

## Water use efficiency, growth and anatomic-physiological parameters of Mediterranean xerophytes as affected by substrate and irrigation on a green roof

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### Abstract

The water use efficiency, growth and anatomic-physiological parameters of Mediterranean medicinal xerophytes on a green roof was investigated, under the effect of water stress and different types of substrate. Rooted cuttings of *Convolvulus cneorum*, *Origanum dictamnus*, *Sideritis athoa*, *Atriplex halimus* and *Lomelosia cretica* were planted on a roof in Athens, Greece. Two substrate types, 10 cm deep, were used, i.e. grape marc compost: perlite: soil: pumice (3:3:2:2, v/v) and a lighter one, i.e. grape marc compost: perlite: pumice (3:3:4, v/v). Two irrigation frequencies were applied during the dry period (summer), normal and sparse, when substrate moisture was 17-20% and 5-11%, respectively. Water use efficiency was increased by sparse irrigation in *A. halimus* and *L. cretica* and by soil substrate in *C. cneorum*. Sparse irrigation reduced dry weight in *O. dictamnus*, *S. athoa* and *L. cretica* and in *A. halimus* in combination with soilless substrate. It increased leaf thickness in all species and reduced  $Chl_a$  in all species, other than *S. athoa*, while in *C. cneorum*, *O. dictamnus* and *L. cretica* this was evident only in the presence of soil in the substrate. In summer, one d before irrigation, stomatal resistance ( $R_{leaf}$ ) was increased and maximum quantum yield of PSII ( $\Phi_{PSII_0}$ ) decreased indicating water limitation, while both parameters were restored to normal levels one d after irrigation indicating that both can serve as sensitive indicators of the onset of water stress.  $\Phi_{PSII_0}$  values, never fell to critical levels, excluding irreversible damage to the photosynthetic apparatus. Therefore, all five plant species are recommended for use on extensive green roofs in semi-arid areas.

**Keywords:** *Atriplex halimus*; *Convolvulus cneorum*; *Lomelosia cretica*; medicinal plants; *Origanum dictamnus*; *Sideritis athoa*

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## Introduction

The indisputable fact of global warming causing constant climate change (Nila *et al.*, 2019; IPCC, 2014; 2007) leads to vegetation changes (Sangüesa-Barreda *et al.*, 2019) and predicts that the next 30 - 90 years, there will be severe and extensive droughts due to water scarcity and increased evaporation (Dai, 2013). Hence, environmental strategies, such as "green constructions" and "green networks", will play a dynamic role in the urban environment, living standards and public health (Cameron *et al.*, 2012; Santamouris, 2014; Kondo *et al.*, 2018; Abass *et al.*, 2020; Feitosa and Wilkinson, 2020; Cristiano *et al.*, 2021). In recent decades, green roofs are the favorite scientific subject of many researchers as a climate adaptation strategy (Andric *et al.*, 2020) and as a dynamic and ecological approach for degraded urban areas (Dunnett and Kingsbury, 2008; Papafotiou *et al.*, 2013; Vijayaraghavan, 2016; Moore *et al.*, 2017; Silva *et al.*, 2018), promoting human, social and environmental sustainability.

Urban culture in private or in public green roofs, is a rational and feasible solution for economical growth, so their techniques and construction materials should be improved and given to the general public (Whittinghill and Rowe, 2012). The design of the green roofs should be based on qualitative and quantitative criteria and should be modified according to the needs of the citizens (Langemeyer *et al.*, 2020). Experiments on the production of herbs and vegetables in extensive green roofs, especially in shallow substrates, have given positive results, either as a complementary source of food production (Walters and Stoelzle Midden, 2018) or helping to manage rainwater in quantity or quality (Whittinghill *et al.*, 2013; 2015; Liu *et al.*, 2021).

In experiments on Mediterranean plant species, a wide range of water use efficiency (WUE) values was observed, which may be due to, water management, plant species, stage of development, as well as environmental factors. Therefore, the study and the improvement of WUE in the Mediterranean should be explored in more detail, considering the lack of water resources and the growth of human population (Katerji *et al.*, 2008). WUE could be maintained at high levels by choosing the right plant species and with the application of the right irrigation methods (Hatfield and Dold, 2019). Deficit irrigation achieves increased WUE without causing serious reduction in yield for various crops (Geerts and Raes, 2009) and microirrigation plays an important role in this (Fan *et al.*, 2018). After all, in green roofs where production is not the first goal of using a plant species, the application of deficit irrigation is even more encouraging.

The substrate materials determine the weight of the green roof construction, which is an important issue in the treatment of old Mediterranean buildings. Due to the fact that soil is heavy for use, researchers are experimenting on creating light substrates (Beattie and Berghage, 2004) often without soil involvement with positive results (Eksi and Rowe, 2019). The participation of organic matter in the substrate helps to retain moisture and provides nutrients to plants. Therefore, the use of compost in green roofs is investigated repeatedly and, in many ways, (Reis *et al.*, 2001; Santos *et al.*, 2008; Tassoula *et al.*, 2015; Papafotiou *et al.*, 2016; Jusselme *et al.*, 2019; Xue and Farrell, 2020). It has recently been shown that the participation of a compost in combination with earthworms apart from promoting plant growth it induced an increase of nectar volume in flowers and in sugars concentration that resulted in greater approach of various and abundant pollinators (Jusselme *et al.*, 2019).

In green roofs, at the eastern Mediterranean that is characterized by intense sunlight and several periods of drought, xerophytes show successful establishment and enhanced growth and development (Papafotiou *et al.*, 2012; 2013; Tassoula *et al.*, 2015; Martinetti *et al.*, 2018). Their acclimation to water stress involves important structures such as pubescence (Karabourniotis *et al.*, 2020a) and calcium oxalate crystals or amorphous calcium carbonate cystoliths (Karabourniotis *et al.*, 2020b). Native plants maintain and increase biodiversity, including pollinators and habitats (Lundholm and Peck, 2008; Cook-Patton and Bauerle, 2012; Benvenuti, 2014), while protecting local character, giving the image of a natural landscape and improving the psychology of employees in their workplace (Loder, 2014). Mediterranean flora includes a very large number of aromatic and medicinal plant species, 500-600 in Greece. Although the climate is ideal for growing many of the above plants, their cultivation is limited and is mainly of local interest. In addition, aromatic and medicinal

plants have low water, nutrient and maintenance requirements and high resistance to the polluted urban environment while having high aesthetic value and they attract insects, especially pollinators for their rich pollen.

