzed with Sigma Stat v. 2.03 (SPSS Inc.). Statistically significant differences between experimental groups were assessed with unpaired Student's t-test and Two-way-ANOVA (factors: substrate depth and plant species). Pairwise differences were tested using Tukey's post hoc test. All results were considered statistically significant at  $P \leq 0.05$ .

## 3. Results

### 3.1 Microclimatic data

Minimum and maximum daily temperatures and precipitation events recorded during the study period are reported in Fig. 1. The mean daily temperature averaged  $20.7 \pm 5.4$  °C, with an absolute minimum of 4.1 °C and an absolute maximum of 36.3 °C recorded on 2 April (spring) and 5 August (summer), respectively. The daily average relative humidity of air ranged between 37% and 89%. The total rainfall was 551 mm, mainly occurring in May (189 mm) and September (162 mm), and nearly absent in July (27 mm). According to the Regional

Meteorological Observatory (<http://www.osmer.fvg.it>) the precipitation anomaly (referred to the 1994–2013 standard period) in the study area was +97% in May and -68% in July, respectively. Despite relatively frequent and abundant spring rainfalls, during the dry period, a total of 35 mm of water was supplied to the experimental modules with irrigation to avoid severe water stress (Fig. 1).



**Fig. 2** Pre-dawn ( $\Psi_{\text{pd}}$ , a) and minimum ( $\Psi_{\text{min}}$ , b) leaf water potential, and leaf conductance to water vapor ( $g_L$ , c) as measured for *C. coggygria* and *P. mahaleb* in 10 cm thick (D-10, black columns) and 13 cm thick (D-13, grey columns) experimental modules on 21 May 2013. Means are reported  $\pm$  SEM. Lettering indicates significant differences among experimental groups (see Tabel 1), while n.s. indicates the lack of significant differences.

### 3.2 Monitoring plant water status and membrane integrity

At the end of May (spring), the water potential at the turgor loss point and the osmotic potential at full turgor were  $-1.73 \pm 0.05$  MPa and  $-1.21 \pm 0.02$  MPa for *C. coggygia*, and  $-2.06 \pm 0.05$  MPa and  $-1.61 \pm 0.09$  MPa for *P. mahaleb*, respectively. During the summer season, *P. mahaleb* apparently adjusted  $\Psi_{\text{tlp}}$  to values of  $-2.59 \pm 0.14$  MPa at the end of August. It was not possible to measure PV curves of *C. coggygia* at the end of August because of lack of leaf rehydration, probably due to extensive drought-induced leaf xylem cavitation and embolism.

The water status of plants growing in the experimental modules was assessed on three sunny days characterized by different substrate moisture conditions, as revealed by volumetric soil moisture content sensors and by  $\Psi_{\text{pd}}$  measurements (Fig. 2-3). On 21 May, *C. coggygia* and *P. mahaleb* showed values of  $\Psi_{\text{pd}}$  higher than  $-0.8$  MPa (Fig. 2a). A significant effect of substrate depth, plant species, and interaction between the two factors was observed on  $\Psi_{\text{pd}}$  values ( $P < 0.05$ , Table 1). In particular, *P. mahaleb* plants growing in 13 cm modules showed a significantly more favorable water status ( $-0.52 \pm 0.04$  MPa) compared to those growing in 10 cm deep modules ( $-0.78 \pm 0.04$  MPa). On the same date, the observed  $\Psi_{\text{min}}$  was relatively high for all plants (about  $-1.10$  MPa, Fig. 2b), while  $g_L$  reached values of about  $580 \text{ mmol m}^{-2} \text{ s}^{-1}$  (Fig. 2c). For  $\Psi_{\text{min}}$  and  $g_L$  no significant effects of substrate depth and plant species were observed ( $P > 0.05$ ).

On 18 June (spring), at the onset of the summer dry period,  $\Psi_{\text{pd}}$  of both shrub species was still relatively high (between  $-1.09$  and  $-1.61$  MPa), while  $\Psi_{\text{min}}$  exceeded the turgor loss point by about  $0.35$  MPa in *C. coggygia* and  $0.02$  MPa in *P. mahaleb*, respectively (data not shown). A sharp (but not significant) decrease of  $g_L$  was recorded under this moderate water deficit condition. However,  $g_L$  showed high intra- and inter-specific variability, with values

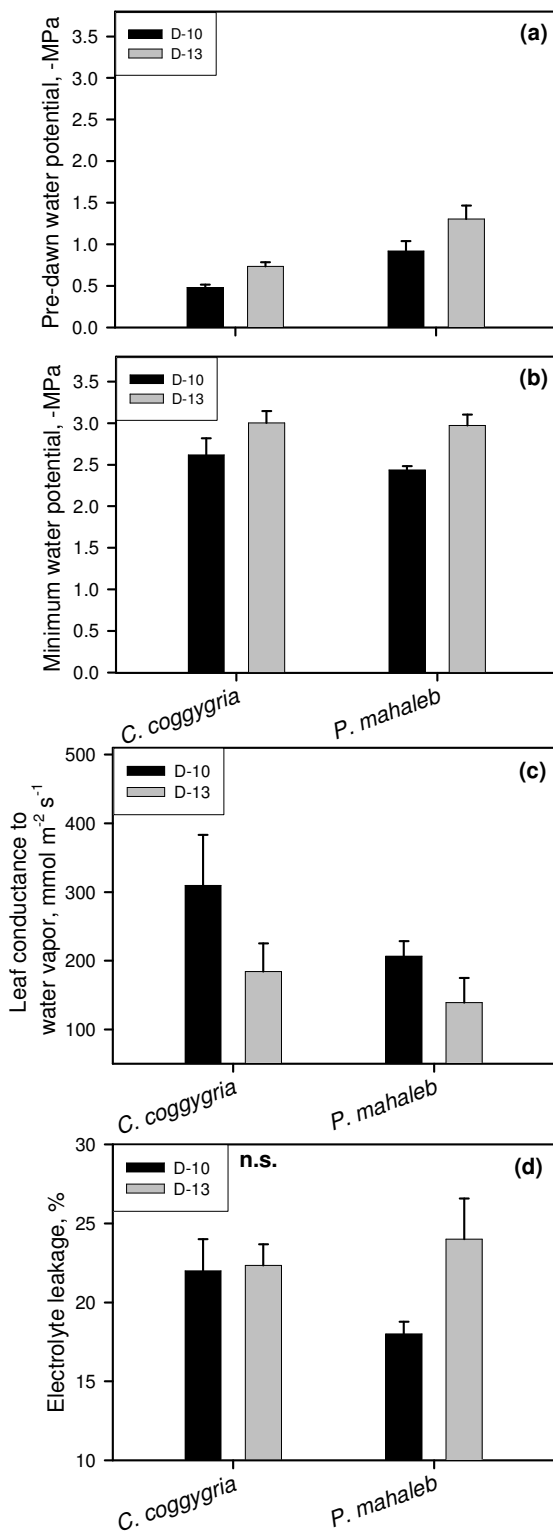
ranging from a minimum of  $32.0 \pm 10.0 \text{ mmol m}^{-2} \text{ s}^{-1}$  to a maximum of  $89.5 \pm 27.5 \text{ mmol m}^{-2} \text{ s}^{-1}$  as recorded for *P. mahaleb* growing in D-13 and *C. coggygia* in D-10 modules. Despite the large difference in terms of  $\Psi_{\text{sub}}$  in D-10 ( $-2.23 \pm 0.90$  MPa) and D-13 modules ( $-1.01 \pm 0.24$  MPa), no statistically significant differences were recorded between experimental groups in terms of plant water status ( $P > 0.05$ ).

On 1 August (summer), a significant effect of substrate depth on  $\Psi_{\text{pd}}$ ,  $\Psi_{\text{min}}$ , and  $g_L$  was observed ( $P \leq 0.05$ , Table 1). Significantly higher (less negative) values of  $\Psi_{\text{pd}}$  were observed in *P. mahaleb* plants grown on 10 cm deep substrate ( $-0.92 \pm 0.12$  MPa) with respect to those growing on 13 cm ones ( $-1.30 \pm 0.16$  MPa, Fig. 3a). Similar but not significant differences were recorded in the case of *C. coggygia* ( $P = 0.13$ ).  $\Psi_{\text{min}}$  dropped below  $-2.4$  MPa in both species, although the water status of plants grown on the shallowest substrate depth was overall more favorable ( $P < 0.05$ , Fig. 3b). The differences recorded among plants of the same species growing in substrates of different thickness were statistically significant only for *P. mahaleb* (*C. coggygia*  $P = 0.06$ ). On the same date,  $g_L$  ranged between 130 and  $300 \text{ mmol m}^{-2} \text{ s}^{-1}$  for the different species, with a significantly higher value (by about 58%,  $P < 0.05$ ) in plants growing in D-10 modules ( $257.9 \pm 38.8 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) with respect to D-13 ( $161.6 \pm 26.7 \text{ mmol m}^{-2} \text{ s}^{-1}$ , Fig. 3c). No significant differences between plant species were observed ( $P > 0.05$ ). Moreover, the electrolyte leakage test (Fig. 3d) revealed slightly lower values (indicating maintenance of cell membrane integrity) for *P. mahaleb* plants growing in D-10 modules ( $18.1 \pm 0.8\%$ ) when compared to values recorded for plants growing in D-13 ones ( $24.0 \pm 2.6\%$ ). No statistically significant influence of substrate depth or plant species was observed ( $P > 0.05$ ).

### 3.3 Plant growth and evapotranspiration rates

Fig. 4 reports plant growth rates (G) as assessed one year after planting. The annual growth

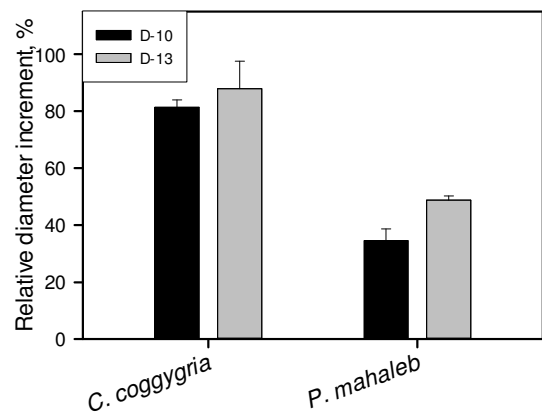




**Fig. 3** Pre-dawn ( $\Psi_{pd}$ , a) and minimum ( $\Psi_{min}$ , b) leaf water potential, leaf conductance to water vapor ( $g_L$ , c), and relative electrolyte leakage (REL, d) as measured for *C. coggygia* and *P. mahaleb* in 10 cm thick (D-10, black columns) and 13 cm thick (D-13, grey columns) experimental modules on 1 August 2013 when substrate water availability was partially restored. For statistical analysis see Table 1. Means are reported  $\pm$  SEM.

ranged between +35 and +88% in terms of increase of the diameter at the root collar. A significant effect of plants species was observed ( $P < 0.05$ ). For *C. coggygia* the average growth was  $84.6 \pm 4.7\%$ , with slightly lower values recorded for plants growing in D-10 modules ( $81.4 \pm 2.5\%$ ) with respect to those growing in D-13 ones ( $87.9 \pm 9.7\%$ ). The *P. mahaleb* annual growth was lower ( $41.7 \pm 3.7\%$ ) if compared to *C. coggygia*. Markedly higher G (by about 41%) was measured for *P. mahaleb* plants growing in thicker substrate ( $48.8 \pm 1.4\%$ ) if compared to plants established on shallower substrate ( $34.6 \pm 4.1\%$ ).

The mean evapotranspiration rates (ET) from experimental modules estimated for the growing season 2013 are reported in Fig. 5. The ET reached a maximum value of  $5 \text{ mm d}^{-1}$  recorded on a hot summer day following a rain event. The mean value was found to be  $1.78 \pm 0.11 \text{ mm d}^{-1}$  and  $2.17 \pm 0.12 \text{ mm d}^{-1}$  for D-10 and D-13 modules, respectively.



**Fig. 4** Relative diameter increment (G) of *C. coggygia* and *P. mahaleb* as estimated one year after planting in 10 cm thick (D-10; black columns) and 13 cm thick (D-13; grey columns) experimental modules. For statistical analysis see Table 1. Means are reported  $\pm$  SEM.

### 3.4 Testing water content recovery of green roof layers

Fig. 6 summarizes the results of experiments designed to estimate the effects of small and large rain events on the substrate water potential ( $\Psi_{sub}$ ), as well as on the water content of the drainage element and water retention layer (AW). After a large rain event, AW was

significantly higher in modules with 10 cm deep substrate than in 13 cm deep ones (t-test  $P < 0.05$ , Fig. 6a). In particular, after a simulated rainfall of 40 mm the AW was about 585% higher in D-10 modules than in D-13, suggesting that a larger water volume was accumulated by the substrate in the modules with thicker substrate depth. Dry substrate subjected to a simulated 5 mm rain event reached  $\Psi_{\text{sub}}$  values of  $-0.62 \pm 0.24$  and  $-1.08 \pm 0.22$  MPa in D-10 and D-13 modules, respectively. Because of high data variability, this difference was not statistically significant. By contrast, significantly higher (less negative, t-test  $P < 0.05$ ) values of  $\Psi_{\text{sub}}$  were found in D-10 ( $-0.04 \pm 0.02$  MPa) modules after 10 mm rain event simulations if compared to data recorded for D-13 ones ( $-0.32 \pm 0.06$  MPa, Fig 6b), indicating larger amounts of water theoretically available to plants. It has to be noted that the mixing of the substrate after the simulation of small rain events (see Material and Methods), could have resulted in the loss of information about different water distribution through the D-10 and D-13 soil profiles.

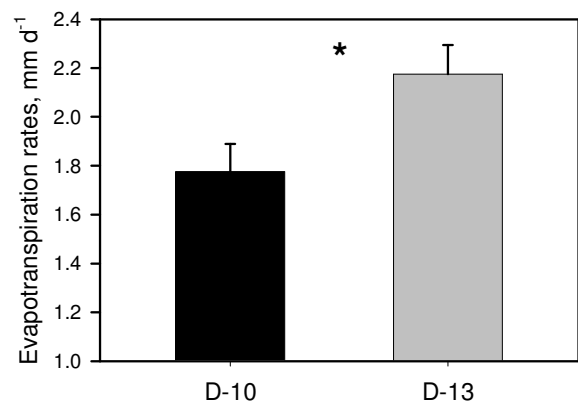
#### 4. Discussion

A monitoring of the physiological status of *C. coggygia* and *P. mahaleb* growing on a green roof revealed that both species are characterized by high resistance to drought and heat stress, and are thus fully suitable for green roof installation in seasonally warm and dry climates. Quite surprisingly, our results revealed that, during hot periods, the water status was more favorable for plants (in particular *P. mahaleb*) established on shallower substrate than in those grown on deeper substrate, probably due to a coordinated effect of reduced plant biomass and faster recharge of water content (and rise of substrate water potential) in modules filled with shallow substrate.

During the first growing season, both *C. coggygia* and *P. mahaleb* showed water deficit symptoms like wilting, leaf chlorosis, and/or partial desiccation. However, the desiccated foliage was quickly replaced in both species by newly sprouted leaves. Plant

mortality rate as recorded one year after planting was less than 20% for both species, considering both D-10 and D-13 modules (data not shown). These results are in accordance with data reported by Nardini *et al.* (2012), where the same species were grown on a 20 cm deep substrate. The resistance of these shrubs to the harsh conditions of a green roof is likely related to their drought resistance strategy, based on an efficient stomatal control of transpiration during dry periods (Nardini *et al.*, 2003). Moreover, the natural habitat of the two species is characterized by environmental conditions that are similar to those commonly found over green roofs, i.e. poorly developed soils with low water storage (Poldini, 2009).

During the spring season, characterized by regular and abundant rainfalls,  $\Psi_{\text{sub}}$  was constantly close to 0 MPa indicating high water availability to plants in all modules. Under these favorable conditions, the substrate likely represented the main source of water for plants and assured high gas exchange rates (Fig. 2). Lower  $\Psi_{\text{pd}}$  (by about 40%) and slightly higher (but not significantly)  $g_L$  (by about 10%) were recorded for plants grown in D-13 modules with respect to D-10 ones, as a likely effect of the higher amount of water stored in the substrate. At the onset of summer drought,

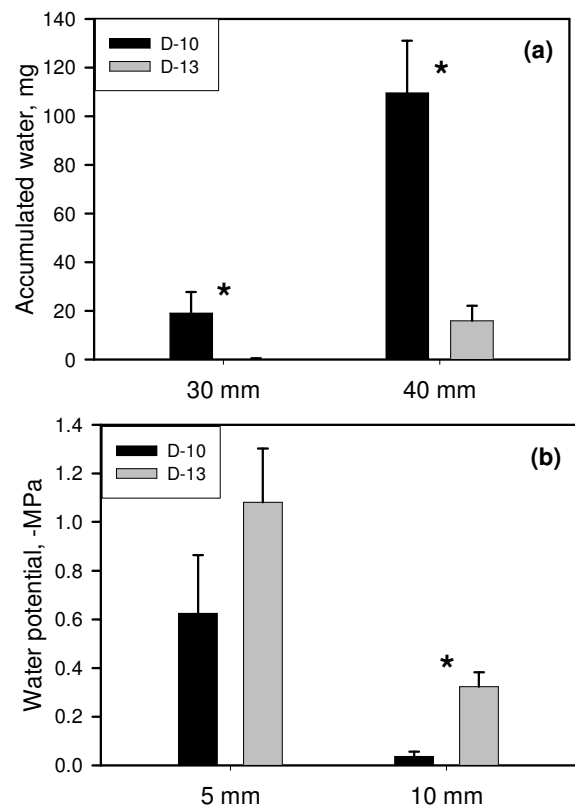


**Fig. 5** Mean evapotranspiration rates (ET) from 10 cm thick (D-10; black column) and 13 cm thick (D-13; grey column) experimental modules estimated for the growing season 2013. Means are reported  $\pm$  SEM. \* indicates statistically significant difference between experimental categories as tested using unpaired Student's t-test ( $P < 0.05$ ).

the water content of the substrate sharply decreased by evapotranspiration processes (Wolf & Lundholm, 2008), as revealed by  $\Psi_{pd}$  dropping below -1 MPa and -2.2 MPa in D-13 and D-10 modules, respectively (data not shown). No appreciable differences between plants of the same species grown on the two substrate depths were highlighted. Under these conditions, partial stomatal closure was observed. The high variability of  $g_L$  recorded in different species/individuals suggested the occurrence of intra- and inter-specific root competition for water (Manoli *et al.*, 2014; Rajcan & Swanton, 2001), as well as a likely partitioning in terms of exploitation of different water sources of the green roof system, i.e. substrate, water retention layer, and drainage layer.

In July, high air temperatures accompanied by absence of rainfall (Fig. 1) led to an intense water deficit, causing partial foliage desiccation. Physiological measurements carried out on a hot summer day when substrate water availability was partially restored (Fig. 3) confirmed previously observed trends in terms of higher (less negative)  $\Psi_{pd}$  and  $\Psi_{min}$  in plants grown on D-10 modules than in those grown on D-13 ones (Fig. 3). A significant effect of the substrate depth on  $\Psi_{pd}$ ,  $\Psi_{min}$ , and  $g_L$  was observed ( $P < 0.05$ ). The  $g_L$  recorded for both *C. coggygia* and *P. mahaleb* was markedly higher (by about 58%) in D-10 than in D-13 modules. The less intense water stress suffered by plants grown on shallower substrate depth was further suggested by electrolyte leakage test, where markedly higher, but not significant, membrane integrity was measured for *P. mahaleb* grown on D-10 than on D-13 modules.

The finding that plants established on 10 cm deep substrate suffered less water stress than those growing on 13 cm substrate is surprising, at first sight, especially considering the seasonal average water potential of the substrate that was lower by about 25% in the former than in the latter group (data not shown). These results might suggest that the main source for root water uptake over long term is not represented by



**Fig. 6** Estimations of water accumulated in the drainage element/water retention layer (AW, a) and the substrate water potential ( $\Psi_{sub}$ , b) recorded for 10 cm thick (D-10; black columns) and 13 cm thick (D-13; grey columns) experimental modules after a large (30 and 40 mm, a) or a small (5 and 10 mm, b) rain event simulation. Means are reported  $\pm$  SEM. \* indicates statistically significant difference between experimental categories as tested using unpaired Student's t-test ( $P < 0.05$ ).

the substrate, but more likely by the water accumulated in the drainage/water retention layers located below the substrate. Savi *et al.* (2013) have recently showed that within only a few months after establishment of sage plant over a green roof, the root system colonizes the cavities of the drainage panel. In fact, it was shown that diurnal substrate temperature fluctuations favored the evaporation of water from the retention layer, the diffusion of water vapor along pressure gradients, and final re-condensation on the surfaces of the drainage panel (Savi *et al.*, 2013). Therefore, we can hypothesize that within 15 months after establishment, the roots of *C. coggygia* and *P. mahaleb* were likely able to extend to the water retention layer as well. Hence, the more favorable water status of D-10 plants

with respect to D-13 ones was a possible consequence of thinner substrate depth favoring faster colonization of the water retention tissue by the roots. The first two years after establishment are very critical for plant survival on green roof installations. In this light, ensuring the largest possible amount of available water to plants is fundamental and the use of reduced substrate depth might be a possible, albeit counter-intuitive solution.

Experiments focused on the analysis of water content/potential recovery of green roof layers upon irrigation provide additional insights into recorded difference in terms of plant water status between the two substrate depths tested. When rain events of 30 and 40 mm were simulated, significantly larger water volumes were accumulated in drainage/water retention layer of D-10 than of D-13 modules (Fig. 6a). This is because a higher amount of water was stored by the substrate in the latter than in the former modules. In a green roof installation, water stored in the substrate is more prone to rapid evaporation, while the water accumulated in the drainage element/water retention tissue is protected from fast evaporation by the substrate layer and is thus potentially available to plants for a longer time. The simulation of 10 mm rainfalls highlighted significantly higher (less negative) substrate water potential in 10 cm deep modules than in 13 cm deep ones. Clearly, the small amounts of water supplied to the two substrates led to higher RWC measured in D-10 modules than in D-13 ones (data not shown), because an equal amount of water was retained by a different substrate volume. As a consequence of the exponential shape of the moisture retention curve of the substrate (relation between RWC and  $\Psi_{\text{sub}}$ , Savi *et al.*, 2014), a small difference in terms of WC translated in the significant difference in terms of water potential observed for D-10 or D-13 substrate (Fig. 6b). Hence, it can be hypothesized that the better water status of D-10 plants with respect to D-13 ones was probably due to the fact that during a dry period small rainfalls improved substrate water potential to a larger

extent in the former group than in the latter enabling the plants to recover earlier a positive water status.

Our data also suggest that shallow substrate improves plant water status by indirectly reducing water consumption by vegetation. Indeed, significantly lower evapotranspiration rates were recorded for D-10 modules ( $1.78 \pm 0.11 \text{ mm d}^{-1}$ ) than for the D-13 ones ( $2.17 \pm 0.12 \text{ mm d}^{-1}$ , Fig. 5). The ET values recorded in our study are in accordance with Berretta *et al.* (2014), who reported maximum ET rates of  $1.83 \text{ mm d}^{-1}$  for an extensive green roof vegetated with *Sedum*, while Schweitzer & Erell (2014) reported water requirements for different species (woody creeping shrubs included) growing on irrigated green roofs to be  $2.6 - 9.0 \text{ mm d}^{-1}$  in a water-limited Mediterranean climate.