*Convolvulus cneorum* L. is used to produce alkaloids in medicine (Palazón *et al.*, 2006) and has been shown to be very effective in curbing particulate air pollution (Shackleton *et al.*, 2012). *Origanum dictamnus* L. known from ancient times, was used for diseases of the stomach and in general the digestive system, spleen, for rheumatism, arthritis and finally for uterine diseases and dystocia (Liolios, 2009). *Sideritis athoa* L. is a powerful antioxidant (Gonzalez-Burgos *et al.*, 2011) and acts against viruses, yeasts and bacteria through its essential oils, mainly apipene and carvacrol (Aligiannis *et al.*, 2001; Fokialakis *et al.*, 2007). *Atriplex halimus* L. is used to treat heart disease, diabetes and rheumatism (Said *et al.*, 2002, 2008). Finally, *Lomelosia cretica* (L.) Greuter and Burdet contains vitamin K1, C, lutein, tocopherol etc. that improve human health and contribute to its recovery (Vardavas, 2006) and fatty acids and sterol, which give it antibacterial properties (Perdetzoglou *et al.*, 1996).

In the present work research was undertaken on the combined effect of substrate type and irrigation frequency on water use efficiency, growth and anatomical and physiological parameters of the five Mediterranean medicinal xerophytes mentioned above, in an extensive type green roof in Athens, Greece, aiming to a sustainable green roof system under harsh environmental conditions.

## Materials and Methods

### *Experimental setup*

The experiments were performed on a second floor, fully exposed flat roof at the Agricultural University of Athens (37° 59' N, 23° 42' E) from July 15 2011 to October 15 2013 (*Convolvulus cneorum*, *Origanum dictamnus*. and *Sideritis athoa*) and from February 15 2013 to September 15 2014 (*Atriplex halimus* and *Lomelosia cretica*). For the first three species, *C. cneorum*, *O. dictamnus*. and *S. athoa*, at the site of the experiments a green roof infrastructure was installed, which included substrate moisture retention and protection of the insulation mat (TSM32; Zinco, Egreen, Athens, Greece) and a drainage element (FD25; Zinco, Egreen), as described by Tassoula *et al.* (2015). The roof was divided with planks to square modules of 50 cm, and in each module the drainage layer was covered by a filter sheet of non-woven geotextile (SF; Zinco, Egreen). On top of it was placed the plant growing substrate. Two rooted cuttings of either *C. cneorum*, or *O. dictamnus*, or *S. athoa*, were planted diagonally in each experimental module. The rooted cuttings of *A. halimus* and *L. cretica* were planted in plastic containers, 60 cm long and 40 cm wide, with the same as above green roof infrastructure fitted. Two types of substrate mix of 10 cm depth were used; one including soil and a soilless, that were made of grape marc compost: perlite: soil: pumice (3:3:2:2, v/v) and grape marc compost: perlite: pumice (3:3:4, v/v), respectively.

### *Irrigation*

The irrigation schedule was applied each dry period from the beginning of May to end of September, by automatic drip irrigation on the surface of the growth substrate, before sunrise, by two drippers placed at equal distances from the center of the container and the two plants (dripper supply 4 L h<sup>-1</sup>). Each irrigation event lasted 60 min to allow water to run off the container. The first week after planting, irrigation was applied every 2 d to those that were planted in summer 2011 and every 4 d to those that were planted in winter 2013, in order for the plants to overcome transplant stress. On July 22, 2011 (*C. cneorum*, *O. dictamnus* and *S. athoa*) and on May 15, 2013 (*A. halimus* and *L. cretica*), plants were irrigated and then were exposed to a preliminary drought experiment for determining the number of d that the plants could withstand between two irrigation events. The daily measurements of the substrate water content (% v/v) recorded as described by Tassoula *et al.* (2015) and the wilting symptoms of each species determined the sparse irrigation frequency. Two irrigation

frequencies were applied during the dry period (May-September), normal and sparse, when substrate moisture was 17-20% and 5-11%, respectively. Substrate water content tests were carried out regularly until the end of September of each year when irrigation stopped. Overall, four treatments were applied (two substrate types combined with two irrigation frequencies). Four modules (containers) per treatment were used for *C. cneorum*, *O. dictamnus* and *S. athoa* and six for *A. halimus* and *L. cretica*, with two plants planted in each module. Based on the above, in the experiments of *C. cneorum*, *O. dictamnus* and *S. athoa*, during the dry period of 2011 and 2012, irrigation was applied either every 5 d (normal) or every 7 d (sparse), while during the dry period of 2013 normal irrigation was determined at 4 d and sparse at 6, as the plants were larger and had higher water needs. In the experiment of *L. cretica* irrigation during the dry period was applied either every 3 d (normal) or every 5 d (sparse) in 2013 and 2014 with the exception of August 2014 where normal irrigation was determined at 2 d and sparse at 4 d. In the experiment of *A. halimus* during the dry season 2013, normal irrigation was applied every 5 d and every 9 d the sparse, while during the dry season 2014, normal irrigation was applied every 4 and every 7 d, respectively.

#### *Meteorological data*

The meteorological data (average monthly air temperature, total monthly rainfall, and average relative humidity for the water stress periods of the experiment, Figure 1) collected from a meteorological cage located near the experimental site, were provided by the Laboratory of General and Agricultural Meteorology of the Agricultural University of Athens.

#### *Chemical and physical properties of the substrates*

The physicochemical properties of the substrates and their elements, as well as the moisture retention curves of the substrates have been described by Tassoula *et al.* (2015). Some further information on pH and Electrical Conductivity (EC) at the beginning and the end of the experimental period is provided in Figure 2. The EC was measured in 1:5 (EC<sub>1:5</sub>) substrate over water ratio where 50 ml of substrate and 250 mL of distilled water were used. The suspension is shaken by hand, 4 times, every 0.5 h for 30 s. After, the extract was obtained, and the EC was determined.

#### *Growth evaluation*

The dry weight of the aboveground part of the plants was measured after oven-drying at 70 °C for 8 d. The increase in plant diameter was calculated by subtracting from the final plant diameter the initial. As plant diameter the average of the biggest diameter and its perpendicular was used.

#### *Water use efficiency evaluation*

WUE was calculated from the quotient of the dry weight of the aboveground part of each plant to its total water consumption (supplied by irrigation) during the experiment (Table 3). Total water consumption of each plant was calculated from the amount of water added in each irrigation event, multiplied by the number of irrigations events during the experiment.

#### *Total chlorophyll concentration (Chl<sub>tot</sub>) evaluation*

Concentration of chlorophylls was measured photometrically. Optical density of 80% acetone extracts of leaf disks was measured at 663, 647 and 720 nm in a UV 160A dual beam spectrophotometer (Shimadzu Co., Tokyo, Japan) according to Lichtenthaler (1987).

#### *Determination of leaf thickness*

The thickness of the leaf was measured under an optical microscope (Zeiss Axiolab, Carl Zeiss, Jena, Germany), from cross sections of fresh leaves taken from the middle of the leaf lamina, with a razor blade. Five leaves per plant, fully exposed to the sun, were selected from four plants per treatment.

#### *Leaf stomatal resistance ( $R_{leaf}$ ) evaluation*

During the periods of water stress application (summer periods) and once during the winter,  $R_{leaf}$  was recorded with an AP4 Porometer (Delta-T devices) on two fully developed young leaves of each plant from 11.00-13.00 HR, as defined by the daily fluctuation of  $R_{leaf}$ .

#### *Maximum quantum yield of PSII photochemistry ( $\Phi_{PSIIo}$ ) evaluation*

$\Phi_{PSIIo}$  was measured in all plants during the periods of water stress application and each February, the d before and the d after the irrigation events, with a Photosynthesis Yield Analyzer (MINI-PAM, Portable Fluorometer, Walz, Effeltrich, Germany). Eight to twelve measurements per treatment were taken as described by Tassoula *et al.* (2015).

#### *Observation / illustration of leaf blade structure*

In mid-July, leaves were collected from all plant species, fully developed, of the same orientation and exposure to the sun and from all operations. In the Electron Microscopy Laboratory, the selected sections were solidified with a suitable cooling gel (Jung, Tissue freezing medium, Leica, Germany) in special metal samplers and placed in the refrigeration microtome (Kryotom, Leica CM1850, Germany), at a temperature of -20 °C. Sections were observed in the optical microscope (Olympus BX 40 Olympus, Japan) through the Cella program (Soft Imagine Systems, Olympus cell family, Japan), while photographing was taken with the Olympus DP71 digital camera, with a resolution of 12.5 Mp in the visible spectrum.

#### *Statistical analysis*

Five multifactorial experiments, one for each plant species, with two factors each were conducted. The two factors were: substrate type (soil and soilless), irrigation frequency (normal and sparse, when substrate moisture was 17-20% and 5-11%, respectively). Therefore, four treatments were applied (2 substrates x 2 irrigations). The containers were arranged following the completely randomized design. The significance of the results was tested by two-way analysis of variance (ANOVA) ( $F$  test, discrete variables followed the normal distribution). The treatment means were compared using Fisher's Least Significant Difference (LSD) or Student's  $t$  at  $P \leq 0.05$ . JMP version 11 statistical software (SAS Institute Inc., Cary, NC) was used.

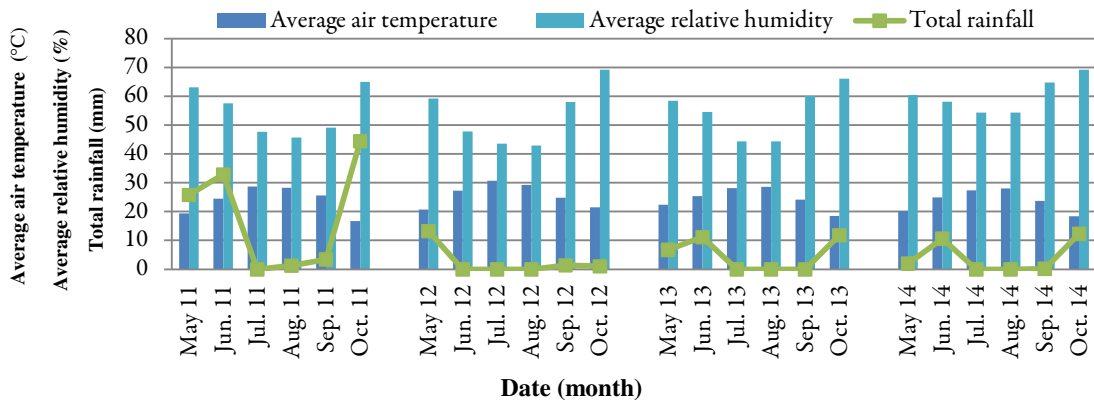
## **Results**

#### *Meteorological data*

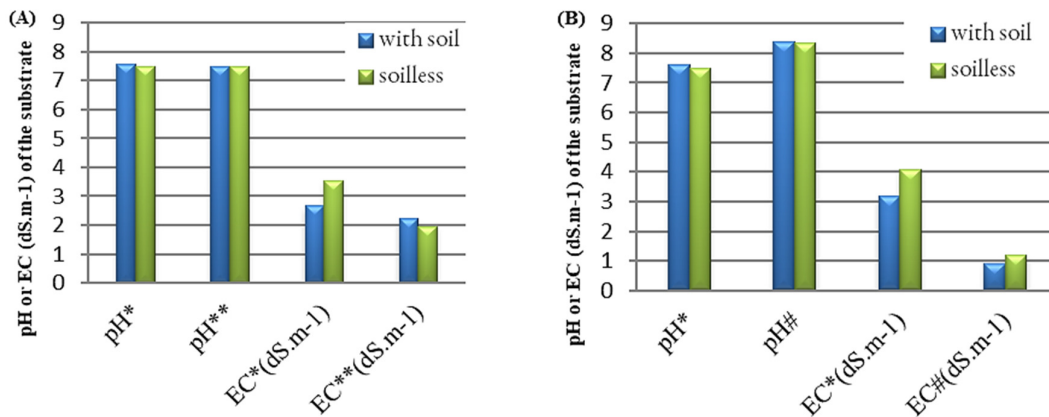
The meteorological data for the periods May to end of September that plants were exposed to deficient irrigation treatment are shown in Figure 1. During this period there were very few incidents of rain, especially in July and August, and they did not affect irrigation treatments.

#### *Chemical properties of substrates*

The  $EC_{1,5}$  of the substrates, was initially elevated due to the presence of compost, but over time, a large decrease was observed (Figure 2).



**Figure 1.** The average monthly air temperature, total monthly rainfall, and average relative humidity for the water stress periods of the experiment (May - Oct. 2011 - 2014)



**Figure 2.** Ph and EC<sub>1.5</sub> values of the substrates, in experiments with *C. cneorum*, *O. dictamnus* and *S. athoa* (A) and *A. halimus* and *L. cretica* (B), at planting (\*) and after (\*\*) 27 or 10 (#) months of culture in (A) or (B) respectively

#### Plant growth

Sparse irrigation adversely affected dry weight in *O. dictamnus*, *S. athoa* and *L. cretica* and in *A. halimus* in combination with soilless substrate, while in *C. cneorum* the soilless substrate had a negative effect (Table 1). The same happened with the increase of the diameter, except for the *S. athoa* that there was no effect of the main factors (Table 2). However, *S. athoa*'s plants grown in the soil substrate presented a greater increase in diameter when irrigated normally (Table 2).