The observed differences in terms of ET between experimental groups might be in part driven by differences in plant biomass. It has to be noted that biomass was not directly measured in this study, but only estimated in terms of plant annual growth (G). G was found to be slightly (but not significantly) higher in D-13 than in D-10 plants. Limited soil depth/volume affects plant growth through mechanical limitations and chemical inhibition of root growth (Semchenko *et al.*, 2007). Plants can sense the available substrate volume and consequently, the developed root/shoot biomass is a function of available rooting volume. Positive correlations between above-ground biomass and evapotranspiration rates have been reported by several authors and for several growth forms (Schweitzer & Erell, 2014; Wolf & Lundholm, 2008). Furthermore, in green roof literature and in agricultural studies it is often reported that substrate depth significantly affects plant development, with final root and shoot biomass being correlated to the available rooting volume (Kotsiris *et al.*, 2012; Razzaghmanesh *et al.*, 2014; Savi *et al.*, 2014; Semchenko *et al.*, 2007).

## 5. Conclusion

In green roof design, the substrate depth should represent a compromise between the ecological needs

of plants and the engineering limits of the building. Substrate depths of at least 15-20 cm are generally recommended for extensive green roofs in a warm arid climate (Benvenuti & Bacci, 2010; UNI 11235, 2007). Our results provide experimental evidence for the possibility to install efficient and fully functional green roofs vegetated with stress-tolerant shrubs in warm sub-Mediterranean areas using only 10 cm deep substrate. Indeed, shallower substrate depths paradoxically translated into less severe water stress experienced by plants, as associated with lower biomass. Moreover, both heavy rainfalls and small precipitations induced better and fastest recovery of favorable water content of both substrate and tissue retention layer when shallow substrate was used. Extensive green roofs based on a combination of reduced substrate depth and drought-tolerant plants may be an optimal, albeit counter-intuitive solution for areas characterized with a climate similar to that of the city of Trieste. Moreover, we highly recommend the installation of a deficit irrigation systems in order to avoid severe drought stress to plants and reconcile vegetation survival over long drought periods with the need to assure water saving in towns located in sub-Mediterranean areas.

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# 4. Plant performance on Mediterranean green roofs: interaction of species-specific hydraulic strategies and substrate water relations

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## ABSTRACT

Recent studies have highlighted the ecological, economical and social benefits assured by green roof technology to urban areas. However, green roofs are very hostile environments for plant growth because of shallow substrate depths, high temperatures and irradiance, and wind exposure. This study provides experimental evidence for the importance of accurate selection of plant species and substrates for implementing green roofs in hot and arid regions, like the Mediterranean area. Experiments were performed on two shrub species (*Arbutus unedo* L. and *Salvia officinalis* L.) grown in green roof experimental modules with two substrates slightly differing in their water retention properties, as derived from moisture release curves. Physiological measurements were performed on both well watered and drought stressed plants. Gas exchange, leaf and xylem water potential, and plant hydraulic conductance were measured at different time intervals following the last irrigation. The substrate type significantly affected water status. *A. unedo* and *S. officinalis* showed different hydraulic responses to drought stress, with the former species being substantially isohydric and the latter one anisohydric. Both *A. unedo* and *S. officinalis* revealed to be suitable species for green roofs in the Mediterranean area. However, our data suggest that appropriate choice of substrate is key to the success of green roof installations in arid environments, especially if anisohydric species are employed.

*Keywords* - anisohydric, arbutus, drought stress, green roof, isohydric, Mediterranean region, sage

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## 1. Introduction

Green roofs are engineered ecosystems designed to favor plant establishment on manufactured layers installed over rooftops, and typically comprise lightweight mineral substrate, drainage and moisture retention layers, and a root-resistant waterproofing barrier (VanWoert *et al.*, 2005; Berndtsson, 2010). Modern green roofs were first developed in the 1960s in Germany and, over the last 15 years, this technology has received increasing attention in several countries of Northern and Central Europe, North America, Australia, Japan and China (Bowler *et al.*, 2010; Dvovak & Volder, 2010; Williams *et al.*, 2010; Chen, 2013). This renewed interest for green roofs is a consequence of recent experimental evidence highlighting the ecological, economical and social benefits provided by this technology to urban areas. In fact, green roofs have been reported to improve urban management of water runoff (e.g. Getter *et al.*, 2007; Lundholm *et al.*, 2010; MacIvor & Lundholm, 2011; Nardini *et al.*, 2012a), reduce the consumption of energy for thermal comfort of buildings (e.g. Theodosiou, 2003; Sailor *et al.*, 2008; Blanusa *et al.*, 2013), mitigate the “urban heat island” effect (Gill *et al.*, 2007; Takebayashi & Moriyama, 2007; Mackey *et al.*, 2012), improve acoustic insulation (Van Renterghem & Botteldooren 2008, 2009), improve air (Rowe, 2011) and water quality (Carter & Jackson, 2007; Berndtsson, 2010) and sequester CO<sub>2</sub> (Getter *et al.*, 2009; Li *et al.*, 2010). Moreover, this technology could prove useful for recycling of waste materials (Solano *et al.*, 2012; Mickovski *et al.*, 2013) and might provide effective instruments to ameliorate the urban appeal, increase the number of recreational spaces, and improve urban biodiversity (Brenneisen, 2006; MacIvor & Lundholm, 2011).

Green roofs are rather hostile environments for plant growth, because of shallow substrate, high temperatures and irradiance, and wind exposure (Getter & Rowe, 2008; Liu *et al.*, 2012). In particular, structural features of buildings frequently require the

use of reduced substrate depths, with predictable impacts on water availability to vegetation. This, in turn, limits the number of species that can thrive over green roofs, especially in hot and arid regions like Mediterranean countries (Fioretti *et al.*, 2010; Nardini *et al.*, 2012b), where drought, high irradiance and temperatures are common stress factors even for natural vegetation (Sanchez-Gomez *et al.*, 2006; David *et al.*, 2007; Nardini *et al.*, 2014). Under these environmental conditions, the plants’ growth over green roofs is particularly challenging and thus requires specific technological and ecophysiological strategies to improve plant survival (Dvorak & Volder, 2013).

In particular, the selection of substrates with high water holding capacity and high amounts of water available to plants is apparently a key requirement to improve the performance of green roofs in arid climates. As an example, Farrell *et al.* (2012) reported a correlation between the survival rate of different succulent species under drought stress and the water holding capacity of different substrates. Similarly, Razzaghmanesh *et al.* (2014) reported significant effects of substrate type on growth and survival of different grass species native to the Australian flora. Moreover, improving water holding capacity of the substrate, amended with different materials, has been reported to be effective in increasing plant survival rates and ameliorating plant water status under drought conditions (Farrell *et al.*, 2013; Papafotiou *et al.*, 2013; Savi *et al.*, 2014).

The selection of drought-resistant plant species is as important as substrate features in order to assure the success of green roofs in arid environments. Specific studies addressing the relative suitability of different plant species for green roof development have appeared in recent years (Dvorak & Volder, 2010; MacIvor *et al.*, 2011; Cook-Patton & Bauerle, 2012; Papafotiou *et al.*, 2013; Van Mechelen *et al.*, 2014), but the most commonly used species are still small succulents, mainly belonging to the genus *Sedum* (Snodgrass & Snodgrass, 2006; Oberndorfer, *et al.*

2007; Rowe *et al.*, 2012). These are characterized by shallow roots, high drought tolerance and relatively fast propagation (Snodgrass & Snodgrass, 2006; Getter & Rowe, 2009; Farrell *et al.*, 2012). By contrast, only few studies have explored the possibility to use alternative plant species over green roofs in arid regions, despite the high number (and drought adaptation) of species native to the Mediterranean region (Benvenuti & Bacci, 2010; Papafotiou *et al.*, 2013; Benvenuti, 2014; Van Mechelen *et al.*, 2014). In particular, the impressive heterogeneity in plant hydraulic strategies and water relations displayed by Mediterranean plants (Nardini *et al.*, 2014; Vilagrosa *et al.*, 2014) might represent an important resource for designing green roofs with specifically requested technical features. As an example, isohydric species that display tight stomatal control of transpiration might help to design green roofs with high resistance against drought, as well as with low irrigation requirements (Rowe *et al.*, 2014). On the other hand, anisohydric species that maximize transpiration and photosynthesis while tolerating very negative water potential values might represent a more interesting choice in order to favor transpirational cooling of buildings (Schweitzer & Erell, 2014) and/or improve the capacity of green roofs to intercept water during intense albeit sporadic rainfall events (Nardini *et al.*, 2012a).

In the present study, we provide experimental evidence for the importance of substrate characteristics, with special reference to water retention properties, to assure sufficient water availability to plants over green roofs under drought stress conditions. Moreover, we provide insights into the importance of species-specific drought resistance strategies and hydraulic properties for selecting Mediterranean native species best suited for specific technical functions and ecological requirements of green roofs. To this aim, experiments were performed using two Mediterranean shrub species: *Arbutus unedo* L. and *Salvia officinalis* L. *S. officinalis* (sage) is a perennial, evergreen, sub-shrub

species widely naturalized even outside its original habitat. *A. unedo* (arbutus) is an evergreen shrub or small tree widely distributed in the Mediterranean Basin (Pignatti, 2002). Both species are well known for their drought tolerance, although a specific comparison of their hydraulic strategies has not been previously performed.

## 2. Materials and methods

Experiments were performed between May and July 2012 on 36 plants of *A. unedo* and 36 plants of *S. officinalis*. Plants were provided at the end of April 2012 by a local nursery and planted in 24 experimental green roof modules with dimensions 75 x 23 x 27 cm (i.e. 12 modules per species, 3 plants per module, Fig. S1). The modules were assembled with the SEIC® extensive system (Harpo Spa, Trieste, Italy). The layering included a water retention geotextile (MediPro MP), a drainage and aeration element (MediDrain MD), a filtering layer (MediFilter MF 1), and 18 cm of one of two different experimental substrates provided by SEIC. Species-specific modules were divided in two main categories on the basis of substrate type tested: substrate A and substrate B. In summary, six modules

	Substrate type A	Substrate type B
Grain size <0.05 (% m/m s.s.)	0	2
Grain size <0.55 (% m/m s.s.)	1	7
Grain size <0.25 (% m/m s.s.)	2	12
Grain size <0.50 (% m/m s.s.)	6	16
Grain size <1.00 (% m/m s.s.)	13	21
Grain size <2.00 (% m/m s.s.)	20	30
Grain size <5.00 (% m/m s.s.)	50	53
Grain size <10.00 (% m/m s.s.)	93	100
Grain size <16.00 (% m/m s.s.)	99	100
Grain size <20 (% m/m s.s.)	100	100
Organic matter (% s.s.)	4.26	6.24
Porosity (% v/v)	65.9	65.7
Electrical conductivity (mS/m s.s.)	20	13
pH	8.9	7.6

**Table 1.** Percentage of different grain sizes, organic matter, porosity and values of electrical conductivity and pH of the two substrate types utilized (i.e. A and B). Data are kindly provided by SEIC.

per species contained substrate A and six modules were filled with substrate B, (Fig. S1).

Both substrates consisted of a mix of mineral material (lapillus, pomix, zeolite) and organic material (peat) with grain size ranging from 0.05 mm to 20 mm. However, substrate A had a lower percentage of grain size ranging from 0.05 and 10 mm, higher electrical conductivity (20 versus 13 mS/m) and pH (8.9 versus 7.6) and lower percentage of organic matter (4.2 versus 6.2 %) than substrate B (Table 1, data kindly provided by SEIC).

The water retention properties of the two substrates were preliminarily measured using a dewpoint potentiometer (WP4, Decagon Devices, Pullman, WA). In particular, the relationships between water content and water potential (pressure–volume curve) of the two substrates were measured to estimate the amount of water available to plants (Whalley *et al.*, 2013). Samples of the two substrates were watered to saturation. After complete drainage of excess water, small samples (a few grams each) were collected and placed in dedicated WP4 sample-holders. Water potential of substrate ( $\Psi_s$ ) was measured in the continuous mode and after each reading, samples were weighed with an electronic balance (Basic BA110S, Sartorius AG, Göttingen, GE) to obtain their fresh weight (FW), and then oven-dried at 70 °C for 24 h.

Samples were weighed again to get their dry weight (DW). Water content (WC) of samples was calculated as  $(FW-DW)/DW$ . Measurements were performed on fully hydrated samples as well as on samples air-dehydrated for increasing time intervals.

Green roof modules were randomly located over the flat rooftop of the Department of Biological and Environmental Sciences, University of Messina. On the basis of irrigation regime, experimental modules were further divided in four experimental groups per species (Fig. S1): three modules per substrate type category were regularly watered to field capacity (well-watered plants: WA and WB), while the other three modules per substrate type category received irrigation up to 75% field capacity (stressed plants: SA and SB). Irrigation was supplied at 48 h intervals for 10 weeks. At the end of the treatment, all plants were irrigated to field capacity and physiological measurements were performed again 24 and 48 h after irrigation.

During the study period, mean air temperatures and relative humidity in the area were  $19 \pm 1$  °C and  $74 \pm 7$  % in May,  $24 \pm 2$  °C and  $75 \pm 5$  % in June, and  $28 \pm 1$  °C and  $74 \pm 5$  % in July, respectively. The total rainfall was 13 mm only. Climatic data were obtained from the weather station Torre Faro, Messina, Italy.

	WA		WB		SA		SB	
	May	July	May	July	May	July	May	July
<i>S. officinalis</i>								
<b>H (cm)</b>	25.8 ± 1.4c	39 ± 2a	26.6 ± 1.3c	40.7 ± 3.7a	26.7 ± 1.2c	29.9 ± 2.0b	26.0 ± 2.5c	30.4 ± 2.2b
<b>Ø (cm)</b>	0.6 ± 0.005b	0.8 ± 0a	0.6 ± 0.007b	0.8 ± 0.007a	0.6 ± 0.01b	0.8 ± 0.003a	0.6 ± 0.006b	0.8 ± 0a
<b>N leaves/plant</b>	94 ± 4.2c	195 ± 12a	94 ± 3.6c	197 ± 8a	100 ± 7c	155 ± 6b	94 ± 3c	142 ± 7b
<i>A. unedo</i>								
<b>H (cm)</b>	43 ± 1.2b	49.3 ± 0.6a	42.5 ± 1.6b	49.7 ± 1.3a	41.7 ± 1.6b	48.8 ± 1.0a	43.3 ± 0.6b	49.8 ± 1.3a
<b>Ø (cm)</b>	0.5 ± 0.005b	0.7 ± 0.005a	0.5 ± 0.005b	0.7 ± 0.002a	0.5 ± 0.002b	0.7 ± 0.03a	0.5 ± 0.01b	0.7 ± 0.008b
<b>N leaves/plant</b>	102 ± 1c	162 ± 3a	102 ± 1c	158 ± 4a	104 ± 1c	128 ± 2b	104 ± 1c	128 ± 1b

**Table 2.** Means ± SD (n=3) of plant height (H), trunk diameter (Ø) and number of leaf per plant (N leaves/plant) as recorded in May and in July (i.e. at the beginning and at the end of treatment irrigation regimes) in plants of *S. officinalis* and *A. unedo* growing in two types of substrate (A and B) and irrigation regimes (W: plants irrigated to field capacity; S: plants irrigated to 75% field capacity) (for details, see text). Different letters indicate, for each measured parameter, statistically different mean values for Tukey pairwise comparison, after performing a 3-way ANOVA test.

At the beginning and at the end of the experiment (i.e. beginning of May and end of July, respectively), 2 plants within each module of *S. officinalis* and 2 plants within each module of *A. unedo* per each experimental group (i.e. WA, SA, WB and SB) were selected and the following parameters were measured: plant height (h), trunk diameter at the root-stem transition zone ( $\emptyset$ ), and total number of leaves per plant (N leaves/plant). During the study period, substrate water status ( $\Psi_s$ ) of both W and S-modules was estimated by measuring the pre-dawn water potential ( $\Psi_{pd}$ ) of six leaves wrapped in cling-film the day before measurements (two leaves per species and per module) and sampled at 0500 h (solar time). Measurements were performed with a pressure chamber (3005 Plant Water Status Console, Soilmoisture Equipment Corp., Goleta, CA, USA), assuming that under nocturnal low transpiration conditions leaf water potential equilibrated with  $\Psi_s$ , so that  $\Psi_{pd} \sim \Psi_s$  (Richter, 1997; Nardini *et al.*, 2003). The indirect estimation of  $\Psi_s$  was preferred to direct sampling of the substrate, in order to avoid the risk of damage to the root system. Measurements of  $\Psi_{pd}$  were performed on the same days selected for gas exchange and midday leaf water potential measurements (see below).

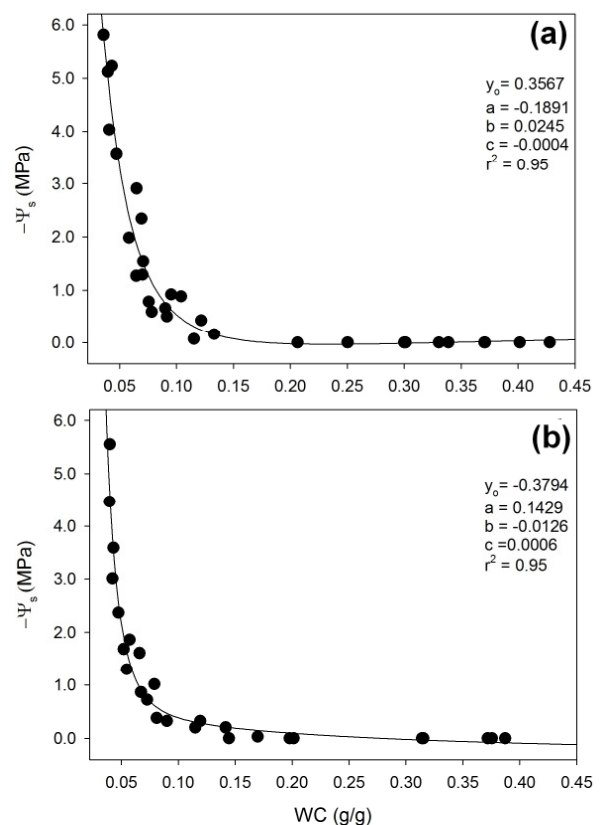
### 2.1 Measurements of leaf gas exchange and water status

At the end of the 10-week treatment period, both 24 h and 48 h after irrigation, maximum leaf stomatal conductance to water vapour ( $g_L$ ) and transpiration rate ( $E_L$ ) were measured between 1200 and 1400 h on leaves of at least one plant per module per experimental group and species using a steady-state porometer (LI-1600, LICor Inc., Lincoln, NE, USA). At the same time, midday diurnal leaf water potential ( $\Psi_{midday}$ ) was estimated using a portable pressure chamber (3005 Plant Water Status Console, Soilmoisture Equipment Corp., Goleta, CA, USA).

In order to quantify eventual acclimation of water relation parameters in terms of leaf water potential at the turgor loss point ( $\Psi_{tlp}$ ), osmotic potential at full turgor ( $\pi_0$ ) and bulk modulus of elasticity ( $\epsilon_{max}$ ), leaf water potential isotherms of leaves of at least one plant per module per experimental group were determined from pressure-volume (P-V) curves (Tyree and Hammel, 1972). Measurements were performed before starting the treatment and repeated at the end of the 10-week period, respectively.

### 2.2. Estimating plant hydraulic conductance ( $K_{plant}$ )

Whole-plant hydraulic conductance ( $K_{plant}$ ) was estimated *in planta* using the Evaporative Flux Method on at least one plant per module per species and per experimental group (Nardini *et al.*, 2003).  $K_{plant}$



**Fig. 1.** Relationships between water potential ( $\Psi_s$ ) and water content (WC) as measured for the substrate A (a) and B (b). Regression curves are expressed by the following function:  $f = y_0 + (a/x) + (b/x^2) + (c/x^3)$ . Coefficient values and correlation coefficients ( $r^2$ ) are reported.

was calculated as:  $E_L / (\Psi_{\text{midday}} - \Psi_s)$  where  $E_L$ ,  $\Psi_{\text{midday}}$  and  $\Psi_s$  were measured as described above. All hydraulic conductance values were corrected to a temperature of 20 °C, to take into account changes in water viscosity.

### 2.3. Statistical Analysis

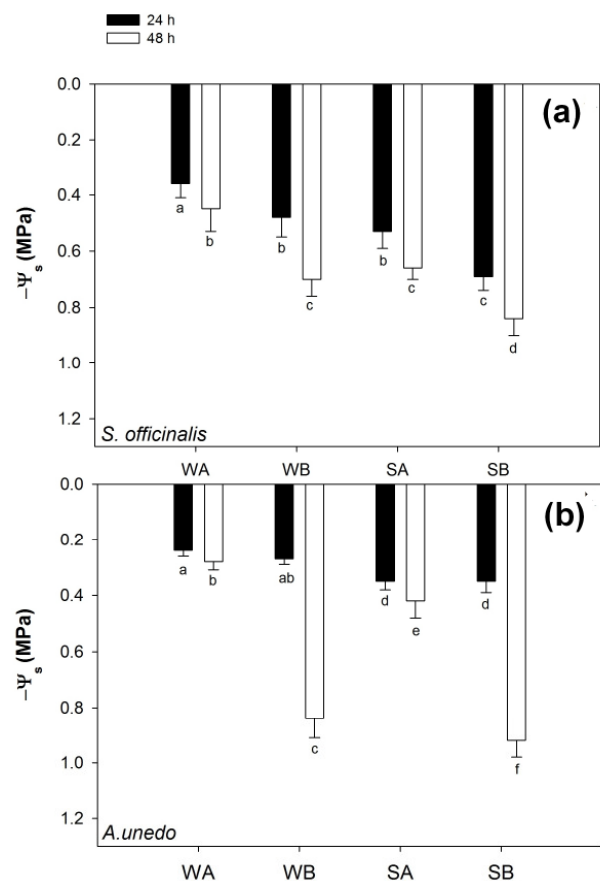
Data were analyzed with the SigmaStat 2.0 (SPSS, Inc., Chicago, IL, USA) statistics package. To test the differences among substrate type and the effects of both irrigation regimes and time after last irrigation on  $\Psi_s$ ,  $g_L$  and  $K_{\text{plant}}$ , a three-way-Anova was performed (soil, irrigation and time as factors) with Type III sums of squares. The same test was used to check the significance of the differences among substrate type and the effects of irrigation regime and time (i.e. May and July) on H,  $\emptyset$  and N leaves/plant. To test the differences among substrate type and effects of irrigation regime on  $\Psi_{\text{tlp}}$ ,  $\pi_o$  and  $\epsilon_{\text{max}}$  a two-way Anova test was performed. Data has been analyzed by nesting the plant observations within each module (n=3). When the difference was significant, a post hoc Tukey's test was carried out. Relationships between the studied characteristics and independent variables were assessed by Pearson's correlations.

### 3. Results

Both irrigation regime and measurement time influenced plant size, as estimated in terms of final plant height and number of leaves per plant in *S. officinalis* but not in *A. unedo* plants (Tabs 2, 4). In fact, in well-watered sage samples (WA and WB), plant height was about 26 cm in May, and increased to about 40 cm by the end of the experimental treatment. By contrast, the size of stressed samples increased by only less than about 30 cm. A different trend was recorded in *A. unedo* plants, where an increase of about 25% in terms of plant height was recorded after 10 weeks in all experimental groups, with no effect of irrigation regime. The increase in the number of leaves per plant during the study period was larger in *S.*

*officinalis* than in *A. unedo*, both in well watered (+100% versus about +60%, respectively) and stressed samples (see below). Moreover, in *S. officinalis* as well as in *A. unedo* the number of leaves per plant was influenced by irrigation regime and time.

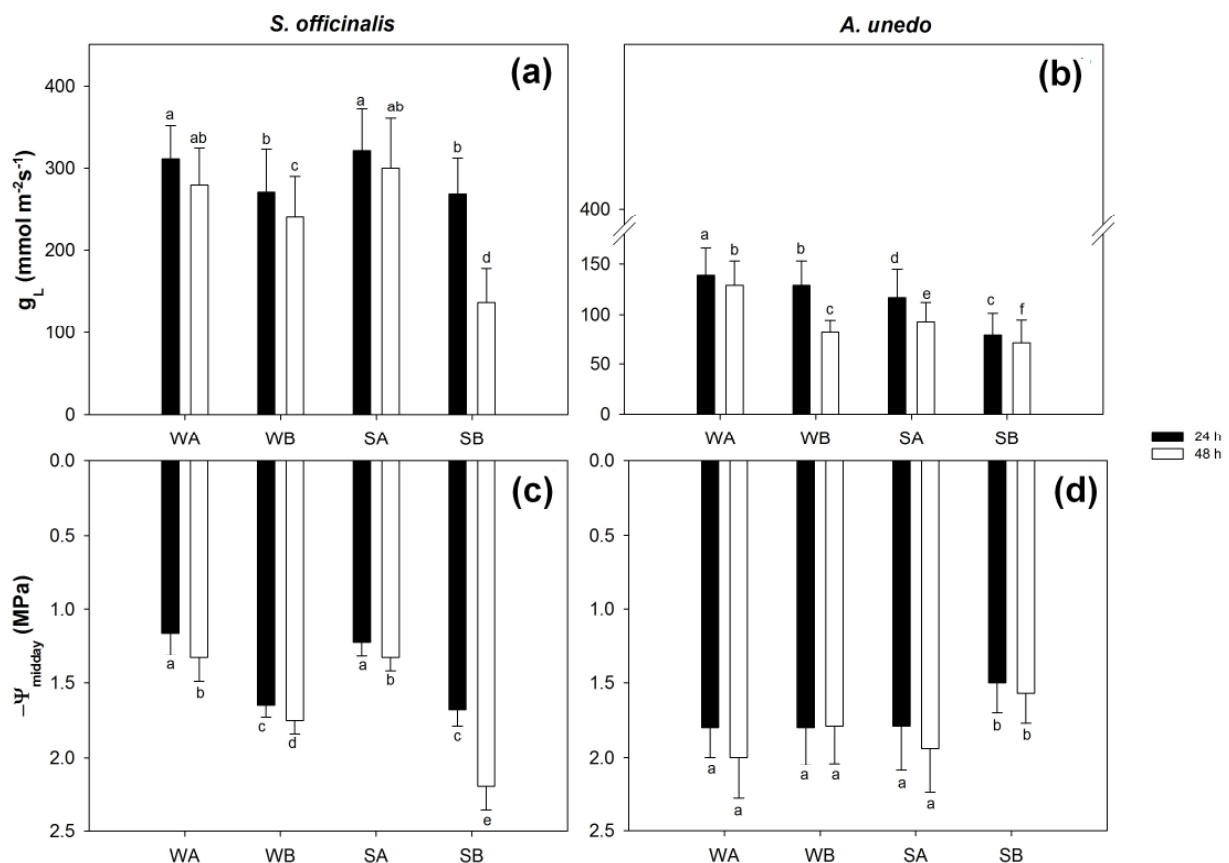
Fig. 1 reports the relationship between soil water potential and water content as measured for substrates A and B. Water content at saturation (SWC) was about 0.43 g g<sup>-1</sup> for substrate A and 0.39 g g<sup>-1</sup> for substrate B. At  $\Psi_s = -1.5$  MPa (i.e. the reference value of permanent wilting point, WWC), water content was about 0.07 g g<sup>-1</sup> for both substrate types. Hence, the amount of water available to plants (AWC) calculated as SWC – WWC turned out to be about 12% higher in substrate A (0.36 g g<sup>-1</sup>) than in substrate B (0.32 g g<sup>-1</sup>).



**Fig. 2.** Substrate water potential ( $\Psi_s$ ) as recorded 24 h and 48 h after irrigation of experimental modules with *S. officinalis* (a) and *A. unedo* (b) plants subjected to two irrigation regimes (W: plants irrigated to field capacity; S: plants irrigated to 75% field capacity). Two substrates were tested (A and B, for details, see text). Different letters indicate statistically different mean values for Tukey pairwise comparison.

In accordance with the above reported differences in terms of SWC and AWC, larger drops of  $\Psi_s$  were recorded within 48 h in modules containing substrate B than modules filled with substrate A, irrespective of the species (Fig. 2). In detail,  $\Psi_s$  values, as recorded 48 h after irrigation, were about -0.5 MPa and -0.7 MPa in WA and SA sage plants, respectively, while values of about -0.7 MPa and -0.9 MPa were recorded in WB and SB samples. Likewise, in WA and SA arbutus plants, 48 h after last irrigation,  $\Psi_s$  values of about -0.3 MPa and -0.5 MPa were recorded in WA and SA samples and values of about -0.9 MPa and -1.0 MPa were found in WB and SB ones. Midday  $g_L$  values recorded in *S. officinalis* growing in modules containing substrate A were higher than values

recorded in samples growing in modules containing substrate B, as recorded 24 h after last irrigation (i.e. about  $300 \text{ mmol m}^{-2} \text{ s}^{-1}$  versus about  $270 \text{ mmol m}^{-2} \text{ s}^{-1}$ ). Moreover, while in WA, WB and SA samples stomatal conductance decreased no more than about 10% within 48 h after last irrigation, in SB samples a decrease of about 50% of  $g_L$  values was recorded 48 h after last irrigation (Fig. 3a). A different trend was recorded in arbutus plants (Fig. 3b) where in samples growing in substrate A,  $g_L$  decreased by about 10% in well watered samples and by about 20% in stressed samples. In WB arbutus plants  $g_L$  decreased by about 40% 48 h after last irrigation with respect to values recorded 24 h before. Moreover, SB samples showed values of  $g_L$  of about  $80 \text{ mmol m}^{-2} \text{ s}^{-1}$  24 h after the last irrigation, and



**Fig. 3.** Leaf conductance to water vapor ( $g_L$ , a and b) and leaf water potential ( $\Psi_{\text{midday}}$ , c and d) as recorded in plants of *S. officinalis* and *A. unedo* growing in the two types of substrate (A and B) and under different irrigation regimes (W: plants irrigated to field capacity; S: plants irrigated to 75% field capacity, for details, see text). Means are given  $\pm$  SD (n=3). Different letters indicate statistically significant differences for Tukey pairwise comparison.

further decreasing to about  $70 \text{ mmol m}^{-2} \text{ s}^{-1}$  48 h after last irrigation. A contrasting behavior was observed in *S. officinalis* and *A. unedo* also in terms of changes in leaf water potential. In WA and SA sage plants,  $\Psi_{\text{midday}}$  showed similar values (i.e. about  $-1.25 \text{ MPa}$ ) and remained quite constant over 48 h after last irrigation (Fig. 3c). By contrast,  $\Psi_{\text{midday}}$  measured in WB and SB samples was about  $-1.7 \text{ MPa}$  in both experimental groups 24 h after last irrigation and, 48 h after last irrigation, midday leaf water potential values remained quite constant in WB plants while decreased to about  $-2.3 \text{ MPa}$  in SB samples. In arbutus plants,  $\Psi_{\text{midday}}$  was maintained constantly around  $-1.8 \text{ MPa}$  in all treatments except in SB samples where values of about  $-1.5 \text{ MPa}$  were recorded (Fig. 3d).

All recorded  $\Psi_{\text{midday}}$  values were within the positive turgor region (Table 2). However, midday leaf water potential of sage plants growing in substrate B was close to the critical turgor loss point. In fact,  $\Psi_{\text{tlp}}$  values of W and S sage samples were about  $-1.8 \text{ MPa}$  and  $-2.3 \text{ MPa}$ , respectively. However, in WA and SA samples,  $\Psi_{\text{midday}}$  values no lower than about  $-1.3 \text{ MPa}$  were recorded while in WB and SB samples  $\Psi_{\text{midday}}$  values were low as about  $-1.72 \text{ MPa}$  and about  $-2.2 \text{ MPa}$ , respectively (Fig. 3c). In arbutus plants,  $\Psi_{\text{tlp}}$  was  $-2.4 \pm 0.1 \text{ MPa}$  and  $-2.6 \pm 0.01 \text{ MPa}$  in WA and WB treatments, respectively, and about  $-3 \text{ MPa}$  in S samples, whereas  $\Psi_{\text{midday}}$  remained above  $-2.0 \text{ MPa}$  (Fig. 3d). Changes in  $\Psi_{\text{tlp}}$  in watered and stressed plants as recorded in both species under study, were

apparently driven by changes in different parameters. Irrigation regimes, in fact, significantly affected only  $\pi_0$  values in sage plants, while more apparent changes in  $\epsilon_{\text{max}}$  values were recorded in arbutus plants (Table 3).

$K_{\text{plant}}$  values changed in response to both type of substrate and time after last irrigation in *S. officinalis* samples (Fig. 4a, Table 4). In WA and SA sage samples and in WB and SB plants,  $K_{\text{plant}}$  decreased over 48 h after the last irrigation. However, 24 h after last irrigation, plants growing in modules containing substrate B showed values of  $K_{\text{plant}}$  lower than samples growing in modules containing substrate A (i.e. about  $8 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$  versus about  $12 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ , respectively). In arbutus,  $K_{\text{plant}}$  was maintained at a constant value of about  $2 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$  in all treatments over 48 h after the last irrigation (Fig. 4b).

When  $g_L$  values were plotted versus the corresponding  $\Psi_s$ , different relationships were observed in sage and arbutus plants (Fig. 5). In sage plants,  $g_L$  values remained quite constant until  $\Psi_s$  was above  $-0.6 \text{ MPa}$ . By contrast, in arbutus plants,  $g_L$  was related to  $\Psi_s$  according to an inverse first order polynomial equation. Likewise, different values of  $K_{\text{plant}}$  as a function of  $\Psi_s$  were recorded in sage plants, while a constant water transport efficiency from root to leaves was recorded in arbutus plants, despite the treatments (Fig. 6).

	$\Psi_{\text{tlp}}$ (-MPa)	$\pi_0$ (-MPa)	$\epsilon_{\text{max}}$ (MPa)		$\Psi_{\text{tlp}}$ (-MPa)	$\pi_0$ (-MPa)	$\epsilon_{\text{max}}$ (MPa)
<i>S. officinalis</i>				<i>A. unedo</i>			
WA	$1.61 \pm 0.01a$	$1.36 \pm 0.14a$	$11.35 \pm 1.4$	WA	$2.41 \pm 0.1a$	$1.96 \pm 0.2$	$22.95 \pm 1.8b$
WB	$1.84 \pm 0.13a$	$1.49 \pm 0.09a$	$13.20 \pm 1.1$	WB	$2.61 \pm 0.01a$	$2.20 \pm 0.2$	$25.30 \pm 3.0b$
SA	$2.40 \pm 0.13b$	$1.73 \pm 0.08b$	$13.03 \pm 1.1$	SA	$2.92 \pm 0.03b$	$2.17 \pm 0.2$	$31.85 \pm 1.1a$
SB	$2.29 \pm 0.16b$	$1.83 \pm 0.04b$	$11.73 \pm 1.2$	SB	$3.03 \pm 0.1b$	$2.11 \pm 0.07$	$34.75 \pm 2.0a$

**Table 3.** Leaf water potential at turgor loss point ( $\Psi_{\text{tlp}}$ ), osmotic potential at full turgor ( $\pi_0$ ) and bulk modulus of elasticity ( $\epsilon_{\text{max}}$ ) as recorded in plants of *S. officinalis* and *A. unedo* growing in two type of substrate (A and B) and irrigation regimes (W: plants irrigated to field capacity; S: plants irrigated to 75% field capacity) (for details, see text). Means are given  $\pm$  SD (n=3). Different letters indicate, for each measured parameter, statistically different mean values for Tukey pairwise comparison, after performing a 3-way ANOVA test.



(a)	S	I	T	SxI	SxT	TxI	SxTxI
<i>S. officinalis</i>							
$\Psi_s$	52.6***	55.3***	35.2***	0.04	2.05	0.074	1.19
$g_L$	477.5***	47.87***	274.86***	79.26***	71.11***	64.25***	57.72***
$\Psi_{min}$	213.9***	15.88***	42.55***	9.44**	2.43	5.36*	11.3**
$K_{plant}$	31.03***	0.061	20.61***	4.65*	0.366	0.791	3.532
H	0.37	28.79***	91.59***	0.417	0.417	29.19***	0.0003
$\emptyset$	0.714	1.4	6555.46***	0.714	1.4	0.714	0.257
N leaves/plant	2.06	61.4***	701.43***	2.915	0.25	76.66***	0.533
<i>A. unedo</i>							
$\Psi_s$	219.1***	31.3***	287.9***	1.597	193.2***	0.13	0.033
$g_L$	58.4***	170.67***	84.15***	3.65	1.44	3.38	23.32***
$\Psi_{min}$	13.98**	6.75*	3.07	1.19	0.101	0.133	0.195
$K_{plant}$	0.07	0.378	2.602	0.289	0.97	3.005	0.088
H	1.37	0.314	180.3***	1.873	0.033	0.00109	0.55
$\emptyset$	0.128	3.872	1889.6***	0.512	2.048	0.032	3.2
N leaves/plant	1.305	275.09***	2000.92***	0.603	1.305	366.51***	1.3

(b)	S	I	SxI
<i>S. officinalis</i>			
$\Psi_{tip}$	0.149	29.8***	2.11
$\pi_0$	4.19	40.69***	0.071
$\epsilon_{max}$	0.182	0.0282	5.97*
<i>A. unedo</i>			
$\Psi_{tip}$	5.98	85.09***	0.591
$\pi_0$	1.17	0.293	2.635
$\epsilon_{max}$	3.87	55.93***	0.125

**Table 4.** Results of: (a) a three-way ANOVA of different measured parameters by soil type, S (i.e. A and B), irrigation regime, I (i.e. samples regularly watered to field capacity and samples watered to 75% field capacity) and time, T (i.e. time after last irrigation for soil water potential  $\Psi_s$ , maximum diurnal leaf conductance to water vapour  $g_L$ , minimum diurnal leaf water potential  $\Psi_{min}$  and plant hydraulic conductance  $K_{plant}$ , and time of year for plant height H, stem diameter  $\emptyset$  and number of leaves per plant (N leaves/plant) treatments; (b) a two-way ANOVA of parameters determined from P-V curves by soil type, S (i.e. A and B) and irrigation treatment, I (i.e. time of the year) recorded in *S. officinalis* and in *A. unedo*. For details, see the text. Numbers represent F values, \*=P<0.05, \*\*=P<0.01; \*\*\*=P<0.001.

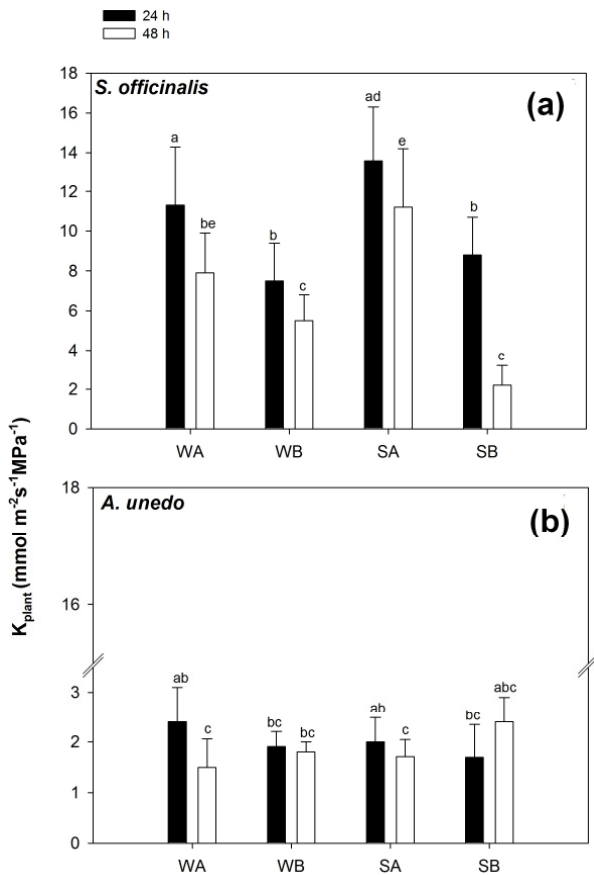
#### 4. Discussion

Our data suggest that the use of species selected from the native flora of the Mediterranean region might be a valuable strategy for implementation of green roof systems in hot and arid areas. On the other hand, our findings reveal that even subtle differences in terms of substrate properties, with special reference to water relation parameters, can have very important consequences for the performance and persistence of vegetation over green roofs.

Substrate A was more suitable than substrate B for installation of efficient and fully functional green roofs in arid-prone areas. This was mainly due to the

higher water retention capability related to the particle size, and especially to the higher amounts of water potentially available to plants (Fig. 1). This feature resulted in the maintenance of higher soil water potential values over 48 h after the last irrigation in plants growing in modules containing substrate A than in samples growing in modules filled with substrate B, as observed in both species, despite their different water relations strategies (Figs. 2, 3).

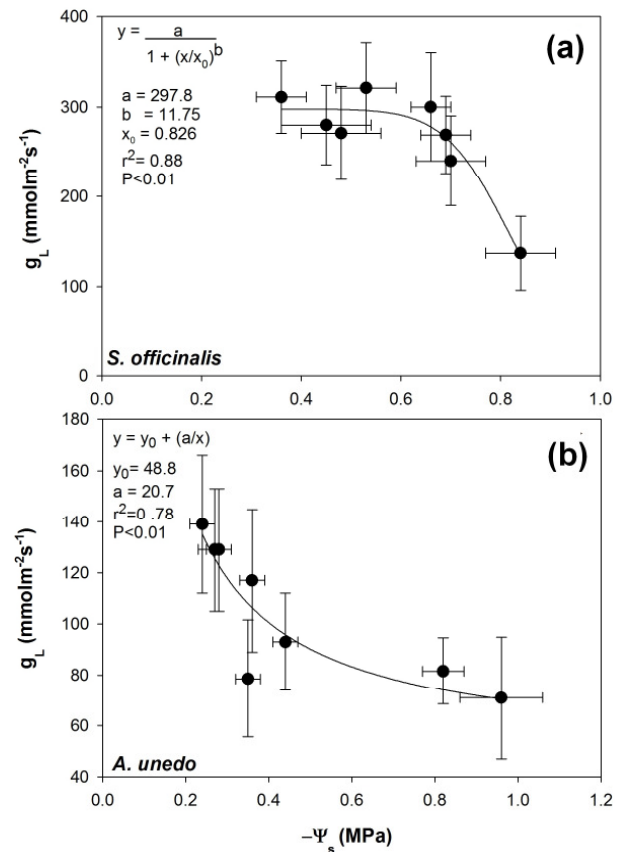
Arbutus and sage plants apparently adopted contrasting strategies to cope with drought stress. On the basis of relationships between  $g_L$  and leaf water potential, it can be suggested that *A. unedo* adopted a



**Fig. 4.** Plant hydraulic conductance ( $K_{\text{plant}}$ ) as recorded in plants of *S. officinalis* (a) and *A. unedo* (b) growing in two types of substrate (A and B) and under different irrigation regimes (W: plants irrigated to field capacity; S: plants irrigated to 75% field capacity, for details, see text). Means are given  $\pm$  SD ( $n=3$ ). Different letters indicate statistically significant differences for Tukey pairwise comparison.

rather typical isohydric behavior, while *S. officinalis* displayed a significant level of anisohydry, although a recent study has highlighted the fact that there might be a continuum of water relations strategies along these two ideal extremes (Klein, 2014). Values of  $g_L$  were lower in arbutus than in sage, even in well watered samples (about 130 versus 300  $\text{mmol m}^{-2} \text{s}^{-1}$ , respectively, Figs. 3a, 3b), and a further reduction of stomatal conductance was observed in arbutus plants under water stress (about 70  $\text{mmol m}^{-2} \text{s}^{-1}$ ). Progressive stomatal closure apparently allowed arbutus plants to limit water loss and maintain relatively stable leaf water potential values both under well-watered and drought stress conditions, especially in samples growing in modules filled with substrate type A (Fig. 3d, 5b). In contrast, *S. officinalis* plants maintained

values of  $g_L$  as high as about 300  $\text{mmol m}^{-2} \text{s}^{-1}$  as long as soil water potential remained above a critical value of about -0.6 MPa (Figs. 3c, 5a). Below this threshold, gas exchange rates were reduced by about 50% (from 300  $\text{mmol m}^{-2} \text{s}^{-1}$  to 150  $\text{mmol m}^{-2} \text{s}^{-1}$ , as recorded in SB samples 48h after last irrigation Fig. 3a). This, in turn, induced statistically significant differences in leaf water potential values as a function of the time after the last irrigation, regime of irrigation and the type of substrate (Fig. 3c, Table 4). The different water use strategies adopted by arbutus and sage plants to face drought stress were also confirmed by the analysis of leaf water potential isotherms. In fact, water-stressed plants of *S. officinalis* lowered the leaf water potential at the turgor loss point by osmotic adjustment. In the case of arbutus, water stress induced a significant increase of the bulk modulus of elasticity ( $\epsilon_{\text{max}}$ , Tabs 3, 4).



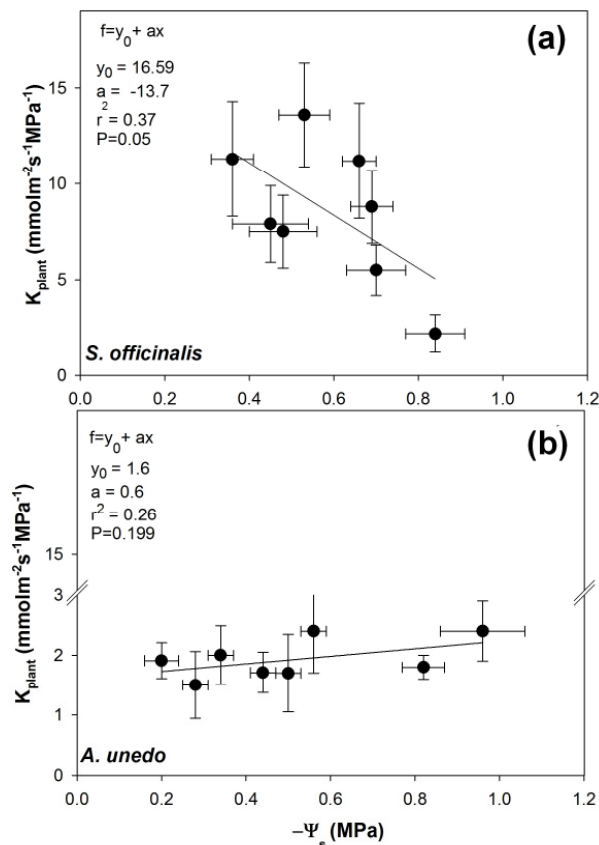
**Fig. 5.** Relationship between maximum leaf stomatal conductance to water vapor ( $g_L$ ) values and substrate water potential ( $\Psi_s$ ) values recorded in plants of *S. officinalis* (a) and *A. unedo* (b) growing in two types of substrate and under different irrigation regimes. Regression equation, coefficient values, P-values and correlation coefficients ( $r^2$ ) are also reported.

Isohydic and anisohydic behavior of different species/genotypes could arise from different stomatal sensitivity to xylem-born ABA (Tardieu & Simmoneau, 1998; Beis & Patakas, 2010; Gallè *et al.*, 2013) and/or to different levels of xylem hydraulic safety/efficiency (Schulz, 2003; Tombesi *et al.*, 2014). Different levels of stomatal control of transpiration under drought stress are known to affect photosynthetic productivity and plant growth (Medrano *et al.*, 2002; Xu & Zhou, 2008). In the present study, the anisohydic behavior recorded in sage plants was coupled to a strong reduction of the number of leaves per plant as recorded in July in stressed versus watered samples (i.e. about 100% versus about 40%). Isohydic and anisohydic behaviors of the two study species were further supported by estimates of plant hydraulic

conductance (Fig. 5). In fact, arbutus plants (isohydic) showed three times lower  $K_{\text{plant}}$  than sage plants (anisohydic, Fig. 4), and this parameter remained quite constant up to 48 h after the last irrigation in samples growing in modules filled with substrate B, despite wide variations in terms of soil water availability (Figs. 2b, 4b, 5b). By contrast,  $K_{\text{plant}}$  of *S. officinalis* strongly changed as a function of  $\Psi_s$  (Figs. 4a and 5a). In other words, the isohydic behavior of arbutus allowed to maintain stable  $K_{\text{plant}}$  values, while anisohydry in sage implied a drop of  $K_{\text{plant}}$  as drought progressed.