#### Water use efficiency

Water use efficiency was increased by sparse irrigation in *A. halimus* and *L. cretica* and by soil substrate in *C. cneorum*, while it was not affected by the main factors in *O. dictamnus* and *S. athoa* (Table 3).

**Table 1.** The effect of the main experimental factors, i.e., irrigation frequency, normal (n) or sparse (s) and substrate type (with soil: 3GC:3P:2S:2Pu or soilless: 3GC:3P:4Pu), and the effect of the experimental treatments, on above ground dry weight (g) of each plant species at the end of the experimental period (Jul. 2011- Oct. 2013 for *C. cneorum*, *O. dictamnus* and *S. athena*; Feb. 2013 - Sept. 2014 for *A. halimus* and *L. cretica*)

Main factor <sup>z</sup>	<i>C. cneorum</i> Oct. 2013	<i>O. dictamnus</i> Oct. 2013	<i>S. athena</i> Oct. 2013	<i>A. halimus</i> Sept. 2014	<i>L. cretica</i> Sept. 2014
Normal	208.8 ± 17,5 a	112.0 ± 10.1 a	257.5 ± 28.9 a	186.2 ± 5.8 a	127.2 ± 6.0 a
Sparse	193.6 ± 21.4 a	62.2 ± 8.5 b	171.2 ± 28.9 b	136.0 ± 6.7 b	96.6 ± 5.2 b
Soil	259.1 ± 17.5 a	95.6 ± 10.3 a	207.6 ± 26.8 a	172.4 ± 6.3 a	112.9 ± 5.6 a
Soilless	143.3 ± 21.4 b	78.5 ± 8.5 a	221.2 ± 30.9 a	149.8 ± 6.1 b	110.9 ± 5.6 a
Firrig	ns	*	*	*	*
Fsubst	*	ns	ns	*	ns
Fir x s	ns	ns	ns	ns	ns
Treatment <sup>y</sup>					
Soil/n	275.9 ± 28,4 a	139.7 ± 17.2 a	282.2 ± a	204.3 ± 7.8 a	134.2 ± 8.5 a
Soil/s	242.4 ± 31,9 a	51.60 ± 11.3 c	133.0 ± b	140.4 ± 10.1 c	91.6 ± 7.4 c
Soilless/n	141.7 ± 15,5 b	84.10 ± 10.6 b	232.8 ± ab	168.1 ± 8.7 b	120.2 ± 8.5 ab
Soilless/s	144.9 ± 12,0 b	72.80 ± 13.4 bc	209.5 ± ab	131.6 ± 8.7 c	101.6 ± 7.4 bc

<sup>z</sup>Mean (± SE) comparison in columns within each main factor with Student's *t* at  $P \leq 0.05$ ; <sup>y</sup>Mean (± SE) comparison in columns within treatments with Student's *t* at  $P \leq 0.05$ ; Means followed by the same letter are not significantly different at  $P \leq 0.05$ ; \* = significant at  $P \leq 0.05$ ; ns = not significant

**Table 2.** The effect of the main experimental factors, i.e., irrigation frequency, normal (n) or sparse (s) and substrate type (with soil: 3GC:3P:2S:2Pu or soilless: 3GC:3P:4Pu), and the effect of the experimental treatments on diameter increase (cm) of each plant species during the experimental period (Jul. 2011- Oct. 2013 for *C. cneorum*, *O. dictamnus* and *S. athena*; Feb. 2013 - Sept. 2014 for *A. halimus* and *L. cretica*)

Main factor <sup>z</sup>	<i>C. cneorum</i>	<i>O. dictamnus</i>	<i>S. athena</i>	<i>A. halimus</i>	<i>L. cretica</i>
Normal	91.2 ± 4.9 a	45.1 ± 1.3 a	49.3 ± 4.4 a	102.9 ± 5.1 a	28.5 ± 0.9 a
Sparse	86.2 ± 5.3 a	34.6 ± 1.3 b	44.7 ± 5.3 a	86.3 ± 5.9 b	25.6 ± 0.9 b
Soil	97.6 ± 4.9 a	41.7 ± 1.3 a	45.2 ± 4.8 a	103.0 ± 5.6 a	26.6 ± 0.9 a
Soilless	79.9 ± 5,3 b	38.0 ± 1.3 a	48.7 ± 5.0 a	86.2 ± 5.3 b	27.5 ± 0.9 a
Firrig	ns	*	ns	*	*
Fsubst	*	ns	ns	*	ns
Fir x s	ns	ns	ns	ns	ns
Treatment <sup>y</sup>					
Soil/n	102.7 ± 8.4 a	49.6 ± 1.2 a	55.8 ± 5.9 a	126.6 ± 7.1 a	28.5 ± 1.3 a
Soil/s	92.4 ± 8.3 ab	33.9 ± 1.6 c	34.7 ± 7.6 b	79.4 ± 8.7 b	24.7 ± 1.3 b
Soilless/n	79.6 ± 5.9 b	40.7 ± 1.8 b	42.7 ± 6.5ab	93.2 ± 7.1 b	28.5 ± 1.3 a
Soilless/s	80.0 ± 3.9 b	35.4 ± 2.7 bc	54.7 ± 7.6 ab	79.2 ± 7.8 b	26.5 ± 1.3 ab

<sup>z</sup>Mean (± SE) comparison in columns within each main factor with Student's *t* at  $P \leq 0.05$ ; <sup>y</sup>Mean (± SE) comparison in columns within treatments with Student's *t* at  $P \leq 0.05$ ; Means followed by the same letter are not significantly different at  $P \leq 0.05$ ; \* = significant at  $P \leq 0.05$ ; ns = not significant

**Table 3.** The effect of the main experimental factors, i.e., irrigation frequency, normal (n) or sparse (s) and substrate type (with soil: 3GC:3P:2S:2Pu or soilless: 3GC:3P:4Pu), and the effect of the experimental treatments, on water use efficiency (WUE) of each plant species at the end of the experimental period (Jul. 201 - Oct. 2013 for *C. cneorum*, *O. dictamnus* and *S. athoa*; Feb. 2013 - Sept. 2014 for *A. halimus* and *L. cretica*)