## 5. Conclusion

Data recorded in the present study suggest that arbutus plants could overcome intense drought conditions and, then, might be more suitable for Mediterranean green roofs than to sage plants. In fact, the higher water use of the latter species might imply the need of additional irrigation to prevent foliage damage and/or desiccation under prolonged drought. In the literature, *A. unedo* is frequently reported to be able to survive even severe drought stress (i.e. Gratani & Ghia, 2002; Munnè-Bosch & Peñuelas, 2004; Castell & Terradas, 2012), as it apparently maintains a positive carbon balance until predawn leaf water potential values of -4 MPa (Filella & Penuelas, 2003). By contrast, sage plants are known to show leaf senescence symptoms when exposed to severe drought conditions (i.e.  $\Psi_{\text{pd}} < -1$  MPa, Munnè-Bosch *et al.*, 2001; Abreu Me & Munnè-Bosch, 2008; Savi *et al.*, 2013). Hence, while arbutus might represent a suitable species for green roofs with very low input of additional irrigation, sage might be more recommendable in order to maximize the transpirational cooling of buildings and/or to favor fast water depletion from substrates, thus improving the effectiveness of green roofs to mitigate water runoff during occasional storms, although the use of this species would probably be possible only when regular albeit low irrigation inputs are guaranteed (Savi *et al.*, 2013). Additional studies focused on testing the



**Fig. 6.** Relationship between plant hydraulic conductance ( $K_{\text{plant}}$ ) values and corresponding substrate water status ( $\Psi_s$ ) recorded in plants of *S. officinalis* (a) and *A. unedo* (b) growing in the two types of substrate and subjected to different irrigation regimes. Regression equation, coefficient values, P-values and correlation coefficients ( $r^2$ ) are also reported.

physiological performance and water requirements of a large number of Mediterranean species over green roofs are required to conclude about possible relationships between plant hydraulic strategies and green roof performance under drought.

### Acknowledgements

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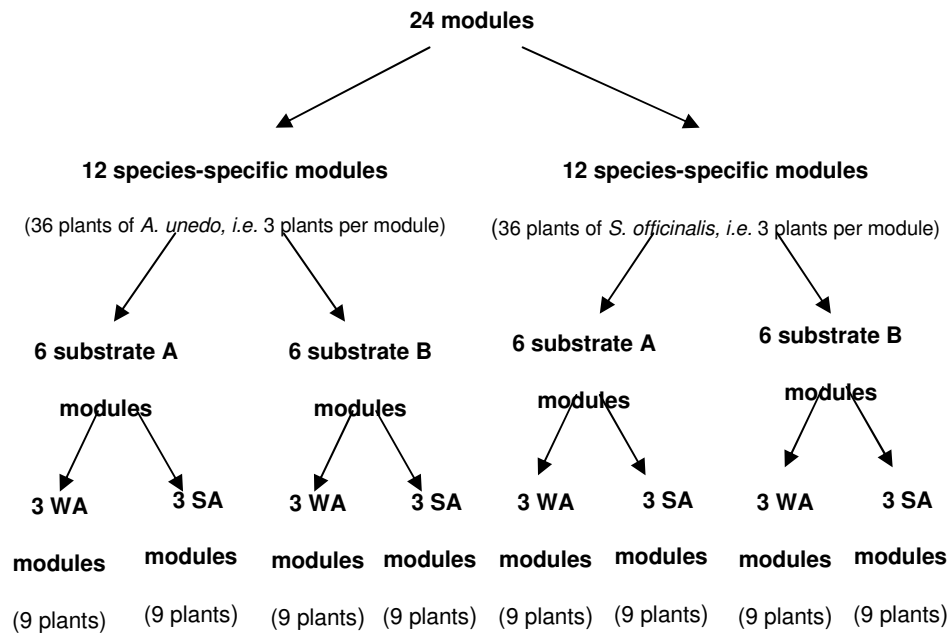
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Supplementary material



**Figure S1.** Schematic representation of the experimental design. 24 modules (75 x 23 x 27 cm) were divided in two groups of 12 modules in which 36 plants of *A. unedo* and 36 plants of *S. officinalis* were planted, respectively (i.e. 3 plants per module). Two type of soils (A and B) and two irrigation regimes (well watered, W and stressed, S) were tested. 12 modules per species were divided in two categories on the basis of substrate type tested: 6 modules per species containing substrate A and the other 6 modules containing substrate B. And, then, they were further divided in four experimental groups on the basis of irrigation regime: 3 modules per substrate type category and regularly watered to field capacity (i.e. WA and WB modules), and 3 modules per substrate type category and receiving irrigation up to 75% field capacity (i.e. SA and SB modules).

## 5. Leaf hydraulic vulnerability protects stem functionality under drought stress in *Salvia officinalis*

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### ABSTRACT

Functional coordination between leaf and stem hydraulics has been proposed as a key trait of drought-resistant plants. A balanced water transport efficiency and safety of different plant organs might be of particular importance for plant survival in the Mediterranean climate. We monitored seasonal changes of leaf and stem water relations of *S. officinalis* L. in order to highlight strategies adopted by this species to survive in harsh environmental conditions. During summer drought, the water potential dropped below the turgor loss point thus reducing water loss by transpiration, while the photosynthetic efficiency remained relatively high. Leaves lost their water transport efficiency earlier than stems, although in both plant organs P50 (water potential inducing 50% loss of hydraulic conductivity) indicated surprisingly high vulnerability, when compared to other drought-tolerant species. The fast recovery of leaf turgor upon restoration of soil water availability suggests that the reduction of leaf hydraulic conductance is not only a consequence of vein embolism, but cell shrinkage and consequent increase of resistance in the extra-xylem pathway may play an important role. We conclude that the drought tolerance of *S. officinalis* arises at least partly as a consequence of vulnerability segmentation.

*Keywords*- Common sage, water relations, aridity, xylem embolism, vulnerability curves, drought resistance

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## 1. Introduction

The structure and efficiency of the water transport system govern the growth and survival of plants by posing a physical limit to stomatal aperture, transpiration rates and photosynthetic productivity (Sperry, 2000). Relatively few studies focused on the hydraulic architecture of plants have been addressed at simultaneously investigating leaf and stem hydraulics (Salleo *et al.*, 2000; Bucci *et al.*, 2012; Nolf *et al.*, 2015; Pivovarov *et al.*, 2014). In addition to roots, leaves represent a significant hydraulic bottleneck, accounting for more than 30% of the total resistance to water flow in the soil-to-leaf pathway (Boyer, 1974; Sack & Holbrook, 2006). It is well known that under water stress leaves often lose a substantial fraction of their hydraulic efficiency at relatively high water potentials (Nardini & Luglio, 2014), when compared to stems that appear to be more resistant to hydraulic dysfunction (Salleo *et al.*, 2000; Bucci *et al.*, 2012; Johnson *et al.*, 2012). Stomatal control of transpiration prevents excessive water loss during arid periods, which otherwise might lead to leaf and stem water potential drop and consequent embolism accumulation in xylem conduits (Sperry, 2000; Sack & Holbrook, 2006). In fact, the lower the pressures in the xylem, the higher is the risk of extensive xylem embolism, which might fully compromise water transport from roots to foliage (Sperry, 2000; Nardini *et al.*, 2014). As a consequence, the likelihood of hydraulic failure, crown die-back, and plant death increases significantly under drought stress (Maherali *et al.*, 2004; McDowell *et al.*, 2011).

Bucci *et al.* (2012) highlighted the protective role of leaf hydraulic systems over stem functionality in six *Nothofagus* species, as leaves were found to lose 50% of hydraulic efficiency at water potential about 2.3 MPa less negative than those inducing a similar hydraulic impairment in stems. It was suggested that the resulting diurnal reduction of leaf hydraulic conductance ( $K_{leaf}$ ) would assure prompt stomatal closure and delay stem water potential drop, thus

preventing extensive xylem embolism build-up. Under severe and prolonged drought, the same mechanism would preserve the functionality of the more carbon-expensive woody portion of the water transport pathway, at the expense of the more disposable leaves (Bucci *et al.*, 2012; Nolf *et al.*, 2015; Nardini *et al.*, 2013). This is consistent with the ‘hydraulic segmentation hypothesis’, suggesting that greater hydraulic resistance and/or vulnerability in leaves may act as a ‘hydraulic fuse’ under extreme drought posing at risk plant survival. In fact, leaf desiccation and shedding play a major role in the survival of several species during intense water deficit, while contributing to nutrient remobilization and limiting large water losses through leaf-level transpiration (Munné-Bosh & Alegre, 2004; Nardini *et al.*, 2013).

Water moves through the leaves both in the vascular system (vein xylem) and in the complex extravascular pathway (Boyer, 1974; Nardini *et al.*, 2010), which includes both bundle sheath and mesophyll cells (Sack & Holbrook, 2006). Leaf xylem embolism is a common event in plants’ life (Lo Gullo *et al.*, 2003; Johnson *et al.*, 2012) and embolism repair has been reported by different studies (Lo Gullo *et al.*, 2003; Nardini *et al.*, 2008; Brodersen *et al.*, 2010; Mayr *et al.*, 2014). For example, air-dehydration of sunflower leaves to a water potential of -1.25 MPa translated in a 46% decrease of  $K_{leaf}$ , but complete and fast recovery (within 10 minutes) of  $K_{leaf}$  was observed when leaves were put in contact with water (Trifilò *et al.*, 2003). The apparently rapid and complete recovery of leaf hydraulic efficiency also suggests that vein embolism might be not the only mechanism underlying drought-induced decline of leaf conductance, as recently suggested by Scoffoni *et al.* (2014). In fact, the extra-xylary pathway represents 30-70% of the total leaf resistance to water flow. Hence, any eventual increase of the extra-xylary pathway resistance might lead to complete leaf hydraulic dysfunction (Sack & Holbrook, 2006; Nardini *et al.*, 2010). Recent studies have demonstrated that the drought-induced reduction

of hydraulic conductance of the mesophyll pathway is triggered not only by changes in aquaporin expression (Sack & Holbrook, 2006; Cochard *et al.*, 2007), but is also a consequence of leaf shrinkage during dehydration (Scoffoni *et al.*, 2014), as drought-induced decline of  $K_{\text{leaf}}$  was shown to be significantly correlated with changes in leaf thickness.

Coordination of water transport efficiency/safety of different organs may be of particular importance for Mediterranean plants facing large root-to-leaf water potential gradients during the prolonged dry summers. On the basis of the above, parameters related to water transport in leaves might have a major influence on the whole-plant success in drought-prone areas. To the best of our knowledge, very little information is available in the literature about the ecophysiological characteristics of *Salvia officinalis* L. (Raimondo *et al.*, 2015), a very common Mediterranean species successfully thriving in habitats characterized by long-term decrease in soil water availability and extremely high air temperatures and irradiance. The aim of this study was to monitor seasonal changes of leaf and stem water relations of *S. officinalis*, to highlight the hydraulic strategy adopted by this species to survive summer drought. We hypothesized that balanced stem and leaf resistance against drought-induced xylem dysfunction enable *S. officinalis* to survive in harsh environmental conditions that characterize its natural habitat. Moreover, we investigated the existence of a possible functional coordination between stem and leaf hydraulics.

## 2. Materials and Methods

### 2.1. The study area

The study was focused on plants of *S. officinalis* growing in natural stands near the village of Prosecco, Trieste (North-East Italy; 45° 41'52"N, 13° 44'90"E; altitude 160 m above sea level). The study site is located in the coastal area and characterized by karstic limestone soils with high water drainage capacity. The vegetation includes a mix of temperate

and Mediterranean species (Pignatti, 2002). The annual mean air temperature of the study area is 12.8°C (min = 3.9 °C in January, max = 22.6 °C in July). The annual rainfall generally exceeds 1300 mm with a relatively dry summer period (July-August = 200 mm, [www.osmer.fvg.it](http://www.osmer.fvg.it), 1 March 2015). Experimental measurements were performed between February and October 2013 and in July-August 2015.

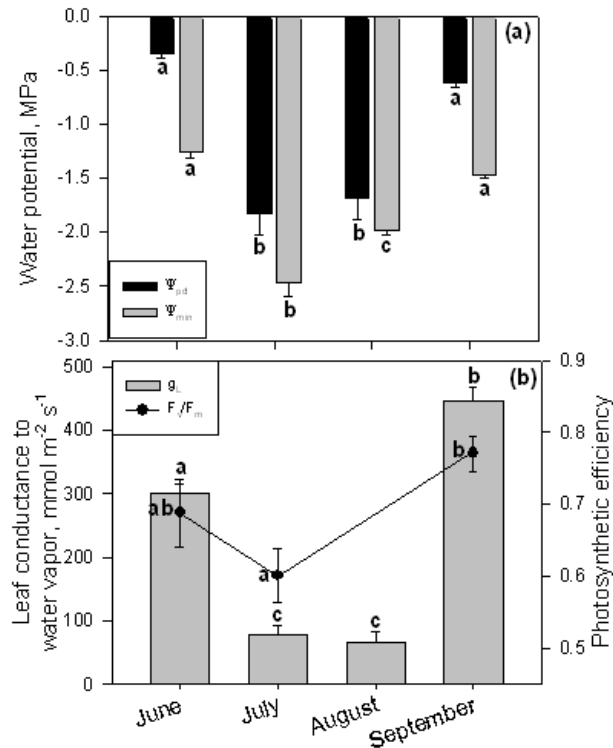
*S. officinalis* (Common sage) is a perennial, evergreen shrub with grayish leaves and woody stems (Pignatti, 2002). It is distributed widely over almost all the Mediterranean basin and it is naturalized even outside the original habitat (Pignatti, 2002).

### 2.2. Pressure-volume traits

From February to October 2013, on a monthly basis, twigs for pressure-volume curve experiments (PV-curve) were excised at pre-dawn and transported to the laboratory with their cut end dipped in water. Fully expanded leaves were immediately detached, wrapped in cling film, and left rehydrating for 30 min with their petioles immersed in distilled water. On the same day of shoot sampling, PV-curves were measured using the bench-dehydration technique and measuring water potential with a pressure chamber (mod. 1505D, PMS Instruments, Albany, Oregon, USA). Water potential ( $\Psi_{\text{leaf}}$ ) and cumulative weight loss (WL) of leaves were measured until the relationship between  $1/\Psi$  and WL became strictly linear indicating the loss of cell turgor. PV-curves were elaborated according to Salleo (1983) in order to calculate leaf osmotic potential at full turgor ( $\pi_0$ ), water potential at the turgor loss point ( $\Psi_{\text{tlp}}$ ), and bulk modulus of elasticity ( $\epsilon$ ).

At the end of experiments, images of fresh leaves were acquired using a scanner and leaf area ( $A_L$ ) was measured with the software ImageJ (<http://rsbweb.nih.gov/ij/index.html>, 1 April 2014). Leaves were oven-dried (24 h, 70°C) in order to get their dry mass (DM) and leaf mass per area (LMA) was calculated as  $DM/A_L$ . PV-curves were also used to calculate leaf capacitance ( $C_L$ ) as the ratio between leaf

water content changes over the corresponding variation of water potential ( $\Delta W/\Delta\Psi$ ).  $C_L$  was normalized by  $A_L$  and used for leaf hydraulic conductance ( $K_{leaf}$ ) calculations on the basis of the rehydration kinetic technique (see below).



**Fig. 1** Pre-dawn ( $\Psi_{pd}$ , black columns) and minimum ( $\Psi_{min}$ , grey columns) water potential (MPa, a), leaf conductance to water vapor ( $g_L$ ,  $mmol\ m^{-2}\ s^{-1}$ , grey columns, b), and photosynthetic efficiency ( $F_v/F_m$ , black dots, b) recorded for *S. officinalis* grown in the natural habitat between June and September 2013. Means are reported  $\pm$  SEM. Lettering indicates significant differences among experimental periods (One-Way ANOVA and Tukey test;  $P < 0.05$ ).

In order to verify if the level of tissue hydration, as reflected in  $\Psi_{leaf}$  at the beginning of PV-curves, has any effect on water relation components (Meinzer *et al.*, 2014), PV-curves were measured and elaborated on leaves collected from plants at different stages of dehydration in summer 2015. Shoots were excised early in the morning, inserted in plastic bags and transported to the laboratory using a cool bag. PV experiments were immediately performed on leaves in their original non-rehydrated conditions ( $\Psi$  ranging between -0.30 MPa and -1.70 MPa). Saturated mass of non-rehydrated leaves for  $\pi_0$  determination was

extrapolated using linear regression on the data above the turgor loss point ( $> \Psi_{tlp}$ ) in plots of cumulative weight loss (WL) *versus*  $\Psi_{leaf}$ . On each sampling date, at least one leaf was artificially rehydrated for 30 min ( $\Psi > -0.3$  MPa) before proceeding with PV-curve elaboration (control leaf).

### 2.3. Leaf and stem hydraulic conductance and vulnerability, wood density

In order to quantify the species' resistance to drought induced xylem embolism, leaf (Brodribb & Holbrook 2003) and stem (Choat *et al.*, 2012) vulnerability curves (VCs) were measured. In September 2013, after abundant late-summer thunderstorms that saturated soil water content, twigs of at least 10 individuals of *S. officinalis* were sampled in the field between 7.00 and 9.00 a.m. and immediately recut under water. Twigs were transported to the laboratory and left overnight with their cut end dipped in water while covered with a black plastic bag in order to allow full hydration and refilling of eventually embolized conduits (Trifilò *et al.*, 2014). Twigs were then bench dehydrated and at regular time intervals three leaves per twig were wrapped in cling film. The twig was enclosed for 20 min in a black plastic bag containing a piece of wet filter paper to stop transpiration. The water potential of two wrapped leaves was measured to estimate initial water potential ( $\Psi_0$ ). The third leaf was cut while keeping the petiole dipped in water and rehydrated for 45 seconds (t) before measuring final water potential ( $\Psi_f$ ).  $K_{leaf}$  was calculated as:  $C_L \times \ln(\Psi_0/\Psi_f) / t$ , and plotted *versus* the corresponding  $\Psi_0$  to build a leaf vulnerability curve (Brodribb & Holbrook, 2003).

Stem vulnerability curve was elaborated with the bench dehydration technique (Sperry *et al.*, 1988). Xylem water potential ( $\Psi_{xylem}$ ) was estimated by measuring  $\Psi$  of two wrapped leaves (see previous paragraph). Twigs dehydrated to progressively lower  $\Psi$  were cut under water between 7<sup>th</sup> and 8<sup>th</sup> internode to a length of 3-4 cm and recut at both ends several times with a razor blade (Venturas *et al.*, 2014). The bark

was removed and samples were connected to a hydraulic apparatus (Xyl'Em, Bronkhorts, Paris, France) and perfused with a 10 mM KCl solution (filtered at 0.45  $\mu\text{m}$ ) under a pressure of 8 kPa in order to record their initial hydraulic conductance ( $K_i$ ). The samples were then flushed for 10 min at high pressure (0.2 MPa) to remove embolism and their conductance was measured again at 8 kPa ( $K_{\text{max}}$ ). The percentage loss of hydraulic conductance (PLC) was calculated with the following equation:  $(1 - K_i/K_{\text{max}}) \times 100$ , and plotted *versus*  $\Psi_{\text{xylem}}$ .

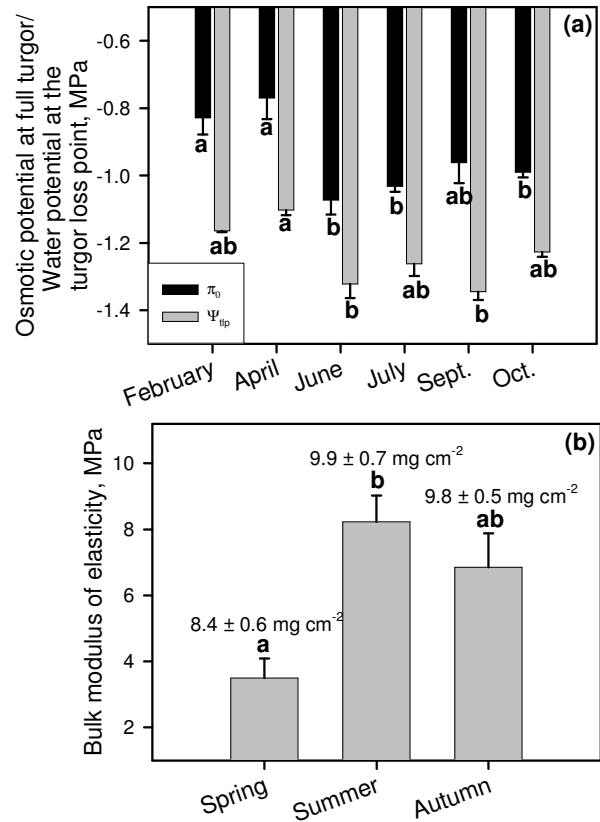
Stem samples of five different *S. officinalis* plants (one sample per plant) were left overnight immersed in water. The bark was removed and the sample fresh volume ( $V$ ) was determined according to Archimedes' principle (Hughes, 2005). Samples were oven dried, their dry mass ( $DM$ ) was recorded, and the wood density ( $\delta_w$ ) was calculated as:  $DM/V$ .

#### 2.4. Leaf shrinkage with dehydration

When summer rains restored soil water availability, shoots from well hydrated plants were collected early in the morning and transported to the laboratory with the cut end dipped in water. Detached leaves were artificially rehydrated (see above) and initial leaf area ( $A_L$ ), leaf thickness ( $T_L$ ), and turgid weight ( $TW$ ) were measured.  $T_L$  was determined by averaging values taken in the bottom, middle, and top thirds of the leaf, using a digital caliper. Leaves were then left to dehydrate on the bench and at regular time intervals  $A_L$ ,  $T_L$ , and fresh weight ( $FW$ ) were measured again followed by  $\Psi_{\text{leaf}}$  determination. The initial ( $V_{L_i}$ ) and final ( $V_{L_f}$ ) leaf volume were calculated as the product of leaf thickness and area, and leaf shrinkage estimated as follows:  $(1 - V_{L_f}/V_{L_i}) \times 100$ . Moreover, the relative water content of all leaves was calculated as  $(FW/TW) \times 100$  and plotted *versus* the corresponding  $\Psi_{\text{leaf}}$ .

#### 2.5. Field measurements

From June to September 2013, on a monthly basis, water status of field growing plants of *Salvia* was



**Fig. 2** Leaf osmotic potential at full turgor ( $\pi_0$ , MPa, black columns), water potential at the turgor loss point ( $\Psi_{tp}$ , MPa, grey columns, a), and bulk modulus of elasticity ( $\epsilon$ , MPa, b), as calculated on the basis of PV-curves measured between February and October 2013. Leaf mass per unit surface area as measured in spring, summer, and autumn is also reported (LMA,  $\text{mg cm}^{-2}$ , b). Means are reported  $\pm$  SEM. Lettering indicates significant differences among experimental periods (One-Way ANOVA and Tukey test;  $P < 0.05$ ).

monitored to record seasonal trends of pre-dawn ( $\Psi_{pd}$ ) and minimum ( $\Psi_{\text{min}}$ ) water potential, leaf conductance to water vapor ( $g_L$ ), and photosynthetic efficiency ( $F_v/F_m$ ). Measurements were performed on selected sunny days between 11.00 a.m. and 1.00 p.m.  $g_L$  was measured on two leaves from each of at least four individuals using a steady state porometer (SC1, Decagon Devices Inc., Pullman, WA, USA). Leaves were then collected, wrapped in cling film and inserted in plastic bags containing a piece of wet filter paper. Leaves were transported to the laboratory in a cool bag and  $\Psi_{\text{min}}$  was measured with the pressure chamber. On the same dates, leaves for  $\Psi_{pd}$  estimation were sampled from the same plant individuals between 6.00 and 7.00

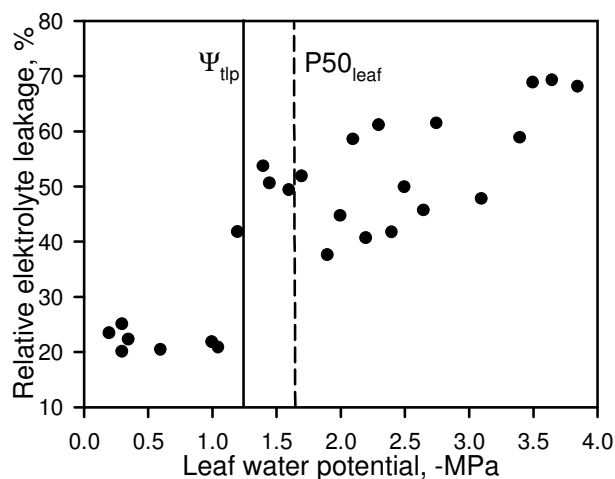
a.m. and measured as described above.

In order to test reliability of field  $\Psi$  measurements and to verify PV-curve elaboration, osmotic potential ( $\pi$ ) of leaves detached from two progressively dehydrating plants was measured at regular time intervals. In July 2015, after a summer rain which restored soil water availability ( $\Psi$  of plants close to 0 MPa) and during subsequent days (plants dehydration), at least three leaves per plant and per day were detached early in the morning (see above).  $\Psi$  of two leaves was measured to estimate  $\Psi_{\text{leaf}}$ . The third fresh leaf was cut in small pieces, sealed in plastic vials, and subjected to three freezing (1 h, -20 °C) and thawing (1 h at room temperature) cycles in order to cause release of cell sap. Osmotic potential of samples was then measured with a dewpoint hygrometer (WP4, Decagon Devices) and correlated with  $\Psi_{\text{leaf}}$ .

In June, July, and September 2013, on the same day-time when  $g_L$  was measured, the photosynthetic efficiency of at least two leaves from each of four individuals was estimated by Chlorophyll *a* Fluorescence emission measurements. Measurements were performed with a portable fluorimeter (Handy PEA, Hansatech, Norfolk, UK) on leaves previously darkened for 30 min to allow oxidation of primary acceptors.  $F_v/F_m$  was recorded as a quantitative measure of the maximum efficiency of PSII.

## 2.6. Estimation of leaf membrane integrity

To evaluate the cell membrane stability of leaf tissue under water deficit stress, electrolyte leakage tests were performed (Beikircher *et al.*, 2013). Overnight rehydrated twigs were bench dehydrated at progressively lower leaf water potential ( $\Psi_{\text{leaf}}$ ). At each target  $\Psi_{\text{leaf}}$  value, 10 leaf discs (0.25 cm<sup>2</sup> each) were cut from 2-3 leaves and inserted in a test tube containing 10 ml of distilled water. Samples were left on a stirrer at room temperature for 3 h and the initial electrical conductivity ( $C_i$ ) of the solution was assessed with a conductivity meter (Twin Cond B-173, Horiba, Kyoto, Japan). The samples were then subjected to



**Fig. 3** Relationship between leaf water potential ( $\Psi_{\text{leaf}}$ , MPa) and relative electrolyte leakage (REL, %), as measured for leaves of *S. officinalis*. The solid and dashed vertical lines represent the water potential at the turgor loss point ( $\Psi_{\text{tlp}}$ ) and leaf water potential inducing 50% loss of hydraulic conductance (P50), respectively.

three freezing and thawing cycles (see above) in order to cause complete membrane disruption and electrolyte leakage. The final electrical conductivity of the solution ( $C_f$ ) was measured, and the relative electrolyte leakage (REL) was calculated as:  $(C_f/C_i) \times 100$ , and plotted *versus*  $\Psi_{\text{leaf}}$ .

## 2.7. Statistics

Statistical analysis were performed with SigmaStat 2.03 (SPSS Inc.). Differences between groups were assessed using One-Way-ANOVA and Tukey's post hoc pairwise comparisons. The significance of correlations was tested using the Pearson product-moment coefficient. Significance was evaluated in all cases at  $P < 0.05$ . Mean  $\pm$  standard error of the mean (SEM) are reported.

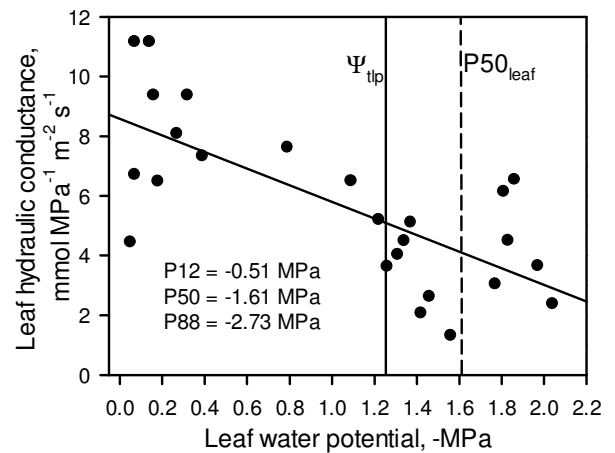
## 3. Results

Fig. 1 reports pre-dawn and minimum water potential, and leaf conductance to water vapor as recorded between June and September 2013. In spring and autumn, high soil water availability ( $\Psi_{\text{pd}} > -0.7$  MPa) ensured a favorable leaf water status ( $\Psi_{\text{min}} > -1.5$  MPa) with consequently high  $g_L$ . In summer, significantly lower  $\Psi_{\text{pd}}$  and  $\Psi_{\text{min}}$  were recorded ( $< -1.7$

MPa) leading to a marked reduction (by about 75%) of  $g_L$ .  $F_v/F_m$  recorded in June and September was higher than 0.7, while in the hot and arid period (July), the same parameter dropped to  $0.6 \pm 0.04$ . A significant recovery in the maximum efficiency of PSII and  $g_L$  was recorded in autumn when late summer thunderstorms restored soil water availability, with both values returning to pre-drought values ( $F_v/F_m$ ) or even surpassing them ( $g_L$ ).

Physiological parameters derived from PV-curves measured between February and October 2013, are reported in Fig. 2. The average  $\pi_0$  over the entire study period was  $-0.98 \pm 0.01$  MPa, while  $\Psi_{tip}$  reached a minimum value of  $-1.35 \pm 0.03$  MPa. The osmotic potential measured with the hygrometer on leaves detached from fully hydrated plants was in agreement with values derived on PV-curves ( $-0.94 \pm 0.06$  MPa). From spring to summer both physiological parameters decreased significantly by about 0.35 and 0.25 MPa for  $\pi_0$  and  $\Psi_{tip}$ , respectively (Fig. 2a). The  $\Psi_{tip}$  during the dry period was  $-1.26 \pm 0.04$  MPa, while the  $\Psi_{min}$  in the same period was  $-2.46 \pm 0.13$  MPa. The decrease in terms of  $\pi_0$  and  $\Psi_{tip}$  was accompanied by a significant increase in  $\epsilon$  (Fig. 2b). In particular, in spring  $\epsilon$  was found to be  $3.5 \pm 0.59$  MPa, while in summer plants apparently adjusted cell wall elasticity and  $\epsilon$  reached  $8.23 \pm 0.8$  MPa. In the second part of the study period,  $\pi_0$ ,  $\Psi_{tip}$ , and  $\epsilon$  underwent slight and not significant fluctuations. In spring the leaf mass per area (LMA) was found to average  $8.5 \text{ mg cm}^{-2}$ , while a slight and not significant increase of the parameter was detected in summer ( $9.9 \pm 0.7 \text{ mg cm}^{-2}$ ).

In August 2015,  $\Psi_{tip}$  (which corresponds to  $\pi_{tip}$ ) was found to be  $-1.33 \pm 0.03$  MPa in accordance with the osmotic potential measured with the hygrometer on leaves at  $\Psi_{leaf} = -1.33$  MPa ( $-1.40$  MPa, data not shown). No pronounced effects of the level of tissue hydration on the first section of PV-curves was observed, since  $\Psi_{tip}$  and  $\pi_0$  remained at about  $-1.30$  MPa and  $-1.10$  MPa, respectively, over a range of initial  $\Psi_{leaf}$  from  $-0.3$  to  $-1.4$  MPa (data not shown).



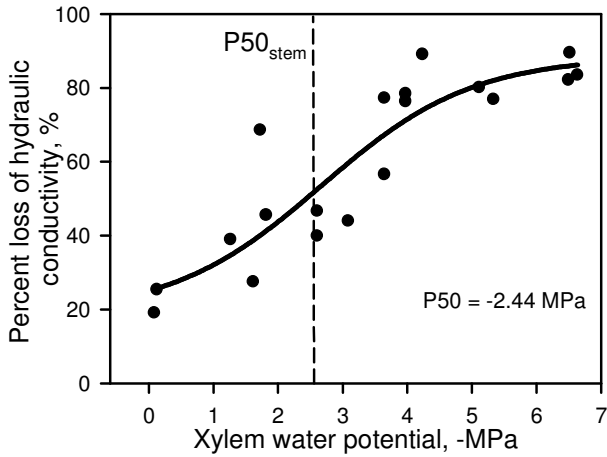
**Fig. 4** Leaf vulnerability curve of *S. officinalis* reporting the relationship between leaf hydraulic conductance ( $K_{leaf}$ ,  $\text{mmol MPa}^{-1} \text{m}^{-2} \text{s}^{-1}$ ), as measured at progressively lower leaf water potential ( $\Psi_{leaf}$ , MPa). Each point represents a different leaf. The linear regression is reported (Pearson's product-moment correlation,  $P < 0.001$ ) together with the calculated  $\Psi_{leaf}$  inducing 12 (P12), 50 (P50, dashed line) and 88% (P88) loss of hydraulic conductance. The solid vertical line represents the water potential at the turgor loss point of the species ( $\Psi_{tip}$ ).

When the initial  $\Psi_{leaf}$  was lower than  $-1.4$  MPa the relationship between  $1/\Psi$  and  $Wl$  was already strictly linear indicating that cell turgor had been previously lost. Physiological parameters for artificially rehydrated leaves (control leaves) did not differ from those of leaves measured in their original non-rehydrated conditions.

The relative electrolyte leakage test suggested that the species maintained leaf membrane integrity ( $REL < 25\%$ ) in the range between 0 and  $-1.25$  MPa, i.e. above  $\Psi_{tip}$  (Fig. 3). The 22.6% of REL recorded for well watered plants ( $\Psi_{leaf} > -0.5$  MPa) is likely due to the leakage caused by the cuttings of the leaf blade and eventual osmotic shock due to the use of aqueous solution. A sharp increase in REL was observed when leaf water potential approached and surpassed  $\Psi_{tip}$ .

Leaf (Fig. 4) and stem (Fig. 5) vulnerability curves of *S. officinalis* were based on 25 and 19 measurements (ranging between 0 and  $-2.2$  MPa for leaves and between 0 and  $-6.5$  MPa for stems), and showed a linear and sigmoidal pattern, respectively.





**Fig. 5** Stem vulnerability curve of *S. officinalis* reporting the relationship between percent loss of hydraulic conductivity (PLC, %), as measured at progressively lower xylem water potential ( $\Psi_{\text{xyL}}$ , MPa). The sigmoidal regression is reported together with the calculated  $\Psi_{\text{xyL}}$  value inducing 50% loss of hydraulic conductivity (P50, dashed vertical line).

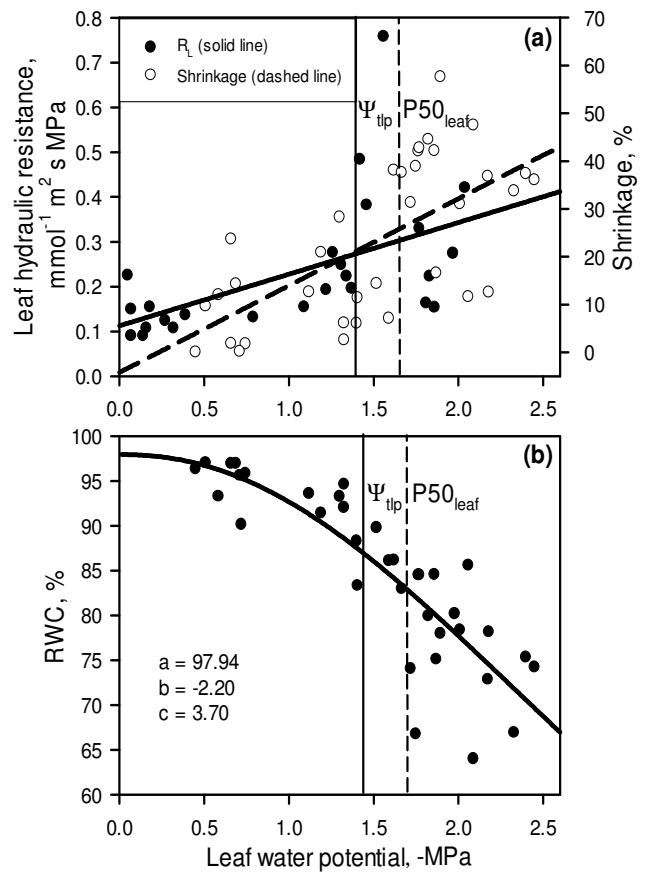
Native embolism of about 20% was observed in sage stems. The leaf maximum hydraulic conductance ( $K_{\text{max}}$ ), calculated as the average of  $K_{\text{leaf}}$  data obtained for well-hydrated leaves ( $\Psi_0 > -0.5$  MPa), was  $8.2 \pm 0.75 \text{ mmol MPa}^{-1} \text{ m}^{-2} \text{ s}^{-1}$ . From VCs the reference parameter P50 ( $\Psi$  inducing 50% loss of hydraulic conductance) was calculated to compare the vulnerability to drought stress of the two organs. Leaf and stem P50 were found to be -1.61 and -2.44 MPa, respectively, i.e. higher vulnerability (by about 0.8 MPa) was recorded for the leaf with respect to the stem. P12 and P88 (water potential inducing 12 and 88% loss of hydraulic conductance) extrapolated from leaf VC were found to be -0.51 and -2.73 MPa, respectively.

Fig. 6a reports the relationship between  $\Psi_{\text{leaf}}$  and leaf hydraulic resistance (calculated as  $R_L = 1/K_L$ ), as well as leaf shrinkage. Both parameters were significantly correlated to  $\Psi_{\text{leaf}}$  ( $P < 0.05$ ) suggesting a simultaneous and coupled increase of  $R_L$  and leaf shrinkage at increasing water deficit conditions. The results of leaf relative water content measured in parallel with  $\Psi_{\text{leaf}}$  are reported in Fig. 6b. The RWC of leaves at  $\Psi_{\text{tip}}$  and P50 was found to be 87% and 83%,

respectively. Moreover, leaves with  $\Psi_{\text{leaf}} = -2.5$  MPa (the lowest  $\Psi$  measured during summer period) reached RWC of about 69%.

#### 4. Discussion

The seasonal monitoring of water status of natural populations of *S. officinalis* highlighted a marked drought tolerance and resilience of the species. In both spring and autumn, the favorable plant water status allowed the maintenance of high  $g_L$ , thus likely assuring high gas exchange rates and  $\text{CO}_2$  uptake.



**Fig. 6** In a relationship between leaf water potential ( $\Psi_{\text{leaf}}$ , MPa) and leaf hydraulic resistance ( $R_L$ ,  $\text{mmol}^{-1} \text{ m}^2 \text{ s MPa}$ , closed circles, solid line) as well as leaf shrinkage (right y axis, open circles, dashed line). In b relationship between leaf relative water content (RWC) and  $\Psi_{\text{leaf}}$ . Regression curve is expressed by the following function:  $y = a \times x^b / (c^b + x^b)$ . Coefficients a, b, and c are reported. The solid and dashed vertical lines represent the water potential at the turgor loss point ( $\Psi_{\text{tip}}$ ) and leaf water potential inducing 50% loss of hydraulic conductance (P50), respectively.

During the summer dry season, both  $\Psi_{pd}$  and  $\Psi_{min}$  dropped below  $\Psi_{tip}$  and  $P50_{leaf}$ . As a consequence, a significant reduction of  $g_L$  was detected. It has been suggested that stomatal closure under water stress conditions is triggered by the coordination between the decrease in leaf hydraulic conductance (both at the vascular and extra-vascular level) and the turgor loss by leaf cells (Brodribb & Holbrook, 2003; Lo Gullo *et al.*, 2003). Moreover, stomatal aperture depends also on other factors such as ion uptake, pH changes in the xylem sap, and chemical signals (Barragán *et al.*, 2012; Davies *et al.*, 2002; Sack & Holbrook, 2006). It has been suggested that different mesophyll cells lose turgor at different  $\Psi_{leaf}$  values (Canny *et al.*, 2012). In particular, guard cells of stomata are able to maintain higher turgor pressure than other epidermal cells, which might delay complete stomatal closure under drought (Frank & Farquhar, 2007). In fact, during summer the water potential of *S. officinalis* was below  $\Psi_{tip}$  even at pre-dawn, but  $g_L$  was still about 25% of that recorded in spring, suggesting low, but probably vital gas exchange rates. Upon restoration of soil water availability after late summer rains, stomatal aperture promptly recovered reaching values even higher than those recorded in spring. This suggests that any eventual impairment to cells or to the water transport system was also efficiently reversed at the end of the summer dry period.

In *S. officinalis*, membrane integrity was apparently not affected by dehydration down to leaf water potential values around -1.25 MPa, while REL sharply increased when leaf water potential dropped below  $\Psi_{tip}$  and  $P50_{leaf}$ . At the peak of seasonal drought stress, a reduction by about 13% of the maximum efficiency of PSII was also observed.  $F_v/F_m$  has been largely used as an indicator of plant stress and the recorded drop suggests the occurrence of reduction of photosynthetic efficiency due to effects of drought stress and excess light energy (García-Plazaola *et al.*, 2008; Huang *et al.*, 2013). However, the maintenance of  $F_v/F_m$  values above 0.6 and the prompt recovery of

this parameter when soil water availability was restored, suggests effective adaptation and acclimation of *S. officinalis* to stress factors that characterize its natural habitat.

The average seasonal  $\Psi_{tip}$  of *S. officinalis* was found to be -1.25 MPa in accordance with previous studies performed on the same species planted on green roofs (Savi *et al.*, 2013, 2014). Indeed, this is a surprisingly high value if we consider that *S. officinalis* is a Mediterranean plant thriving in extremely harsh edaphic and climatic conditions. No evidence of artificial rehydration-induced variation of  $\Psi_{tip}$  and  $\pi_0$  was observed in this species (Meinzer *et al.*, 2014), and the physiological parameters exhibited apparent low plasticity in response to changes in tissue hydration over short timescales.  $\Psi_{tip}$  is classically recognized as a major physiological trait underlying species' drought tolerance, with direct impacts on metabolism, cellular integrity, and whole plant performance (McDowell *et al.*, 2011; Bartlett *et al.*, 2012; Ding *et al.*, 2014). In fact, Bartlett *et al.* (2012) reported clear biome-related trends in terms of  $\Psi_{tip}$ , with average values of this parameter ranging from -1.5 MPa in tropical wet forests to -2.5 MPa for Mediterranean and dry temperate areas. Hence, the turgor loss point of sage plants is much closer to values expected for mesophytes than to those typical of xerophytes, raising questions about the reliability of PV-curve extrapolated traits in this species and/or possible functional significance of such extreme leaf symplastic vulnerability. Also, despite some seasonal adjustment of  $\Psi_{tip}$  occurring in *S. officinalis* during drought progression (about 0.25 MPa), this was lower than typically recorded in Mediterranean species and generally averaging 0.7 MPa (Dichio *et al.*, 2003). On the basis of the above, and considering the large difference recorded between field measured  $\Psi_{min}$  and  $\Psi_{tip}$  ( $\Delta = 1.2$  MPa), questions on the validity of  $\pi_0$  and/or  $\Psi_{tip}$  measurements and interpretation are unavoidable. In fact, the difference between  $\Psi_{min}$  and  $\Psi_{tip}$  probably did not cause a significant decrease of

leaf symplastic water content and plasmolysis, since the RWC reduction in the range between full turgor and -2.5 MPa (about 30%) was not large enough to entirely explain such gap. The PV analysis has been frequently questioned in the past. Moreover, a recent study based on micromechanical analysis of leaf cells suggested that the majority of published PV curves result in errors of at least 0.1 MPa in derived osmotic potential and turgor pressure (Ding *et al.*, 2014). The error increases with decreasing cell size leading to an overestimation of both  $\pi_0$  and  $\Psi_{tip}$ . The authors proposed that small cell size in leaves (width of palisade mesophyll cells  $< 14 \mu\text{m}$ ) represents an adaptation allowing some plants to endure negative values of  $\Psi_{leaf}$  with relatively little water loss. Anatomical analysis of *S. officinalis* leaves highlighted an average diameter of palisade cells of about  $9 \mu\text{m}$  (data not shown). According to Ding *et al.* (2014), these cell dimensions would allow substantial negative turgor pressure (of about 1 MPa) to build up under drought, further favored by increased cell wall rigidity (Oertli, 1986; Rhizopoulou, 1997; Ding *et al.*, 2014). We conclude that PV-curve parameters derived for *S. officinalis* and other species with small mesophyll cells should be interpreted with caution, taking into account the possibility that negative  $P_t$  may develop in these cells.

In *S. officinalis*,  $\Psi_{tip}$  was correlated to  $\pi_0$  and  $\epsilon$  suggesting that seasonal adjustments in terms of drought tolerance in this species were conferred by both active solute accumulation (osmotic adjustment, Bartlett *et al.*, 2012) and increasing cell wall rigidity (elastic adjustment, Salleo, 1983; Bartlett *et al.*, 2012). Both increasing and decreasing  $\epsilon$  have been suggested to be adaptive in dry habitats (Salleo, 1983; Abrams, 1990; Bartlett *et al.*, 2012). In our study, higher cell wall rigidity in summer might have allowed tolerance of negative turgor pressure (see above), while preventing large fluctuations in tissue RWC and ensuring, at the same time, prompt stomatal closure even for small changes in water content (Salleo, 1983;



**Fig. 7** Pictures of *S. officinalis* plants with wilted and folded leaves, i.e. when thresholds represented by  $\Psi_{tip}$  and  $P_{50_{leaf}}$  were surpassed (a), and the recovery of leaf turgor occurring within 1-2 hours after a single rain event (b).

Oertli, 1986; Abrams, 1990; Niinemets, 2001). As a likely consequence of solute accumulation, increasing cell wall rigidity, and low or null cell turgor limiting the expansion of leaves, a slight increase of LMA was detectable during the dry season (Fig. 3b). LMA has been associated with  $\epsilon$ ,  $\pi_0$ , and  $\Psi_{tip}$  (Bartlett *et al.*, 2012), and positively correlated to leaf longevity (Niinemets, 2001). On the other hand, values of LMA and  $\epsilon$  recorded for *S. officinalis* were markedly lower if compared to data obtained for other species living in dry environments (Bartlett *et al.*, 2012; Scoffoni *et al.*, 2014). In habitats characterized by prolonged summer drought, the maintenance costs of leaves could exceed the replacement costs. The lower biomass investment required per unit leaf area of *S. officinalis* if compared to other drought adapted species, might represent an advantage as, at the expense of the more disposable leaves, it allows higher carbon investments in the long-

lived woody portion of the water transport pathway. In addition, leaves with lower LMA and  $\epsilon$  may contribute to greater water storage capacitance after stomatal closure (Ogburn & Edwards, 2010).