Main factor <sup>z</sup>	<i>C. cneorum</i> Oct. 2013	<i>O. dictamnus</i> Oct. 2013	<i>S. athoa</i> Oct. 2013	<i>A. halimus</i> Sept. 2014	<i>L. cretica</i> Sept. 2014
Normal	0.61 ± 0.06 a	0.33 ± 0.03 a	0.76 ± 0.16 a	0.9 ± 0.04 b	0.29 ± 0.02 b
Sparse	0.79 ± 0.06 a	0.26 ± 0.03 a	0.71 ± 0.14 a	1.30 ± 0.05 a	0.38 ± 0.02 a
Soil	0.91 ± 0.06 a	0.31 ± 0.03 a	0.69 ± 0.13 a	1.15 ± 0.05 a	0.34 ± 0.02 a
Soilless	0.50 ± 0.06 b	0.27 ± 0.03 a	0.77 ± 0.17 a	1.08 ± 0.05 a	0.34 ± 0.02 a
Firrig	<i>ns</i>	<i>ns</i>	<i>ns</i>	*	*
Fsubst	*	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Fir x s	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Treatment <sup>y</sup>					
Soil/n	0.81 ± 0.09 ab	0.41 ± 0.06 a	0.83 ± 0.18 a	0.97 ± 0.06 b	0.31 ± 0.03 bc
Soil/s	1.01 ± 0.09 a	0.21 ± 0.04 b	0.55 ± 0.18 a	1.33 ± 0.07 a	0.36 ± 0.02 ab
Soilless/n	0.42 ± 0.09 c	0.25 ± 0.04 b	0.68 ± 0.26 a	0.89 ± 0.06 b	0.28 ± 0.03 c
Soilless/s	0.57 ± 0.09 bc	0.30 ± 0.04 ab	0.87 ± 0.21 a	1.26 ± 0.06 a	0.40 ± 0.02 a

<sup>z</sup>Mean (± SE) comparison in columns within each main factor with Student's *t* at  $P \leq 0.05$ ; <sup>y</sup>Mean (± SE) comparison in columns within treatments with Student's *t* at  $P \leq 0.05$ ; Means followed by the same letter are not significantly different at  $P \leq 0.05$ ; \* = significant at  $P \leq 0.05$ ; *ns* = not significant

#### Total chlorophyll concentration ( $Chl_{tot}$ )

Sparse irrigation also reduced  $Chl_{tot}$  of all plant species, other than *S. athoa*, while in *C. cneorum*, *O. dictamnus* and *L. cretica* this effect was evident only in the presence of soil in the substrate. In *C. cneorum*, *O. dictamnus* and *S. athoa* the combination of soilless substrate and sparse irrigation led to lower total chlorophyll concentrations (Table 4).

**Table 4.** The effect of the main experimental factors, i.e., irrigation frequency, normal (n) or sparse (s) and substrate type (with soil: 3GC:3P:2S:2Pu or soilless: 3GC:3P:4Pu), and the effect of the experimental treatments, on total chlorophyll concentration ( $Chl_{tot}$ ) in the middle of the dry season (July 2013 or 2014)

Main factor <sup>z</sup>	<i>C. cneorum</i> July 2013	<i>O. dictamnus</i> July 2013	<i>S. athoa</i> July 2013	<i>A. halimus</i> July 2014	<i>L. cretica</i> July 2014
Normal	30.2 ± 2.3 a	24.9 ± 2.08 a	-	38.0 ± 1.7 a	12.4 ± 6.1 a
Sparse	16.1 ± 2.3 b	13.0 ± 2.08 b	-	17.7 ± 1.7 b	3.4 ± 6.1 b
Soil	26.6 ± 2.3 a	23.5 ± 2.08 a	-	28.4 ± 1.7 a	12.4 ± 6.1 a
Soilless	19.7 ± 2.3 b	14.5 ± 2.08 b	-	27.2 ± 1.7 a	3.4 ± 6.1 b
Firrig	*	*	-	*	*
Fsubst	*	*	-	<i>ns</i>	*
Fir x s	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>
Treatment <sup>y</sup>					
Soil/n	29.5 ± 3.2 a	27.4 ± 2.9 a	23.7 ± 3.1 a	38.7 ± 2.4 a	20.8 ± 8.6 a
Soil/s	23.7 ± 3.2 a	19.6 ± 2.9 a	26.3 ± 3.1 a	18.0 ± 2.4 b	3.9 ± 8.6 b
Soilless/n	30.0 ± 3.2 a	22.4 ± 2.9 a	27.8 ± 3.1 a	37.0 ± 2.4 a	4.0 ± 8.6 b
Soilless/s	8.4 ± 3.2 b	6.5 ± 2.9 b	4.9 ± 3.1 b	17.3 ± 2.4 b	2.8 ± 8.6 b

<sup>z</sup>Mean (± SE) comparison in columns within each main factor with Student's *t* at  $P \leq 0.05$ ; <sup>y</sup>Mean (± SE) comparison in columns within treatments with Student's *t* at  $P \leq 0.05$ ; Means followed by the same letter are not significantly different at  $P \leq 0.05$ ; \* = significant at  $P \leq 0.05$ ; *ns* = not significant

*Leaf thickness*

The thickness of the leaf was increased by sparse irrigation in all plant species. In *O. dictamnus* it was positively affected by the soil substrate as well (Table 5).

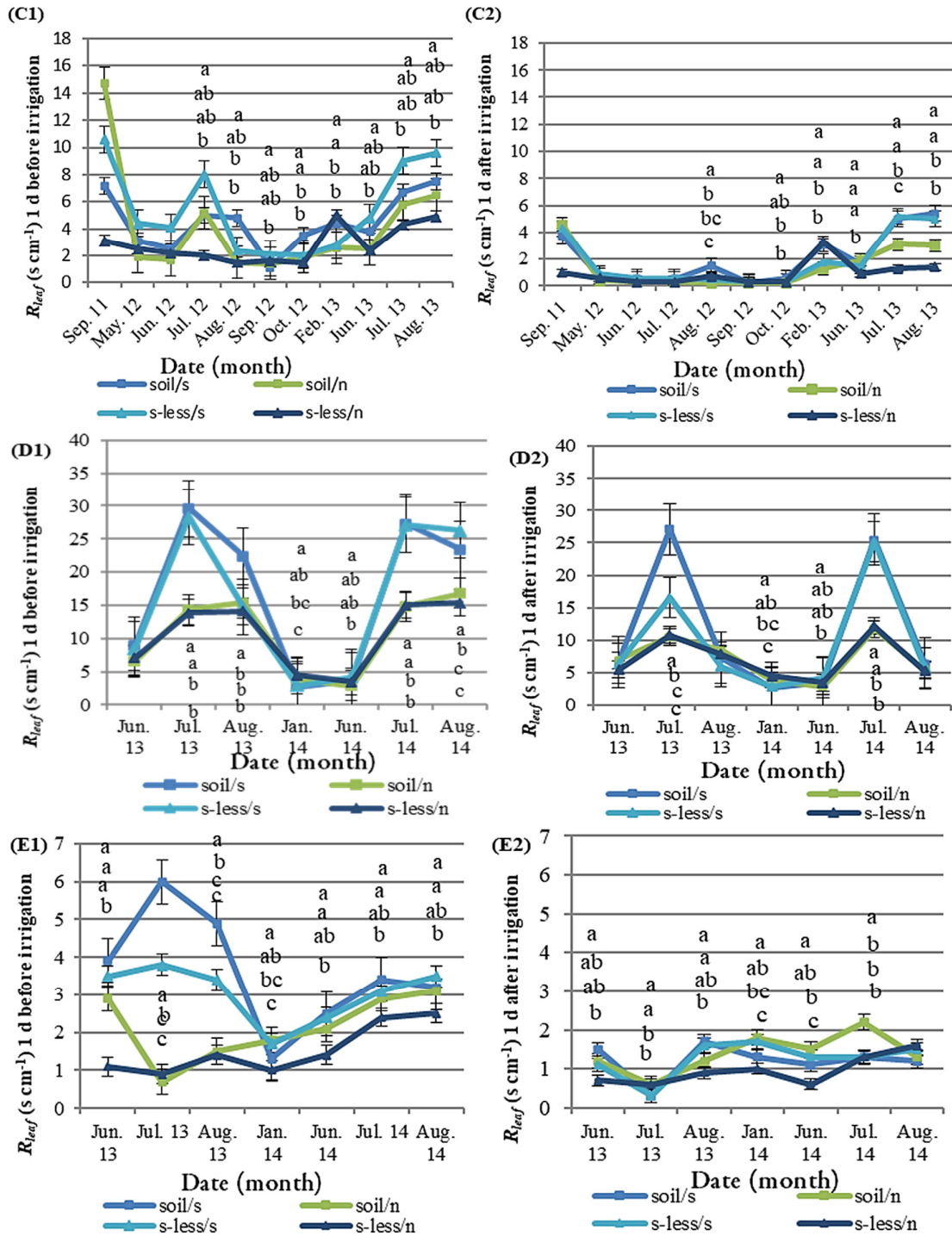
**Table 5.** The effect of the main experimental factors, i.e., irrigation frequency, normal (n) or sparse (s) and substrate type (with soil: 3GC:3P:2S:2Pu or soilless: 3GC:3P:4Pu), and the effect of the experimental treatments, on leaf thickness ( $\mu\text{m}$ ) in July 2012, 2013 or 2014

Main factor <sup>z</sup>	<i>C. cneorum</i> July 2013	<i>O. dictamnus</i> Sept. 2012	<i>S. athoa</i> July 2012	<i>A. halimus</i> July 2014	<i>L. cretica</i> July 2014
Normal	693.2 $\pm$ 10.7 b	350.0 $\pm$ 14.9 b	351.7 $\pm$ 10.5 b	360.0 $\pm$ 7.9 b	603.0 $\pm$ 3.9 b
Sparse	710.9 $\pm$ 10.7 a	492.5 $\pm$ 14.9 a	464.2 $\pm$ 11.2 a	422.5 $\pm$ 7.9 a	623.0 $\pm$ 3.9 a
Soil	708.0 $\pm$ 10.7 a	462.5 $\pm$ 14.9 a	366.7 $\pm$ 11.2 a	380.0 $\pm$ 7.9 a	611.0 $\pm$ 3.9 a
Soilless	696.1 $\pm$ 10.7 a	380.0 $\pm$ 14.9 b	449.2 $\pm$ 10.5 a	402.5 $\pm$ 7.9 a	615.0 $\pm$ 3.9 a
<i>F</i> <sub>irrig</sub>	*	*	*	*	*
<i>F</i> <sub>subst</sub>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>	<i>ns</i>
<i>F</i> <sub>ir x s</sub>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Treatment <sup>y</sup>					
Soil/n	697.0 $\pm$ 15.2 ab	315.0 $\pm$ 21.2 c	350.0 $\pm$ 15.9 b	345.0 $\pm$ 11.2 b	600.0 $\pm$ 5.6 c
Soil/s	719.0 $\pm$ 15.2 a	610.0 $\pm$ 21.2 a	383.3 $\pm$ 15.9 b	415.0 $\pm$ 11.2 a	622.0 $\pm$ 5.6 ab
Soilless/n	689.5 $\pm$ 15.2 b	385.0 $\pm$ 21.2 b	353.3 $\pm$ 13.8 b	375.0 $\pm$ 11.2 b	606.0 $\pm$ 5.6 bc
Soilless/s	702.7 $\pm$ 15.2 ab	375.0 $\pm$ 21.2 bc	545.0 $\pm$ 15.9 a	430.0 $\pm$ 11.2 a	624.0 $\pm$ 5.6 a

<sup>z</sup>Mean ( $\pm$  SE) comparison in columns within each main factor with Student's *t* at  $P \leq 0.05$ ; <sup>y</sup>Mean ( $\pm$  SE) comparison in columns within treatments with Student's *t* at  $P \leq 0.05$ ; Means followed by the same letter are not significantly different at  $P \leq 0.05$ ; \* = significant at  $P \leq 0.05$ ; *ns* = not significant

*Leaf stomatal resistance (R<sub>leaf</sub>)*

In all five plant species, stomatal resistance ( $R_{\text{leaf}}$ ) was increased especially in the middle of the summer one d before irrigation in sparsely irrigated plants indicating water limitation. In autumn, its values were normal again, indicating the end of water stress (Figure 1). There was no water limitation observed in any plant species during winter (Figure 3).