The leaf  $K_{\max}$  recorded for *S. officinalis* was in accordance with values reported in the literature for woody species growing in dry habitats (Nardini & Luglio, 2014). On the other hand,  $P50_{\text{leaf}}$  resulted only moderately negative (-1.6 MPa) if compared to other drought tolerant species, where this parameter ranges between -2 and -4 MPa and averages approximately -2.5 MPa in the Mediterranean biome (Nardini & Luglio, 2014). In Mediterranean climatic conditions, such a low leaf resistance in terms of  $P50_{\text{leaf}}$  and  $\Psi_{\text{tip}}$  seems paradoxical and unlikely to represent a functional advantage. However, it is worth noting that when surpassing critical thresholds represented by  $\Psi_{\text{tip}}$  and  $P50_{\text{leaf}}$ , leaves of *S. officinalis* appeared deeply wilted and folded (Fig. 7a). This can be interpreted as a defense mechanism, as the exposed leaf surface area is drastically reduced and the hairy abaxial leaf blade can efficiently reflect the excess light energy and reduce water loss by transpiration (Pérez-Estrada *et al.*, 2000; Holmes & Keiller, 2002). Hence, our findings suggest that the precocious reduction of  $K_{\text{leaf}}$  and cell turgor may serve in this species as a mechanism for limiting the amount of incident solar radiation and consequent injuries on photosystems ( $F_v/F_m > 0.6$ ). The transpirational water loss is controlled by  $g_L$  reduction which prevents, at the same time, a sharp stem  $\Psi$  drop. Regular visual assessments of the turgor status of *S. officinalis* in the natural habitat have pointed out the surprisingly fast (within 1-2 hours) recovery of turgor in wilted leaves after even small rain events (Fig. 7b). Similarly, an apparent rapid recovery of  $K_{\text{leaf}}$  has been reported in leaves of several species under controlled experimental conditions (Lo Gullo *et al.*, 2003; Trifilò *et al.*, 2003). This phenomenon has been mainly attributed to refilling of embolized conduits (Sack & Holbrook 2006). However, the extremely fast recovery of sage leaf turgor when water availability was

restored, may indicate that the drought-induced reduction of  $K_{\text{leaf}}$  was not only a consequence of leaf vein embolism (Scoffoni *et al.*, 2014). The significant correlation between leaf hydraulic resistance and  $\Psi_{\text{leaf}}$ , as well as cell shrinkage and  $\Psi_{\text{leaf}}$  (Fig. 5a) suggests that the drop in  $K_{\text{leaf}}$  shown by the vulnerability curve could also arise from the loss of connectivity among leaf cells and consequent increase of resistance in the extra-xylem water pathway (Sancho-Knapik *et al.*, 2011; Scoffoni *et al.*, 2014; Bouche *et al.*, 2015). Simulations of water potential gradients in transpiring leaves suggested that because of the high hydraulic resistance of the protoplasts (Boyer, 1974), the most negative  $\Psi$  develops at the distal end of the hydraulic pathway (leaf mesophyll), while xylem tensions rarely reach pressures that would induce embolism (Scoffoni *et al.*, 2014). In this light, the drought-induced reduction of leaf hydraulic conductance observed in *S. officinalis*, can be interpreted as a 'safety hydraulic fuse', as it prevents the water potential drop in the xylem that would lead to embolism build-up and catastrophic xylem hydraulic failure.

The  $P50_{\text{stem}}$  of *S. officinalis* (-2.44 MPa) was lower than  $P50_{\text{leaf}}$  ( $\Delta = 0.83$  MPa) but still higher than values reported for stems of other drought-adapted species as reviewed by Maherali *et al.* (2004) and Nardini *et al.* (2014), suggesting  $P50_{\text{stem}}$  values averaging -5.0 MPa. The  $P50_{\text{stem}}$  is largely used as a predictor of species' drought tolerance (Choat *et al.*, 2012), but in the case of *S. officinalis* this would not explain the ecology of the species. The safety margins toward massive embolism formation calculated as the difference between  $\Psi_{\text{min}}$  and  $P50_{\text{stem}}$  (Choat *et al.*, 2012) was found to be slightly negative (-0.02 MPa) at the peak of the summer drought. Data reported in the literature suggest that about 70% of woody plants generally operate with narrow safety margins and could easily surpass critical xylem water potential pressures facing potential risk of hydraulic failure (Choat *et al.*, 2012; Nolf *et al.*, 2015; Savi *et al.*, 2015). The partial  $\Psi$  rise during night-time ( $\Psi_{\text{pd}}$ ) and the fast

recovery of leaf turgor after rain events, might indicate that the stem xylem pathway was likely not deeply impaired in sage. In addition to the fundamental role played by leaves in preventing excessive stem  $\Psi$  drop, we can hypothesize that high sapwood capacitance could also contribute to conferring hydraulic safety (Meinzer *et al.*, 2009). Indeed, species with low wood density (*S. officinalis*  $\delta_w = 0.4 \text{ g cm}^{-3}$ ) are generally characterized by high sapwood capacitance, possibly contributing to embolism avoidance via transient release of stored water to buffer fluctuations in xylem tension (Meinzer *et al.*, 2009).

On the basis of our results, we suggest that drought tolerance of *S. officinalis* is the result of peculiar anatomical and physiological traits, partly unexpected in a Mediterranean plant. Apparently, rather than investing carbon for the construction of a more embolism resistant stem water transport pathway, sage plants rely on unusually high leaf hydraulic vulnerability to isolate and protect the xylem under conditions of extreme aridity.

## 5. Conclusion

Our results contribute to the understanding of the functional meaning of coordination of leaf and stem hydraulics, supporting the view that leaves may act as a ‘safety hydraulic fuse’ to prevent catastrophic stem hydraulic dysfunction. The ability to survive water stress by maintaining the functionality of stem hydraulic system is apparently more important for plants thriving in the extreme Mediterranean habitat, than the achievement of high gas exchange and photosynthetic rates.

$\Psi_{\text{tip}}$ ,  $P50_{\text{leaf}}$ , and  $P50_{\text{stem}}$  are widely used for comparisons of drought resistance among species and across biomes. Nevertheless, despite their utility as indices of resistance to loss of cell turgor and hydraulic efficiency, in some cases like the one reported in this study, they have to be interpreted with caution taking into consideration that they could not have a specific physiological relevance when considered outside the

context of the overall adaptation mechanisms conferring hydraulic safety and assuring survival to plant species growing in arid habitats.

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## 6. Composition and performance of succulent and herbaceous plant covers of green roofs in response to microclimatic factors

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### HIGHLIGHTS

- Green roof technology is still under-represented in drought-prone areas
- Early establishment and ecology of succulent and herbaceous vegetation were monitored
- CAM metabolism allowed succulent species to thrive in the harsh environment
- Four herbaceous communities (for a total of 30 species) could be distinguished
- The possible use of a succulent/herbaceous mix in arid climate deserves further studies

### ABSTRACT

One of the most critical steps in green roof installation is the selection of appropriate plant species to optimize technical and ecological functions such as thermal insulation of buildings, stormwater run-off reduction, habitat restoration, and biodiversity conservation. Experimental green roof modules settled in a sub-Mediterranean climate were vegetated with succulent (8 cm deep substrate) or herbaceous plants (8 and 10 cm deep substrate). The vegetation composition as well as the efficiency in terms of evapotranspiration during the dry season were monitored over the first year following installation. Native succulent species were suitable for the harsh environmental conditions likely due to their CAM metabolism and ability to reallocate water in response to drought stress. In herbaceous modules, four plant communities (for a total of 30 species) could be distinguished in different times of the season in terms of species composition and ground cover. The change in plant community composition was apparently correlated with changes in multiple environmental factors such as substrate water content, air temperature, and water pressure deficit. C<sub>4</sub> plants proved to be particularly suitable for sub-Mediterranean roof greening. Our results also suggest that the association of succulent and herbaceous plants might ensure a tradeoff between low water use for survival under critical conditions and high water use for storm-water runoff mitigation under optimal conditions. Hence, further research is needed to test the strategy of integration of these two different plant functional groups for implementation of Mediterranean green roofs.

*Keywords* - plant communities, Mediterranean climate, water use complementarity, C<sub>4</sub> and CAM metabolism, vegetation resilience

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## 1. Introduction

In recent years, green infrastructures have gained importance as effective tools to mitigate the impact of climate change in cities and help restore the ecological functions of urban habitats (Gill *et al.*, 2007). In particular, green roofs play an important role in the mitigation of the urban heat island effect (Gago *et al.*, 2013), in the reduction of stormwater run-off (Czemiel Berndtsson, 2010) and pollutants (Yang *et al.*, 2008), as well as for habitat recreation, biodiversity conservation, and restoration of ecological connectivity in cities (Dvorak & Volder, 2010).

One of the most critical steps in green roof installation is the selection of an appropriate set of plant species (Dvorak & Volder, 2010). This is particularly relevant if reduced substrate depths are to be used in areas characterized by a warm, dry climate. In fact, substrate depth is an important factor affecting the performance of plants colonizing green roofs (Papafotiou *et al.*, 2013). Physiological requirements of plants in terms of substrate depth must be reconciled with structural limits of the buildings and installation costs, both limiting the amount of substrate that can be used (Benvenuti & Bacci, 2010). Hence, suitable species for roof greening must be able to tolerate very harsh environmental conditions in terms of drought duration and intensity, coupled to high temperatures and irradiance, as well as wind exposure (Oberndorfer *et al.*, 2007). Fast rooting ability, rapid spread and high soil cover are also desired plant features in order to improve the technical performances of green roofs such as thermal insulation and consequent energy conservation, stormwater management etc. (Getter & Rowe, 2006).

Different criteria have been proposed for the successful selection of species for green roofs (Lundholm, 2006; Farrell *et al.*, 2013; Van Mechelen *et al.*, 2014b; Lundholm *et al.*, 2015). For example, Lundholm (2006) suggested to base plant selection on the study of the flora of natural ecosystems with environmental conditions similar to those of green

roofs, i.e. cliffs and rocky soils (habitat template hypothesis). Furthermore, Farrell *et al.* (2013) developed a plant selection model evaluating water use strategies of 12 granite outcrop species under contrasting water availability. The study pointed out that the ideal species have to be characterized by morpho-physiological traits that allow a tradeoff between low water use for survival under critical conditions, and high water use for storm water runoff mitigation under optimal conditions. Finally, Van Mechelen *et al.* (2014b) showed that the study of plant physiological traits as drought adaptation and regeneration capacity can be used to select suitable plant species and optimize green roof performance in Mediterranean countries.

Recently, it was demonstrated that both irrigation and/or substrate amendment can significantly improve plant survival over shallow substrates (Savi *et al.*, 2014; Schweitzer & Erell, 2014), but an appropriate selection of drought-tolerant species remains a key target for the installation of fully functional green roofs in arid-prone areas (Van Mechelen *et al.*, 2014a; Raimondo *et al.*, 2015). In addition to the limits imposed by environmental conditions, species selection should also optimize green roofs in terms of habitat restoration and biodiversity conservation (Gedge & Kadas, 2005). Dvorak & Volder (2010) highlighted the importance of using native species in roof greening, to ensure more relevant functional and ecological benefits in the framework of urban conservation biology. In recent years, great attention has been paid to the reconstruction over green roofs of typical rural landscapes and synanthropic habitats, like meadows and brown-fields (Nagase & Dunnett, 2013; Benvenuti, 2014). These habitats result from the interaction between natural ecosystems and human activities and they all support high levels of biodiversity.

The urban areas, in particular those located in Mediterranean regions, are currently threatened by landscape conversion and climate changes (Underwood

*et al.*, 2009; Fischer & Schär, 2010), and hence might be among the major beneficiaries of the multiple benefits offered by the green roof technology. In particular, the floristic diversity of the Mediterranean region represents an important resource for efficient green roof establishment in this region (Van Mechelen *et al.*, 2014a). Benvenuti & Bacci (2010) monitored 20 Mediterranean xerophytes colonizing two experimental green roofs (15 and 20 cm substrate thickness). Almost all selected species showed excellent performances in terms of growth, ground cover, and flowering during the hot season in both substrate depths. Nonetheless, the number of Mediterranean species specifically tested for their performance on green roofs is still quite limited (Van Mechelen *et al.*, 2014a). In some recent papers, Van Mechelen *et al.* highlighted that 79% of the species growing on rocky soils in south France have never been used on green roofs (Van Mechelen *et al.*, 2014a) and identified 34 newly potential green roof species (Van Mechelen *et al.*, 2014b).

The vegetation composition of green roofs can affect evapotranspiration, which is a key parameter providing both thermal and hydrological services. Lundholm *et al.* (2010) evaluated the functional performances of green roofs planted with monocultures or mixtures, concluding that some mixtures outperformed the best monocultures in terms of evapotranspiration. In a recent study, Klein & Coffman (2015) found that the high evapotranspiration rate of grass and wildflower species can positively affect the surface energy balance of green roofs in extreme climatic conditions. On other hand, the lower evapotranspiration rate of succulent species and their moderate groundcover, if compared to herbaceous cover, might decrease the ability of a green roof to mitigate stormwater runoff (Nagase & Dunnett, 2012).

The present study is aimed at contributing to the optimization and diffusion of low maintenance green roofs in drought-prone regions, starting from the analysis of vegetation patterns in experimental green roof modules installed in a sub-Mediterranean area. In

particular we monitored: I) the survival and coverage of native crassulacean species over one year; II) the early establishment and development of an autochthonous semi-spontaneous herbaceous cover over the spring-autumn period; III) the efficiency in terms of evapotranspiration of succulent and herbaceous plant cover during a summer dry season.

## **2. Materials and methods**

### **2.1. Study area**

The study was carried out from April 2012 to October 2013 on the rooftop of a building located in the main campus of the University of Trieste (Trieste, Italy; 45°39'40" N, 13°47'40" E; altitude 125 m asl). The climate of Trieste is characterized by warm and dry summers and relatively mild winters. Climate data for the period 1995-2012 (<http://www.osmer.fvg.it/>) report a mean annual temperature of 15.7 °C, with the coldest and warmest monthly average temperature of 6.8 °C and of 25 °C recorded in January and July, respectively. The proximity of the sea reduces the diurnal thermal excursion to an annual average of 6 °C. The cumulative annual rainfall is 843 mm, with a maximum between September and November (290 mm) and two relatively dry periods in January-February (105 mm) and July (55 mm).

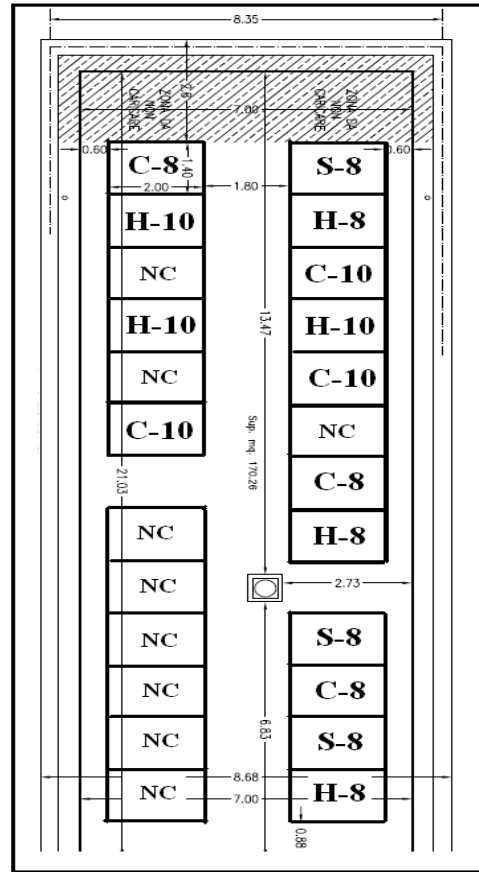
### **2.2. Experimental modules and plant material**

The experimental set-up installed in April 2012 consisted of 15 experimental modules (Fig. 1). Each module measured 2 × 1.25 m and contained a complete layering of materials provided by SEIC verde pensile (Harpo Spa, Trieste, Italy), including a root resistant and waterproof 1.5 mm thick PVC membrane (Harpoplan ZDUV 1.5), a moisture retention layer with water holding capacity of 15 L/m<sup>2</sup> (Idromant 4), a drainage layer made of plastic profiled elements (MediDrain MD 40, water retention 4 L/m<sup>2</sup>), a filter membrane (MediFilter MF1), and SEIC substrate for extensive green roof installations (dry bulk density:

848 kg/m<sup>3</sup>). Several cavities of the drainage plastic elements were pierced to obtain holes of 4 mm in diameter (340 holes/m<sup>2</sup>), to improve the amount of water available to plants (Savi *et al.*, 2013). The substrate was a blend of pomix, lapillus and zeolite (grain size 0.05-20 mm), enriched with 2.9% organic matter (peat), with total porosity = 67.35%, pH = 6.8, drainage rate = 67.4 mm/min, water content at saturation = 0.44 g/g, cation exchange capacity = 23.8 meq/100 g and electrical conductivity = 9 mS/m. Experimental modules were divided into two groups filled with either 8 cm (9 modules) or 10 cm (6 modules) deep substrate (Fig. 1). The two substrate depths were chosen on the basis of the Italian national guidelines (UNI 11235:2007) recommending for green roof installation in semi-arid climate minimum substrate depths of 8 cm and 10 cm for succulent and herbaceous plants, respectively. Each experimental module was equipped with a volumetric soil moisture content sensor (EC-5, Decagon Devices Inc.). Calibration relationships for sensors installed in sub-samples of substrates were used to convert values of volumetric soil water content (VWC, V/V) to values of water content (WC, g/g) and water potential ( $\Psi$ , -MPa, for details see Savi *et al.*, 2015).

In the mid of April 2012, modules were greened with two different types of plants, i.e. succulents on 8 cm (S-8) and herbaceous plants on both 8 cm (H-8) and 10 cm (H-10). Each combination of plants and substrate depth was replicated 3 times, and 3 additional modules for each category of substrate depth were left bare of vegetation (control modules; C-8, C-10; Fig. 1). The modules vegetated with succulents were divided by plastic wires into 25 x 25 cm squares used for plants ground cover determination and monitoring.

The succulent species used were native to the natural habitats surrounding Trieste. Rooted cuttings of the following species were collected and randomly transplanted (400 g m<sup>-2</sup>) in the experimental modules: *Hylotelephium telephium* (L.) H Ohba sl, *Sedum album*



**Fig. 1** Schematic representation of the experimental set-up. 9 green roof modules were filled with 8 or 10 cm deep substrate and vegetated with succulent (S-8) or herbaceous species (H-8, H-10). 3 additional modules for each category of substrate depth were left bare of vegetation (control modules; C-8, C-10). NC = other experimental modules not considered in the present study.

L., *Sedum dasyphyllum* L., *Sedum pseudorupestre* Gallo, *Sedum sexangulare* L. and *Sempervivum tectorum* L. (Pignatti, 1982).

The herbaceous cover was obtained by spreading a mixture of seeds and hay (265 g m<sup>-2</sup>) collected in a local barn (in March 2012) and obtained from pasture grassland mowing by farmers. The grasslands belong to the association Arrhenatheretum which develop on limestone soils, have anthropogenic origin and had been largely fertilized and periodically mown (pH range: slightly acid-slightly basic; Poldini, 1989). The characteristic species are *Achillea millefolium* L., *Medicago lupulina* L., *Plantago* sp., *Poa pratensis* L., *Trifolium* sp, *Vicia* sp, etc. (Poldini, 1989).

During the study period, the experimental modules were irrigated only occasionally during extreme and prolonged dry periods (for a total of six times), i.e. when the substrate water potential dropped below -3 MPa.

Air temperature and humidity (EE06-FT1A1-K300, E+E Elektronik), precipitation (ARG 100 Raingauge, Environmental Measurements Limited), wind direction and speed (WindSonic 1, Gill Instruments), and irradiance (MS-602, EKO Instruments) were collected by a weather station installed nearby the experimental modules. The water pressure deficit (VPD) was calculated daily between 12.00 and 14.00 h with the following equation:  $VPD = E_0 \times (1 - RH)$ , where  $E_0$  is the saturation vapor pressure at a definite air temperature and RH the air relative humidity.

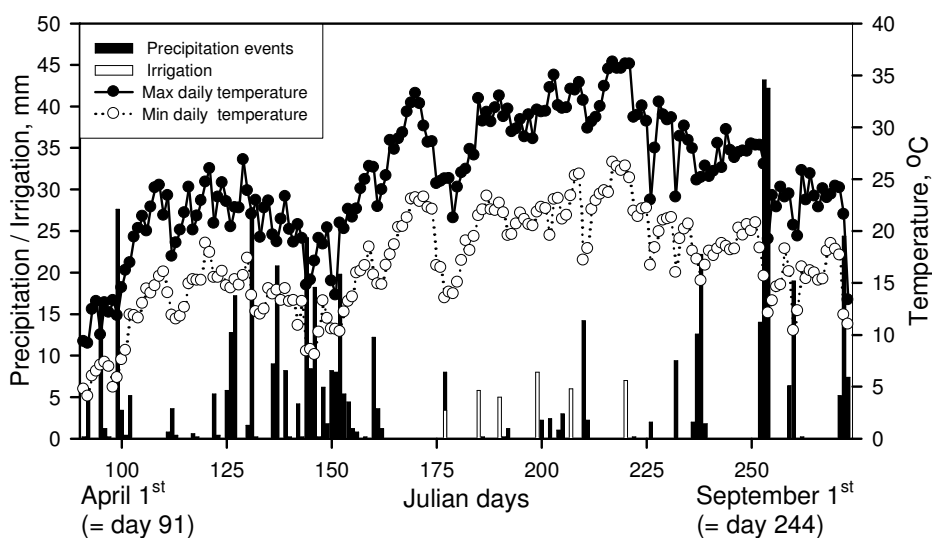
### 2.3. Monitoring vegetation cover and dynamics

The total ground area covered by the succulent species (i.e. area covered by vegetation/total module area) was monitored at regular intervals from August

2012 to October 2013 by analysing digital images of the 25 x 25 cm squares (see above) using the software ImageJ (ImageJ 1.46r, NIH, USA). Three digital images of randomly selected squares were acquired for each replicate. The species composition of herbaceous flora was monitored from April to September 2013. The species were identified on the basis of Pignatti (1982). Species nomenclature follows Conti *et al.* (2005). The plant ground cover of the herbaceous modules was estimated on a monthly basis by visual assessment.

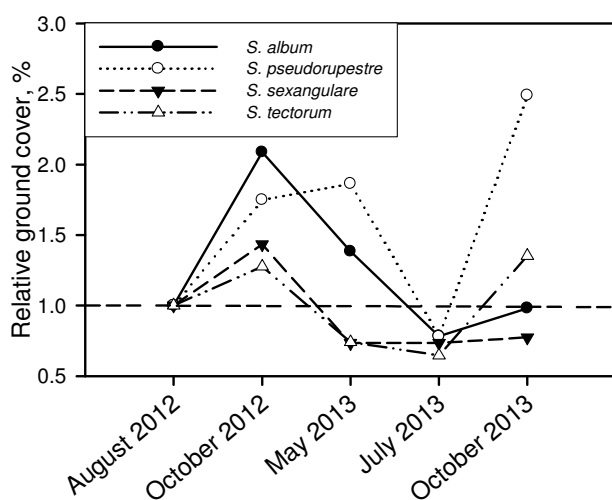
### 2.4. Succulent species photosynthetic metabolism

Some succulent plant species can engage CAM metabolism and their performance in harsh green roof environmental conditions could be influenced by the capacity to switch between  $C_3$  and CAM photosynthesis. To identify the photosynthetic metabolism preferentially engaged by the succulent species, carbon isotopic composition ( $\delta^{13}C$ ) was measured to discriminate between  $C_3$  and CAM metabolism (Osmond *et al.*, 1975; Silvera *et al.*, 2010;



**Fig. 2** Precipitation events (black columns), supplementary irrigation (white columns), minimum (white circles) and maximum (black circles) daily temperatures recorded over the rooftop between April 1st and September 30th 2013.