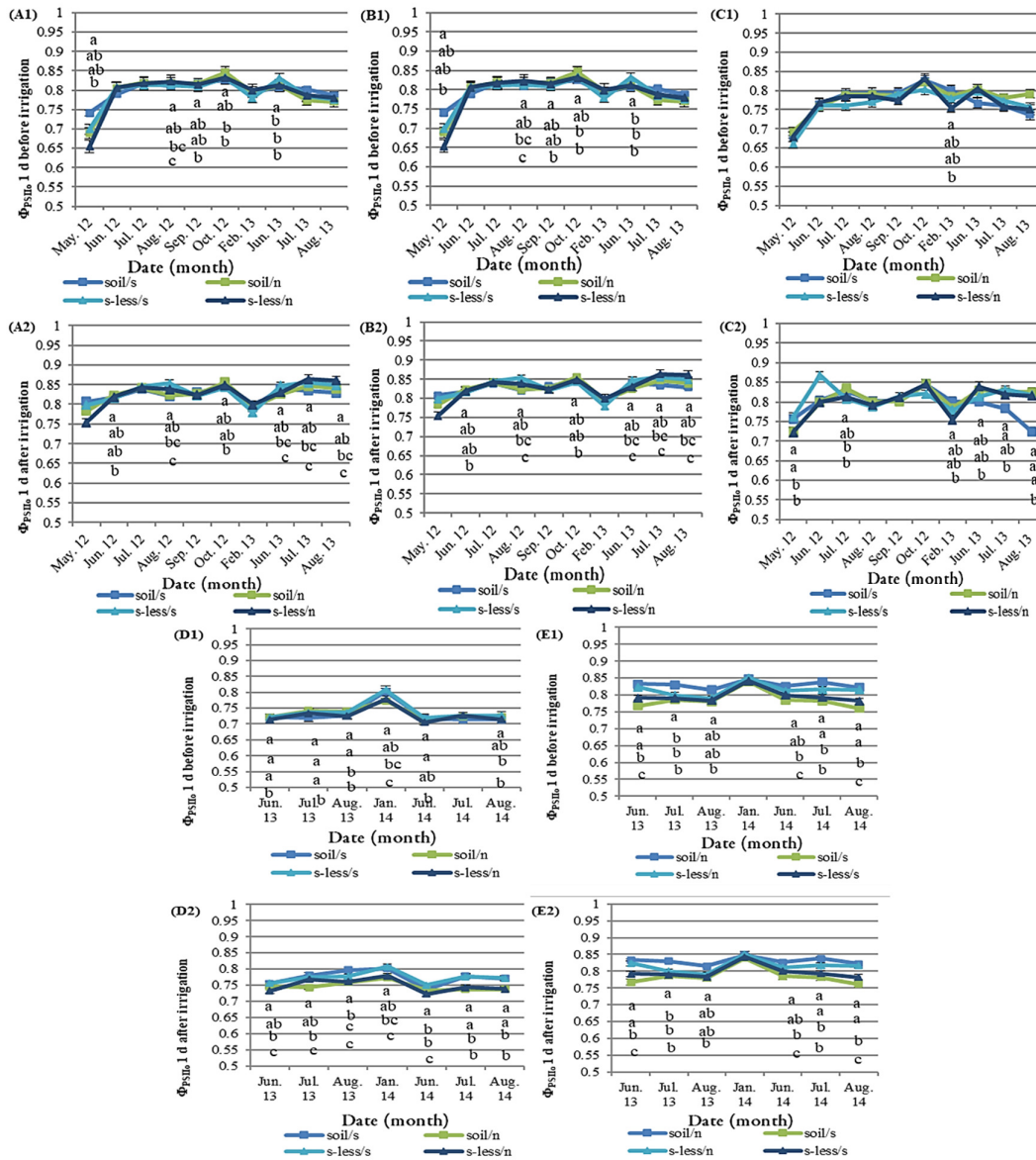


**Figure 3.** Effect of the experimental treatments that consist of combinations of irrigation frequency (n = normal or s = sparse) and substrate type (soil = 3GC:3P:2S:2Pu or soilless = 3GC:3P:4Pu) on plants'  $R_{leaf}$  ( $s\ cm^{-1}$ ) 1 d before and 1 d after an irrigation event during the water stress application periods and once in the winter (Feb. 2013 or Jan. 2014) in (A) *C. cneorum*, (B) *O. dictamnus*, (C) *S. athena*, (D) *A. halimus* and (E) *L. cretica*.

Mean comparison at each date (month) with Fisher's Least Significant Difference (LSD) at  $P < 0.05$ . GC = grape marc compost; P = perlite; S = soil; Pu = pumice

Maximum quantum yield of PSII photochemistry ( $\Phi_{PSIIo}$ )

Generally, after the application of irrigation the maximum quantum yield of PSII ( $\Phi_{PSIIo}$ ) was slightly higher in all treatments. One d before irrigation, the sparsely irrigated plants had the lowest  $\Phi_{PSIIo}$  values, which never fell to critical levels. This implies that there was no irreversible damage to the photosynthetic apparatus. One d after an irrigation event,  $\Phi_{PSIIo}$  values completely returned to the normal levels (Figure 4). In winter and spring, the  $\Phi_{PSIIo}$  in *C. cneorum*, *O. dictamnus* and *S. athena* there was an indication that it had the lowest values, while in *A. halimus* and *L. cretica* the values measured were at the highest range (Figure 4).

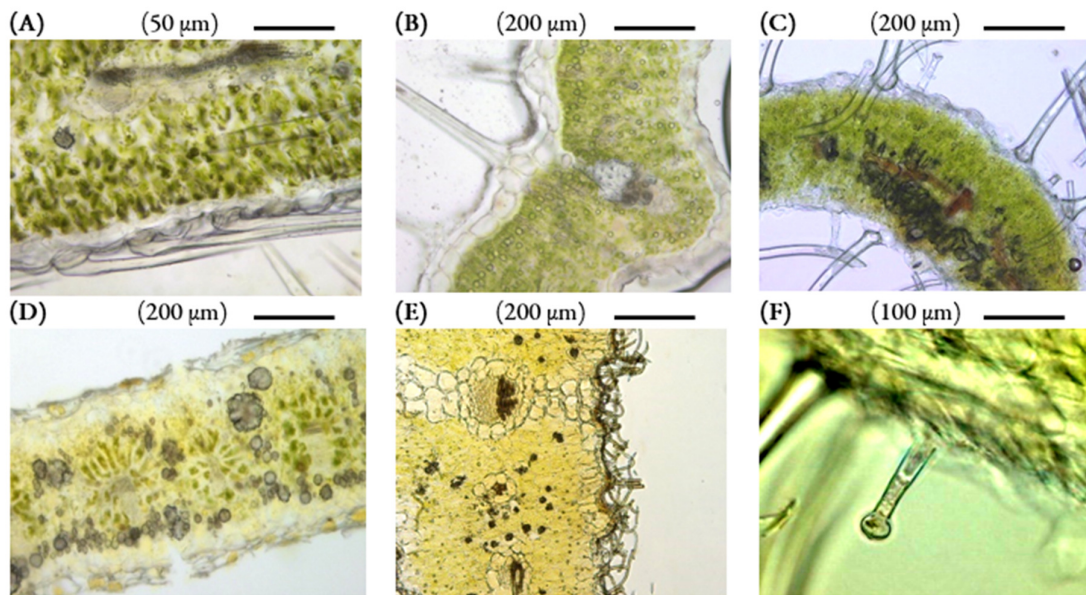


**Figure 4.** Effect of the experimental treatments that consist of combinations of irrigation frequency (n = normal or s = sparse) and substrate type (soil = 3GC:3P:2S:2Pu or soilless = 3GC:3P:4Pu) on plants'  $\Phi_{PSIIo}$  1 d before and 1 d after an irrigation event during the water stress application periods and once in the winter (Feb. 2013 or Jan. 2014) in (A) *C. cneorum*, (B) *O. dictamnus*, (C) *S. athena*, (D) *A. halimus* and (E) *L. cretica*