Cernusak *et al.*, 2013). On June 17<sup>th</sup> (high substrate water availability) and July 17<sup>th</sup> (water stress) 2013, 5 g of leaves sampled from different individuals of *S. album* and *S. sexangulare* were collected in each module for a total of three samples per species. *S. album* and *S. sexangulare* were selected due to their good ground cover and survival capabilities. The samples were dried at 70 °C for 24 h, grinded and sent for mass spectrometry analysis to ISO4 Snc (Torino, Italy).



**Fig. 3** Relative ground cover (%) trends of *S. album*, *S. pseudoruprestre*, *S. sexangulare* and *S. tectorum* in the study period between August 2012 and October 2013.

### 2.5. Estimation of evapotranspiration rates

In order to evaluate eventual differences in terms of evapotranspiration of experimental vegetation types, the substrate water content (WC) was monitored on an hourly basis by volumetric soil moisture content sensors (see above). On the basis of the dry mass of substrate ( $M_s$ ) contained in modules with different substrate depth (204 and 270 kg in D-8 and D-10, respectively), the WC data recorded at 00.00 h were used to calculate the total amount of water, expressed in liters, contained in the substrate of each module ( $WC1 = WC \times M_s$ ). The daily water loss from each experimental module was calculated as the difference

between the water content (WC1) at 00.00 h (midnight) and the water content at 00.00 h of the following day ( $WC1_{+24h}$ ), as  $(WC1 - WC1_{+24h}) / A$ , where A is the area of experimental modules (2.5 m<sup>2</sup>). The volume of water lost in 24 h was interpreted as evapotranspiration (ET) in vegetated modules or as simple evaporation (E) in control modules (bare substrate only). Transpiration (T) was estimated as  $T = ET - E$ . Only days characterized by the absence of rain events were considered.

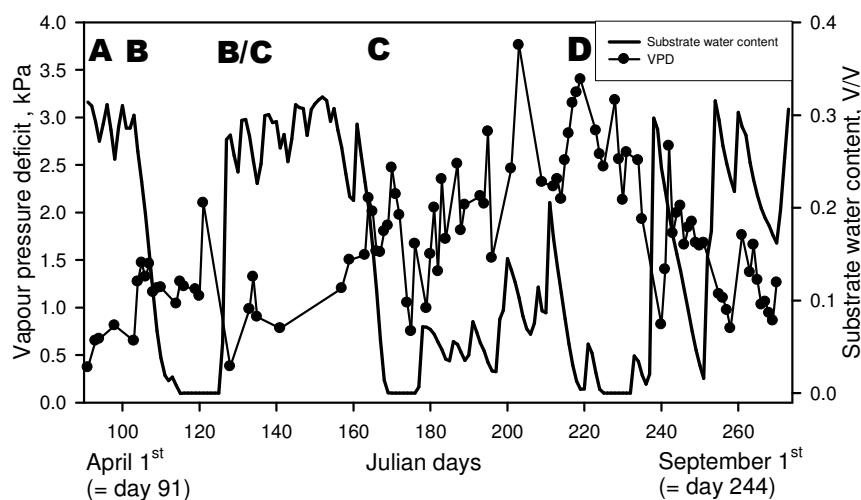
### 2.6. Statistics

Statistic analysis was performed using the software Sigma Stat v. 2.03 (SPSS Inc.). Statistically significant differences ( $P < 0.05$ ) between experimental groups (normality of data satisfied) were assessed with Student's t-test and ANOVA, followed by Tukey's HSD post hoc test. The variability of data is expressed as standard error of the mean (SEM).

## 3. Results

### 3.1. Climatic data

Fig. 2 reports minimum and maximum daily temperatures and precipitation events recorded during the period April-September 2013 (when species composition of herbaceous flora was monitored) over the green roof, as well as the amount of water supplied with irrigation. The daily mean temperature averaged  $20.7 \pm 5.4$  °C, with an absolute minimum and maximum of 4.1 °C (April 2<sup>nd</sup>) and 36.3 °C (August 5<sup>th</sup>), respectively. The total rainfall was 551 mm, falling mainly in May (189 mm) and in September (162 mm) and almost absent in July (26.6 mm). The historical climatic data for the study area over the same period are 21 °C and 529 mm for the mean air temperature and rainfalls, respectively (<http://www.osmer.fvg.it>). During the dry period, irrigation provided a total of 35.2 mm.



**Fig. 4** Water pressure deficit (VPD, black dots) and substrate water content (VWC, solid line) measured over the rooftop between April 1st and September 30th 2013. Bold letters indicate the succession of four different plant communities observed during the study period.

### 3.2. Propagation, ground cover and metabolism of succulent species

Significant fluctuations in vegetation ground cover were observed over the entire study period (Fig. 3). The ground cover assured by succulent species, as estimated at different stages of the 15 months monitoring, is expressed as relative to the value recorded at the beginning of the study period (relative ground cover, %).

The estimation of *H. telephium* ground cover was not always possible due to its growth form, mainly developing in height, while *S. dasyphyllum* was neglected, because it disappeared within few weeks after planting. *S. album*, *S. pseudorupestre*, *S. sexangulare*, and *S. tectorum* showed similar increase/decrease trends of ground cover during the study period, although the magnitudes of these changes were species-specific.

During the start-up observation period (between August and October 2012), the total ground cover in experimental modules significantly increased up to  $41.9 \pm 6.9\%$  (+68%,  $P < 0.05$ ). In particular, the largest increase was recorded for *S. album* (+109%) and the lowest for *S. tectorum* (+28%, Fig. 3, Table 1).

The total plant cover showed a highly significant decrease (52.6%,  $P < 0.001$ ) in winter, spring and early summer. In particular *S. album* and *S. sexangulare* ground cover significantly decreased by 62.5% and 48.8%, respectively ( $P < 0.05$ ). Only *S. pseudorupestre* showed a weak increase in cover (by about 6%) during winter and spring, followed by a sharp decrease (-58%,  $P < 0.05$ ) in summer (Fig. 3).

During the late summer, characterized by frequent thunderstorms, highly significant increase ( $P < 0.001$ ) of ground cover (by about 50%) was observed (Table 1). In particular, *S. pseudorupestre* and *S. tectorum* showed a marked increase in growth by 220% and 110%, respectively.

A significant difference ( $P < 0.001$ ) was found in terms of  $\delta^{13}\text{C}$  values recorded for *S. album* ( $-23.2 \pm 0.9\text{‰}$ ) and *S. sexangulare* ( $-26.2 \pm 0.5\text{‰}$ , data not shown). Leaf  $\delta^{13}\text{C}$  values did not show considerable differences between the samples collected in the mid of June and July.

### 3.3 Diversity and dynamics of herbaceous cover

The sowing of local seeds mixture led to the development of a dense vegetation cover within a short time interval (30 days). Species determination was performed between April and September 2013. In some



cases, species identification was difficult, because of roof microclimatic conditions that did not allow the complete development of plants up to flowering. Plant individuals devoid of diacritical characters were not considered in the survey. During the whole study period, a total of 30 species (Table 2) were identified in both 8 and 10 cm deep modules, with a prevalence of pioneer and ruderal species. Therophytes and hemicryptophytes were the dominant life-forms, representing 63% and 30% of the species, respectively.

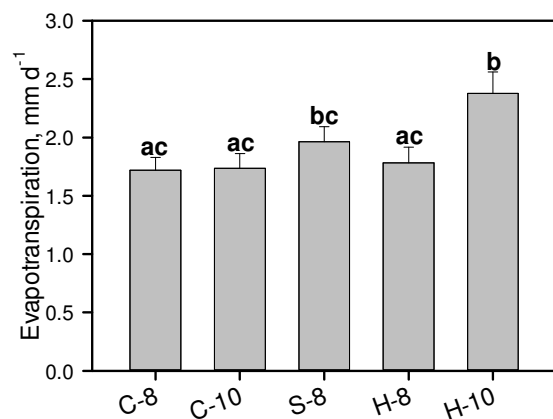
During seasonal drought progression, four different plant communities could be described (A, B, C, and D) based on species composition and ground cover assessed at different monitoring times (succession in time, Table 2). A high percentage of identified plant species were representative for the *Arrhenatheretum* grasslands used for seed collection. The series of plant communities was apparently driven by changes in multiple environmental factors, i.e. substrate water content, vapor pressure deficit (VPD) and daily temperature fluctuations (Fig. 2 and 4). The abundance of species per plant community varied between 4 and 21.

In early spring, with high water availability and relatively low air temperatures (5-15 °C) and VPD, synanthropic therophytes (7 species: community A) were the dominant life-form (Table 2), with an estimated ground cover ranging between 20 and 50%.

The following rapid increase of air temperatures (10-25 °C) led to the development of community B (Fig. 4), characterized by the highest biodiversity (21 species) and ground cover (> 90%). Dominant species belonged to the genus *Medicago* and *Vicia* (Fabaceae).

After a short drought period (substrate WC close to zero), *Medicago* and *Vicia* species desiccated leaving space to perennial xerophytes of arid, moderately disturbed habitats (6 species: community C), with a ground cover not exceeding 50% (Fig. 4, Table 2).

At the end of July, characterized by extreme drought, VPD and maximum daily temperatures up to 35 °C, only four species characterized by C<sub>4</sub> photosynthetic metabolism were found (community D, Fig. 4). Initially, their ground cover did not exceed 10%, but after some rainfalls and supplementary irrigation, values close to 50% were reached, mainly due to the growth of a few *Portulaca oleracea* plants.



**Fig. 5** Average evapotranspiration rates recorded for control modules (C-8 and C-10), succulent (S-8) and herbaceous (H-8 and H-10) vegetation during the growing season (April-September 2013). Error bars represent the SEM (n=96). Different letters indicate a statistically significant difference (P<0.05) according to the one-way ANOVA test followed by Tukey test.

#### 3.4. Estimation of evapotranspiration

Fig. 5 reports the average evapotranspiration rates (ET) from different experimental groups as estimated over the 2013 growing season. 8 and 10 cm deep control modules did not differ in terms of evaporation rates. H-10 modules had significantly higher ET (by about 35%,  $2.38 \pm 0.18 \text{ mm d}^{-1}$ ), if compared to H-8 ones ( $1.78 \pm 0.13 \text{ mm d}^{-1}$ , Fig. 5) and the data differed from both control modules (C8 and C-10, bare substrate), as well. Overall, ET of the vegetated modules (succulent and herbaceous vegetation) was significantly higher (by about 18%, P<0.05) when compared to the controls (data not shown). The evapotranspiration rates in modules S-8 averaged  $1.96 \pm 0.13 \text{ mm d}^{-1}$ .

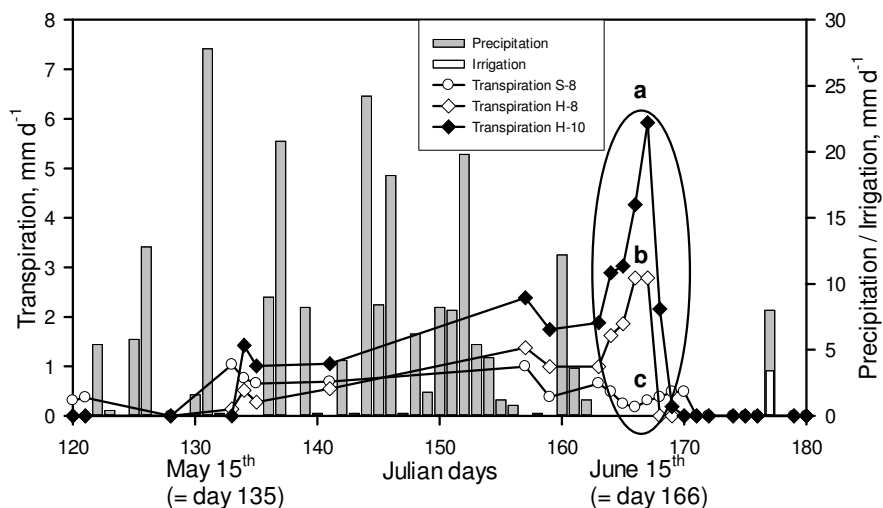
Fig. 6 reports the transpiration trends during May and June 2013. The transpiration rates (T) of succulent (S-8) and herbaceous (H-8 and H-10) vegetation were statistically different ( $P < 0.05$ ) and, generally, increased after rain events and decreased (close to  $0 \text{ mm d}^{-1}$ ) during dry periods. At the beginning of the dry period, maximum transpiration was reached in H-10 modules ( $6 \text{ mm d}^{-1}$ ), while a simultaneous transpiration drop (min  $0.2 \text{ mm d}^{-1}$ ) was recorded for S-8 modules (Fig. 6,  $P < 0.05$ ).

#### 4. Discussion

The succulent and herbaceous vegetation types showed different responses to the severe environmental conditions of the experimental green roof modules. The summer drought and maximum substrate temperatures (about  $46 \text{ }^{\circ}\text{C}$ ) recorded in our study reflected the typical conditions of Mediterranean green roofs (Fioretti *et al.*, 2010; Olivieri *et al.*, 2013).

Under such conditions, the succulent species showed a high survival rate over the entire study period, with the exception of *S. dasyphyllum* which disappeared within the first weeks after transplant. A fast decline of *S. dasyphyllum* was also observed by

Rowe *et al.* (2012) on experimental green roofs with 2.5 and 7.5 cm substrate depths, probably because this chasmophytic species does not find its ecological requirements in the open habitat of a green roof. Moreover, in its natural habitat *S. dasyphyllum* has probably not developed a high inter-specific competitiveness, which represent an essential plant characteristic for establishment and survival in a green roof ecosystem. During the first growing season, other *Sedum* species and *S. tectorum* displayed high growth rates, with a consequent significant increase of their relative cover (Fig. 3, Table 1). This fast cover increase may have been favored by the relatively low inter-specific competition at the initial growth stages (Emilsson, 2008). The capacity to rapidly spread over the substrate is a desired and important feature of plant species to be used for roof greening (Monterusso *et al.*, 2005), because the vegetation cover limits weed development, reduces substrate erosion and increases the functional benefits of green roof installations (Van Woert *et al.*, 2005). In this sense, *S. album* was the best performer among succulents (109% of ground cover increase after the transplanting), in agreement with Emilsson (2008) and Rowe *et al.* (2012).



**Fig. 6** Transpiration trends in succulent modules S-8 (white circles) and herbaceous H-8 and H-10 modules (white and black diamond, respectively) during the months of May and June 2013. Red ellipsis suggests an opposite and complementary water use between succulent and herbaceous vegetations ( $P > 0.05$ ). Precipitation events (black columns) and supplementary irrigations (white columns) are also reported.

During the study period, the succulent cover showed considerable fluctuations, mainly related to climatic factors such as temperature and water availability. The frost events of the winter period, relatively rare or exceptional in areas with a true Mediterranean climate, significantly impacted the biomass of *S. album*, *S. sexangulare*, and *S. tectorum*, leading to a significant decrease of total plant cover. The high vulnerability of the genus *Sedum* to frost damage has been previously reported (Boivin *et al.*, 2001). On the other hand, *S. pseudorupestre* showed a ground cover increase of 6% during the same period, reflecting species resistance to low winter temperature due to its mountain-Mediterranean distribution (Pignatti, 1982). A significant decrease of plant cover (by about 30%) was observed in dry months, suggesting that crassulacean species are able to survive but not ensure a suitable ground cover in Mediterranean climatic conditions. However, the significant ground cover increase observed in the following months, when late summer thunderstorms restored substrate water availability, suggests a fast response of succulent plants to changing microclimatic conditions. The leaf  $\delta^{13}\text{C}$  of *S. album* and *S. sexangulare* were in accordance with data recorded in natural habitats for the same species (Osmond *et al.*, 1975). The value of -23‰ recorded for *S. album* suggests a stronger contribution of CAM metabolism to  $\text{CO}_2$  fixation in this species with respect to *S. sexangulare* (-26‰; Silvera *et al.*, 2010), and this might explain the better performance of this species under the microclimatic conditions of our green roof installation. In fact, it has been hypothesized that the

ability of *Sedum* species to switch between  $\text{C}_3$  and CAM photosynthesis is the reason for their success as green roof plants, allowing them to grow quickly when water is abundant (typical of  $\text{C}_3$ ), and survive drought (typical of CAM; Butler & Orians, 2011). The survival of succulent species during dry periods can also be guaranteed by their ability to reallocate water to vital plants tissues. In fact, Teeri *et al.* (1986) observed that *Sedum rubrotinctum* preserved turgid and vital apical portions, while the basal portions were wilted. In our study, both CAM metabolism and water reallocation might explain the biomass decrease and survival during the dry period.

The sowing of a local seed mixture over bare substrate allowed to obtain a lush herbaceous cover within a short time interval. Most of the 30 identified species were pioneer, ruderal, and sinanthropic. In a recent study, a similar dominance of ruderal plants over a green roof obtained with the same greening method was observed (Nardini *et al.*, 2012). Overall, several plant species representative of Arrhenatheretum grasslands were identified, but it was not possible to distinguish sowed species from those eventually colonizing our modules by natural seed dispersal. Indeed, an important component of green roof vegetation is represented by spontaneous species already present in neighboring areas (Madre *et al.*, 2014). In fact, Dunnett *et al.* (2008) identified 35 wild colonizing species on an experimental green roof, the majority of which was typical of cultivated and disturbed adjacent areas. On the basis of the above, we assume that the floristic composition observed over a

	Ground cover, %					
	<i>H. telephium</i>	<i>S. album</i>	<i>S. pseudorupestre</i>	<i>S. sexangulare</i>	<i>S. tectorum</i>	Total
<b>August 2012</b>		8.8 ± 4.3	2.9 ± 0.8	10.5 ± 2.5	2.8 ± 0.9	24.9 ± 5.5
<b>October 2012</b>		18.4 ± 3.4	5.0 ± 1.7	15.0 ± 2.7	3.5 ± 1.2	41.9 ± 4.4
<b>May 2013</b>	0.9 ± 0.6	12.2 ± 3.2	5.4 ± 1.5	7.7 ± 2.1	2.1 ± 1.0	28.2 ± 3.7
<b>July 2013</b>	1.2 ± 0.6	6.9 ± 1.9	2.3 ± 0.5	7.7 ± 2.0	1.8 ± 0.5	19.9 ± 3.2
<b>October 2013</b>	2.6 ± 1.8	8.6 ± 1.3	7.2 ± 2.7	8.1 ± 2.9	3.7 ± 0.7	30.2 ± 2.0

**Table 1** Average ground cover (%) of the five succulent species and total succulent ground cover estimated in experimental modules in August and October 2012 and May, July and October 2013.

herbaceous green roof modules may reflect the early stages of a primary succession, which are characterized by the dominance of pioneer therophytes, chaotic interactions between species and limited intra- and inter-specific competition (Schulze *et al.*, 2005). Moreover, the prevalence of therophytes and

hemicryptophytes species identified in our study (93%) is in accordance with the typical composition of spontaneous urban flora (Sukopp & Werner, 1985).

The prevalence of annual plants observed in our study might represent a significant advantage for roof greening, leading to the reduction in management

Species	Family	Lifeform	Photosynthetic metabolism
<b>Plant community A</b>			
<i>Cardamine hirsuta</i> L.	Brassicaceae	T	C3
<i>Calepina irregularis</i> (Asso) Thell.	Brassicaceae	T	C3
<i>Cerastium glomeratum</i> Thuill.	Caryophyllaceae	T	C3
<i>Erodium cicutarium</i> (L.) l'Hér	Geraniaceae	T	C3
<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	T	C3
<i>Senecio vulgaris</i> L.	Asteraceae	T	C3
<i>Veronica persica</i> Poir.	Plantaginaceae	T	C3
<b>Plant community B</b>			
<i>Achillea millefolium</i> L.	Asteraceae	H	C3
<i>Arabidopsis thaliana</i> (L.) Heynh	Brassicaceae	T	C3
<i>Calepina irregularis</i> (Asso) Thell.	Brassicaceae	T	C3
<i>Capsella bursa-pastoris</i> (L.) Medik	Brassicaceae	H	C3
<i>Cerastium glomeratum</i> Thuill.	Caryophyllaceae	T	C3
<i>Euphorbia helioscopia</i> L.	Euphorbiaceae	T	C3
<i>Erodium cicutarium</i> (L.) l'Hér	Geraniaceae	T	C3
<i>Lamium purpureum</i> L.	Lamiaceae	T	C3
<i>Medicago lupulina</i> L.	Fabaceae	T	C3
<i>Medicago sativa</i> L.	Fabaceae	H	C3
<i>Myosotis ramosissima</i> Rochel	Boraginaceae	T	C3
<i>Vicia hirsuta</i> (L.) Gray	Fabaceae	T	C3
<i>Vicia sativa</i> L.	Fabaceae	T	C3
<i>Veronica persica</i> Poir.	Plantaginaceae	T	C3
<i>Plantago lanceolata</i> L.	Plantaginaceae	H	C3
<i>Poterium sanguisorba</i> L.	Rosaceae	H	C3
<i>Senecio vulgaris</i> L.	Asteraceae	T	C3
<i>Silene vulgaris</i> (Moench) Garcke	Caryophyllaceae	H	C3
<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	H	C3
<i>Thlaspi perfoliatum</i> (L.) F.K.Mey.	Brassicaceae	T	C3
<i>Trifolium repens</i> L.	Fabaceae	Ch	C3
<b>Plant community C</b>			
<i>Lolium perenne</i> L.	Poaceae	H	C3
<i>Orlaya grandiflora</i> (L.) Hoffm.	Apiaceae	T	C3
<i>Petrorhagia saxifraga</i> (L.) Link s.l.	Caryophyllaceae	H	C3
<i>Plantago lanceolata</i> L.	Plantaginaceae	H	C3
<i>Silene latifolia</i> Poir.	Caryophyllaceae	H	C3
<i>Silene vulgaris</i> (Moench) Garcke	Caryophyllaceae	H	C3
<b>Plant community D</b>			
<i>Amaranthus retroflexus</i> L.	Amaranthaceae	T	C4
<i>Cynodon dactylon</i> (L.) Pers	Poaceae	G	C4
<i>Portulaca oleracea</i> L.	Portulacaceae	T	C4
<i>Setaria viridis</i> (L.) P.Beauv.	Poaceae	T	C4

**Table 2** List of plant species, and relative families identified in sowed modules. The life forms of species (chamaephyte-Ch, geophytes-G, hemicryptophytes-H and therophytes-T) and their photosynthetic metabolism (C3 or C4) are also reported. Species identification was performed between April and September 2013.

costs due to lower levels of imposed management practice. In fact, annual plants germinate, grow and flower under favorable conditions, while they lie dormant as seeds during unfavorable conditions (Schulze *et al.*, 2005). Similarly, a recently developed screening procedure for plant selection suitable for Mediterranean roof greening indicated annuals as a promising life form that has, until now, rarely been considered (Van Mechelen *et al.*, 2014 b).

The species abundance in plant communities varied between 4 and 21. The number of identified species was in accordance with Köhler (2006), that recorded a number of 8-25 species for each survey for a total of 110 species during 20 years-long monitoring in Berlin.

In early spring, the dominance of *Medicago* and *Vicia* species might have favored the accumulation of nitrogen in the substrate, leading to the development of a self-sufficient green roof in terms of fertilization. In fact, the use of Fabaceae species is well known to significantly decrease the need of fertilizers (Jensen *et al.*, 2011). For example, *Medicago sativa* is able to fix 350 kg N/ha in a year, *Trifolium repens* 545 kg N/ha, and *Vicia villosa* 138 kg N/ha (Carlsson & Huss-Danell, 2003; Anugroho *et al.*, 2009).

The increase in temperature and aridity led to the development of the plant community D, based exclusively on C<sub>4</sub> species. The abundance of C<sub>4</sub> species across biomes and habitats is generally positively correlated to the increase in environmental temperature and aridity (Pyankov *et al.*, 2010). Enhanced photosynthetic rates and water use efficiency under drought conditions makes the C<sub>4</sub> plants particularly suitable for Mediterranean roof greening, also taking into account that most European C<sub>4</sub> species are found in the Mediterranean region and they represent an important fraction of the overall biodiversity (Pyankov *et al.*, 2010).

The mean evapotranspiration rates in vegetated modules averaged 2 mm/d, in accordance with Köhler (2006). The average contribution of the

vegetation to ET did not exceed 20%, indicating that a relevant amount of water was lost by evaporation from the substrate. We suggest that the use of mulching of organic material, gravel or recycled materials to limit the evaporation loss might significantly improve water availability in Mediterranean green roofs, while also limiting weeds growth (Nagase *et al.*, 2013).

The minimum and maximum ET were recorded for herbaceous vegetation grown on 8 (1.78 ± 0.13) and 10 cm (2.38 ± 0.18) deep substrate, respectively. We hypothesize that this difference of ET might be an effect of the smaller plant biomass accumulated in modules with the shallower substrate, in agreement with a recent study by Savi *et al.* (2014). Similarly, the ET of herbaceous flora grown on 10 cm deep substrate seemed to outperform (although not significantly) the succulent vegetation, probably due, in addition to the bigger plant biomass, to reduced stomatal control of transpiration.

Transpiration trends in succulent and herbaceous modules showed an opposite and complementary exploitation of available water between these two different vegetation types. In fact, Korner *et al.* (1979) recorded the lowest values of leaf conductance to water vapor in succulent species and the highest ones in herbaceous C<sub>3</sub> species. The functional diversity of plants reduces inter-specific competition and increases the complementary use of resources (Gross *et al.*, 2007; Lundholm *et al.*, 2010). For example, Butler & Orians (2011) reported that *S. album* increases the performance of neighboring plants during summer water deficit, reducing the temperature of the substrate and the evaporation.

## 5. Conclusion

Our study provides insight into important relationships between plant diversity and vegetation development over green roofs, and related technical functions under the harsh environmental conditions of sub-Mediterranean climate. Native succulent species, with the exception of the chasmophytic species *Sedum*

*dasyphyllum*, resulted suitable to the environmental conditions of a Mediterranean green roof. The suitability of these species can be explained mainly by their facultative CAM metabolism and ability to reallocate water in response to environmental conditions.

The sowing of a local seed mixture allowed to obtain a lush herbaceous cover. Microclimatic fluctuations led to the development of a series of herbaceous communities and ensured an overall high biodiversity level. The prevalence of annual plants observed in our study suggests that this life form could carry significant advantages for roof greening as, for example, reduced management costs. In particular, C<sub>4</sub> plants proved to be particularly suitable for Mediterranean roof greening, and future research should investigate a wider range of Mediterranean C<sub>4</sub> species.

Moreover, our results may suggest that the association of succulent and herbaceous plants might ensure an optimal tradeoff between low water use for survival under critical conditions and high water use for stormwater runoff mitigation under optimal conditions, thanks to the transpiration complementarity. Hence, future efforts are needed to test the combination of these two functional groups over sub-Mediterranean green roofs.

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## 7. Drought versus heat: what's the major constraint to Mediterranean green roofs?

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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 depths, shrub species, water status, mortality

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## 1. 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 & Bacci, 2010; Cao *et al.*, 2014; Vijayaraghavan & 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 & 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 & 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 & 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 & 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 ( $\Psi_{\text{tlp}}$ , Bartlett *et al.*, 2012). Low  $\Psi_{\text{tlp}}$  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 & 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 & 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 eleven 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](http://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. Mean annual rainfall is 869 mm, with relatively dry periods in July and January-February.

The experimental extensive green roof was composed of 10 modules, each covering an area of 2.5 m<sup>2</sup>. Modules were built with a six-layer system by SEIC (Harpo Spa, Italy), consisting of: a waterproof/root resistant membrane, a moisture retention layer, a drainage layer, a filter membrane, and substrate (for technical details on materials see Savi *et al.*, 2015). 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, Italy) installed at the maximum substrate depth and 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, we selected 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 pyrastrer* Burgsd., *Spartium junceum* L., Conti *et al.*, 2008). The 2-3 year-old potted plants were provided by either a public (Regional Forestry Service, Tarcento) or a private nursery (Vita Verde, Bologna). 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, and maintained nearby experimental modules for additional physiological measurements (see below). During the study period, plants received natural rainfall and additional emergency irrigation only during severe drought (about 25 mm over the whole summer season).

Species	$\Psi_{\text{tlp}}$	$\pi_0$	P50	Growth, %	
	-MPa	-MPa	-MPa	D-10	D-13
<i>C. salvifolius</i>	1.64 ± 0.14	1.28 ± 0.05	4.40	59.3	128.5
<i>C. coggygria</i>	1.89 ± 0.22	1.32 ± 0.18	3.9	81.1	87.0
<i>E. majus</i>	1.90 ± 0.17	1.44 ± 0.17	2.76	47.8	103.4
<i>L. vulgare</i>	1.75 ± 0.12	1.15 ± 0.09	5.00	74.6	106.1
<i>P. spina-christi</i>	2.02 ± 0.1	1.51 ± 0.03	2.13	30.4	34.9
<i>P. angustifolia</i>	2.49 ± 0.02	1.78 ± 0.16	2.7	41.3	25.0
<i>P. lentiscus</i>	2.69 ± 0.15	2.23 ± 0.08	1.6	0.0	15.9
<i>P. mahaleb</i>	2.15 ± 0.12	1.55 ± 0.14	5.0	34.4	48.5
<i>P. pyrastrer</i>	2.32 ± 0.29	1.68 ± 0.28	1.7	x	x
<i>S. officinalis</i>	1.26 ± 0.04	1.03 ± 0.02	2.51	122.2	72.9
<i>S. junceum</i>	1.02 ± 0.16	0.69 ± 0.14	3.66	202.6	219.1

**Table 1.** Leaf water potential at turgor loss point ( $\Psi_{\text{tlp}}$ , MPa), osmotic potential at full turgor ( $\pi_0$ , 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.

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

## 2.2. Plant water status

Plant water status was assessed in terms of pre-dawn ( $\Psi_{\text{pd}}$ ) and minimum ( $\Psi_{\text{min}}$ ) 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). At 5.00 a.m., 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  $\Psi_{\text{pd}}$  was measured with a pressure chamber (mod. 1505D, PMS Instruments, 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, USA). After  $g_L$  measurements, leaves were sampled and transported to the laboratory for  $\Psi_{\text{min}}$  determination as described above.

## 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 ( $\Psi_{\text{leaf}}$ ) was measured with the pressure chamber, followed by fresh weight measurements (FW). Leaves were left dehydrating on the bench and sequential measurements of  $\Psi_{\text{leaf}}$  and FW were performed until the relationship between  $1/\Psi_{\text{leaf}}$  and cumulative water loss became linear. PV-curve elaboration (Tyree & Hammel, 1972) led to the extrapolation of the osmotic potential at full turgor ( $\pi_0$ ) and the water potential at turgor loss point ( $\Psi_{\text{tlp}}$ ).

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  $\mu\text{m}$  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 ( $F_{\text{max}}$ , 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  $\text{PLC} = 1 - (F/F_{\text{max}}) \times 100$ . 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, USA). The diameter was re-measured in September 2014 ( $D_f$ ) and the relative diameter increment was calculated as:  $G = (D_f/D_i) - 1 \times 100$ . 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 2015 on the basis of visual assessments. Desiccated plants without vital buds were considered dead. 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.

Species	$\Psi_{pd}$ , -MPa				$\Psi_{min}$ , -MPa				$g_L$ , mmol m <sup>-2</sup> s <sup>-1</sup>			
	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
<i>C. salviifolius</i>	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
<i>C. coggygria</i>	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
<i>E. majus</i>	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
<i>L. vulgare</i>	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
<i>P. spina-chris</i>	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
<i>P. angustifolia</i>	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
<i>P. lentiscus</i>	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
<i>P. mahaleb</i>	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
<i>S. officinalis</i>	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
<i>S. junceum</i>	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

**Table 2.** Pre-dawn ( $\Psi_{pd}$ ) and minimum ( $\Psi_{min}$ ) leaf water potential (MPa), and leaf conductance to water vapor ( $g_L$ , mmol m<sup>-2</sup> s<sup>-1</sup>) 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.

### 2.5. Root vulnerability to heat stress

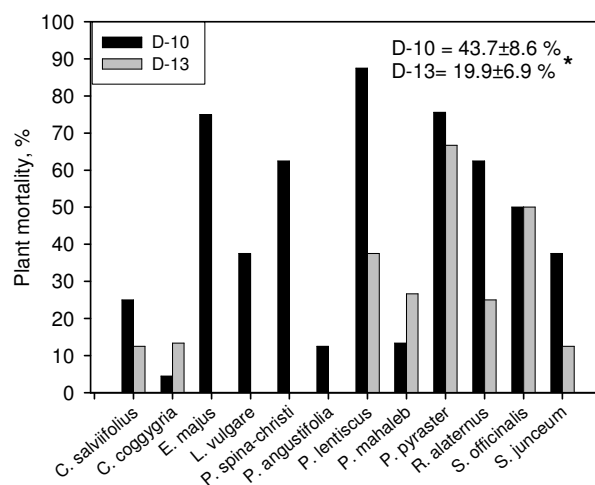
On the basis of the significant differences found in substrate temperature and plant mortality between D-10 and D-13 modules (see Results), a laboratory experiment was performed in September 2015 to evaluate species-specific vulnerability of roots to heat stress. Root cell membrane stability at high temperatures was estimated with electrolyte leakage tests. Four potted plants per species were gently eradicated 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 & 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, Japan). Both T and C samples were then subjected to 3 freezing-thawing cycles (1 min in liquid N<sub>2</sub> followed by 30 min at room temperature) and the final electrical conductivity was measured ( $C_f$ ). The relative leakage ratio was calculated as:  $REL = (C_f / C_i) \times 100$ . 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. All results were considered statistically significant at  $P \leq 0.05$ . Means are reported  $\pm$  standard error of the mean (SEM).

### 3. Results

Table 1 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  $\Psi_{tp}$  and  $\pi_0$  of the study species were  $-1.92 \pm 0.15$  MPa and  $-1.42 \pm 0.12$  MPa, respectively. The species with the lowest (more negative) values of  $\Psi_{tp}$  and  $\pi_0$  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. pyraeaster* 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. Indeed the lowest G was recorded in *P. lentiscus* and the highest in *S. junceum* (see Supporting information, Table 1b). A positive correlation was also observed between  $\Psi_{tp}$  or  $\pi_0$  and



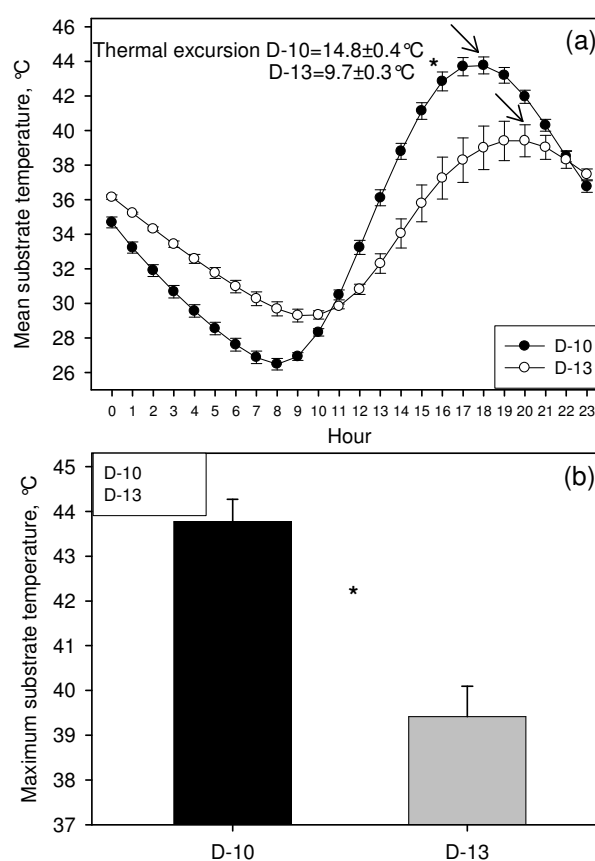
**Fig. 1.** Plant mortality (M, %) of the 11 study species growing in 10 cm (D-10, black columns) and 13 cm (D-13, gray columns) deep green roof modules. The average plant mortality calculated for 10 or 13 cm thick substrate (n=11) is also reported. \* indicates statistically significant difference between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

plant water status as recorded in June and August, in both D-10 and D-13 modules (Table 2). Overall, species characterized with lower  $\Psi_{\text{tip}}$  and  $\pi_0$  showed more negative  $\Psi_{\text{pd}}$  and  $\Psi_{\text{min}}$ , as well as lower  $g_L$  values. For example, in June *S. junceum* had the most favorable water status, while the lowest values of  $\Psi_{\text{pd}}$ ,  $\Psi_{\text{min}}$ , 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  $\Psi_{\text{min}}$  of -4.2 MPa ( $\Psi_{\text{tip}}=-2.49$  MPa) and a  $g_L$  of about  $110 \text{ mmol m}^{-2} \text{ s}^{-1}$  (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  $\Psi_{\text{min}}$  for all shrubs recorded in June was found to be  $-1.16 \pm 0.07$  and  $1.39 \pm 0.10$  MPa for D-10 and D-13 plants, respectively ( $P=0.08$ ). Moreover, the  $\Psi_{\text{pd}}$  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 dead 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). The lowest mortality rate was recorded for *P. angustifolia* (no dead plants in D-13), while the highest rates were found in *P. pyraeaster* (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  $\Psi_{\text{tip}}$  and  $\pi_0$ . 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 (Supporting information, Table 1b).

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  $29.3\text{--}39.2$  °C for the 13 cm deep substrate (Fig. 2a). The average daily thermal excursion 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 (8.00 p.m.) if compared to the shallower ones (6.00 p.m.). 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 ( $43.8 \pm 0.49$  °C) and D-13 ( $39.4 \pm 0.68$  °C) modules (Fig. 2b).



**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 excursion 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,  $P<0.05$ ).



Figure 3 summarizes the results of experiments designed to estimate the root vulnerability to heat stress. Cell membrane sensitivity to high temperatures, estimated as  $\Delta\text{REL}$ , 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.  $\Delta\text{REL}$  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

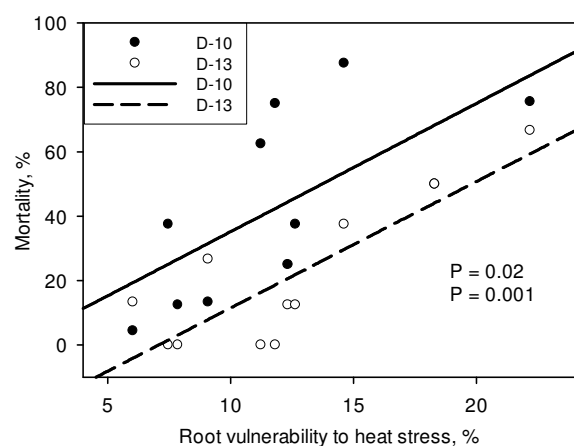
Our results provide experimental evidence that species-specific functional traits are useful and reliable proxies of plant performance on green roofs installed in Mediterranean-climate regions. In particular, our data suggest that traits conferring resistance to drought and high substrate temperatures 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  $\Psi_{\text{tlp}}$ ,  $\pi_0$ , 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  $\Psi_{\text{tlp}}$  and  $\pi_0$  are considered reliable indicators of drought tolerance (Bartlett *et al.*, 2012). In fact, our data show that  $\Psi_{\text{tlp}}$  sets the limit that can be reached by  $\Psi_{\text{pd}}$  and  $\Psi_{\text{min}}$ . Progressively more negative  $\Psi_{\text{tlp}}$  allowed some species to reach and tolerate more negative  $\Psi_{\text{pd}}$  and  $\Psi_{\text{min}}$ , thus extending the time interval for maintenance of stomatal aperture, photosynthetic carbon gain, and growth (Sack & Holbrook, 2006; Lenz *et al.*, 2006). The highly significant positive correlation between  $\Psi_{\text{tlp}}$  or  $\pi_0$  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  $\Psi_{\text{tlp}}$  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  $\Psi_{\text{leaf}}$  drop under drought (Farrell *et al.*, 2013).

Plants with more negative  $\pi_0$  also displayed significantly lower growth rates in both 10 and 13 cm deep modules. 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  $\pi_0$ , 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



**Fig. 3.** Relationship between root vulnerability to heat stress ( $\Delta\text{REL}$ , %) 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.

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,  $\Psi_{pd}$  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  $\Psi_{tp}$  and  $\pi_0$ ). These results 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 nor the major cause of plant failure on Mediterranean green roofs.

Xylem hydraulic vulnerability as estimated in terms of P50 was correlated with  $\Psi_{pd}$  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  $\Psi_{leaf}$  while maintaining positive safety margins (calculated as  $P50 - \text{seasonal minimum } \Psi_{leaf}$ ) towards massive embolism formation (Choat *et al.*, 2012; Nardini *et al.*, 2014a). The reduced  $\Psi_{leaf}$  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. pyraeaster* ( $P50 = -1.70$  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 excursion 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 ( $\Delta$ REL) was estimated after a 45 °C treatment. Interestingly  $\Delta$ REL 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*. 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 & 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).

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. It 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 & 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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## Supporting information

(a)	$\Psi_{pd}$ , -MPa				$\Psi_{min}$ , -MPa				$g_L$ , mmol m <sup>-2</sup> s <sup>-1</sup>			
	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
$\Psi_{tp}$	<b>0.67 *</b>	<b>0.71 *</b>	<b>0.73 *</b>	-0.15	<b>0.76 **</b>	<b>0.94 ***</b>	<b>0.82 **</b>	<b>0.76 **</b>	<b>0.67 *</b>	<b>0.67 *</b>	<b>0.8 **</b>	<b>0.7 *</b>
$\pi_0$	<b>0.78 **</b>	<b>0.77 **</b>	<b>0.7 *</b>	-0.067	<b>0.86 **</b>	<b>0.96 ***</b>	<b>0.81 **</b>	<b>0.72 *</b>	<b>0.67 *</b>	0.56	<b>0.82 **</b>	0.56
<b>P50</b>	<b>0.73 *</b>	0.61	0.43	-0.12	0.42	0.45	0.45	0.43	<b>0.67 *</b>	0.31	0.28	-0.17
<b>M-10</b>	<b>-0.7 *</b>	-0.57	0.14	-0.47	-0.57	-0.33	0.013	-0.24	-0.64	-0.21	-0.11	0
<b>M-13</b>	-0.2	-0.06	0.08	-0.15	-0.24	0.076	-0.009	0.4	0.33	0.4	0.22	0.64
<b>G-10</b>	<b>0.67 *</b>	x	0.48	x	<b>0.81 **</b>	x	0.61	x	0.64	x	<b>0.79 **</b>	x
<b>G-13</b>	x	<b>0.73 *</b>	x	-0.11	x	<b>0.74 **</b>	x	0.51	x	0.5	x	0.08

(b)	Growth		Mortality	
	D-10	D-13	D-10	D-13
$\Psi_{tp}$	<b>0.89 ***</b>	<b>0.83 **</b>	-0.22	-0.12
$\pi_0$	<b>0.89 ***</b>	<b>0.84 **</b>	-0.35	-0.2
<b>P50</b>	0.24	0.43	<b>-0.73 **</b>	-0.42

**Table 1.** 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 ( $\Psi_{tp}$ ), osmotic potential at full turgor ( $\pi_0$ ), water potential inducing 50% loss of stem hydraulic conductivity (P50), plant mortality (M), relative diameter increment (G), pre-dawn and minimum water potentials ( $\Psi_{pd}$ ,  $\Psi_{min}$ ), and leaf conductance to water vapor ( $g_L$ ), as measured in 10 and 13 cm deep green roof modules. \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ .

## 8. GENERAL CONCLUSIONS

Green roofs are engineered ecosystems characterized by a complex ecology and functionality, in particular when they are installed in Mediterranean-type ecosystems, where high temperatures and prolonged drought make plant life over rooftops challenging. The studies described and discussed in this thesis suggest a combination of strategies that can be used to optimize the drought-resistance of green roofs and encourage, as a consequence, a widespread installation of the technology in water-scarce environments.

Two of our main assumptions (see Thesis aims and structure) were confirmed, while the results related to the third assumption opened new insights into the precautions needed in the planning process of the overall green-roof design and during the installation phase.

In particular, our experimental data provided evidence for the possibility to efficiently install green roofs vegetated with stress-tolerant shrubs using 10 cm deep substrate only (hypothesis 1). Indeed, the reduced substrate volume paradoxically translated into less severe water stress experienced by plants, as a consequence of reduced plant biomass and a more efficient recovery of the water content of the system.

Moreover, our results demonstrated that polymer hydrogel amendments have the potential to significantly improve the amount of water available to vegetation, reducing, at the same time, the water stress suffered by plants at the establishment phase (hypothesis 2). In particular, plant water status was most effectively improved when reduced substrate depths were used, which also limited the biomass accumulation during early growing stages. However, it was observed that the high water retention capacity of the substrate-hydrogel blends was significantly reduced over a relatively short-time interval. Hence, future efforts should be invested in the study of physical-chemical characteristics of different hydrogel molecules, taking into consideration their interactions with potential green roof substrates, while testing water holding capabilities of the mixtures over medium and long time-spans.

We initially assumed that the process of species selection (in particular shrubs) for roof greening in arid-prone areas should be based on the knowledge of the species-specific resistance to drought stress (hypothesis 3). This third hypothesis was only partially confirmed by our experimental data. In fact, the results highlighted that traits reflecting species drought tolerance can be conveniently used as predictors of plants water needs and consumption, as well as indicators of their growth rate. But, the plants survival over shallow green roofs is principally influenced by the substrate temperature reached during the hot summer season. Hence, the resistance of the plant root system to heat stress represents the real driver behind species performance on extensive green roofs and the most important factor influencing vegetation survival on installations established in Mediterranean climate. In conclusion, the species-specific root resistance to heat stress turned out to be an easy and relatively inexpensively measurable trait, but a reliable predictor of plant suitability. Therefore, being the substrate temperature a crucial environmental factor affecting the overall green roof functionality, the study of species-specific root resistance to heat should be included in the screening procedure for plant selection for roof greening in warm and dry climates. The creation and constant update of a database of drought and heat tolerance traits for a wide range of species and growth forms is essential to optimize the planning process and plant selection for green roof installations.

Additional studies focused on hydraulic strategies, drought-resistance and, in particular, heat-resistance of a larger number of Mediterranean species potentially suitable for roof greening will ensure the overall improvement of the



installations efficiency, as well as the optimization of provided technical benefits. Moreover, taking into consideration the major constrain to Mediterranean green roofs represented by heat stress, further experiments should test possible solutions to increase the albedo of green roof systems with shallow substrates, to reduce heat transmission to the substrate. In summary, the optimal design for green roofs in arid-prone areas should include a shallow substrate with high water holding capacity capable to buffer temperature peaks, vegetated with carefully selected species with low growing rates, capable to save water, and to tolerate extreme below-ground temperatures.

In conclusion, the study presented in this PhD thesis underlines the importance to further extend our knowledge on the different components of an extensive green roof settled in the Mediterranean area. Our findings showed that the substrate characteristics and vegetation assemblages could be further optimized, taking into consideration the multitude of intercorrelations and reciprocal effects that link all green roof elements in an absolute and complete system. In fact, a green roof is not simply an ensemble of layers, but a complex system in which each element plays a fundamental role to ensure the functionality, efficiency, and sustainability of the whole system.

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**Battipaglia G, Savi T, Ascoli D, Castagneri D, Esposito A, Mayr S, Nardini A.** Effects of prescribed burning on ecophysiological, anatomical and stem hydraulic properties in *Pinus pinea* L. *Tree Physiology*, Under review.

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*Da človek postane nekdo potrebuje dobrega učitelja. Hvala Mojemu Učitelju in vsem, ki so na katerikoli način pripomogli k dosegu tega, poslednjega, cilja.*