Mean comparison at each date (month) with Fisher's Least Significant Difference (LSD) at  $P \leq 0.05$ . GC = grape marc compost; P = perlite; S = soil; Pu = pumice

*Observation / illustration of leaf blade structure*

The observation of the leaf blade sections in the optical microscope revealed some notable structures. In the plant species *C. cneorum* (Figure 5A) oblique non-glandular mechanical hairs of particularly long length as well as a carbon cellular inclusion can be seen and in *O. dictamnus* (Figure 5B) irregularly distributed liposomes and hairs are distinguished. In *S. athoa* (Figure 5C) numerous hairs, glands and liposomes appear, while in *A. halimus* (Figure 5D) the "Krantz" type anatomy of the leaf and carbon cellular inclusions in a straight line is evident. In *L. cretica* (Figure 5E) appear carbon cellular inclusions and solid hairs and in Figure 5F the anatomy of *S. athoa*'s glandular hair is clearly visible.



**Figure 5.** Transverse section of leaf blade in (A) *C. cneorum*, (B) *O. dictamnus*, (C) *S. athoa*, (D) *A. halimus*, (E) *L. cretica* and (F) transverse section of *S. athoa* leaf blade, showing the anatomy of the plant hair

**Discussion***Meteorological data*

All periods when irrigation treatments were applied were characterized by high atmospheric temperatures, low atmospheric humidity, as well as almost no rainfall, especially in July and August (Figure 1), a common situation for Greek and in general east-Mediterranean summers. Visual observations of all the plants showed that during the dry season the size of the leaf decreased, in an attempt by the plants to reduce their surface area and thus reduce respiratory water losses. The reduction of the leaf-blade area was reflected in the increase in the leaf-blade thickness (Table 5). This behavior was more pronounced in *O. dictamnus* leaves that appeared after defoliation due to drought, and in *S. athoa*, which sometimes also showed leaf lamina rolling. Therefore, all five species have morphological characters that contribute to a strong limitation of respiratory water losses (Figure 5).

*Chemical and physical properties of substrates*

The Electrical conductivity ( $EC_{1.5}$ ) of the substrates, was initially elevated due to the presence of compost, but over time, a large decrease was observed (Figure 2 A, B) that could be attributed to salt leaching due to autumn and winter rainfalls, in agreement with previous studies (Papafotiou *et al.*, 2005; 2013). Thus,

at the end of the first series of experiments (*C. cneorum*, *O. dictamnus* and *S. athoa*), the EC<sub>1.5</sub> of the soilless substrate was similar to that of the soil substrate (Figure 3A). The substrates had a similar pH at planting and at the end of the experiment (Figure 2A, B), thus possibly excluding a different nutrient environment in plants of the two substrate types. Grape marc compost had adequate quantities of nutrients (Tassoula *et al.*, 2015) to support growth of all five plant species during the experimental period. The two substrates had similar water retention curves with the difference that the substrate with soil held more water at saturation and at low suctions (0-40 cm) (Tassoula *et al.*, 2015).

The saturated weights of the both substrates (Tassoula *et al.*, 2015) are lower than those considered acceptable for extensive green roofs (Dunnett and Kingsbury, 2008).

### *Plant growth*

Establishment and growth of all plant species in the green roof were successful under all experimental treatments. All five species survived the harsh conditions of winter as well as the summer drought period. Although there were periods of growth inhibition, which differed little from species to species, at the end of the experiments a satisfactory degree of plant growth (Tables 1, 2) and an excellent appearance of the green roof was observed, as the plants were overlapping the experimental modules. In two out of the five species, i.e., *C. cneorum* and *A. halimus*, canopy diameter and dry weight was promoted by soil amended substrate, in three out of the five species, i.e., *O. dictamnus*, *A. halimus* and *L. cretica* these growth parameters were promoted by normal irrigation, while the combination of soil substrate with normal irrigation strongly promoted growth in *O. dictamnus* and *A. halimus* (Tables 1, 2). Soil's beneficial effect on growth may be due to soil ability to retain more moisture (Nektarios *et al.*, 2011; Tassoula *et al.*, 2015). In an experiment concerning *Thymus citriodorus* using the same substrates, plants' horizontal diameter and dry weight of canopy appeared to be positively affected by the presence of soil in the substrate, regardless of irrigation (Papafotiou and Adami, 2020). However, due to the fact that natural soils are heavy for use in green roofs, researchers are experimenting to develop light substrates (Beattie and Berghage, 2004; Papafotiou *et al.*, 2018; Eksi and Rowe, 2019). Among the co-cultivated species *C. cneorum*, *O. dictamnus* and *S. athoa*, the latter two developed a similar horizontal diameter, while the highest diameter, about twice in most treatments, was presented by *C. cneorum* (Table 1). However, *C. cneorum* in most treatments showed a similar or lower dry weight to *S. athoa*, and *O. dictamnus* showed the lowest dry weight compared to all species (Table 2). The high dry weight of *S. athoa* was probably due to its mostly woody shoots, compared to the other two species. Among the co-cultivated *A. halimus* and *L. cretica*, the former had the highest diameter and dry weight, being a fast-growing bush, in contrast to *L. cretica* that is a small rather slowly growing shrub. Sparse irrigation adversely affected plant diameter and dry weight in *O. dictamnus*, *A. halimus* and *L. cretica* but the plants had satisfactory canopy growth (Tables 1,2) considering that in green roofs the maximum growth is not intended. Throughout the experimental period, regardless of season, a large part of the lower leaves that came in contact with the substrate dried and fell off, while there was a constant growth of new foliage. The reduced growth of some of the plants in the present study under sparse irrigation, without being a negative element for the green roof, is a common effect of water stress on plant growth (Flexas *et al.*, 2002; Singh *et al.*, 2019).

A key factor for the growth of all five plant species could be the use of grape marc compost containing nutrients in adequate quantities for the growth of these species. Its high content of K could be a reason they coped well with the harsh green roof's conditions (Cakmak, 2005; Papafotiou *et al.*, 2013). In the present study, the decomposition of the compost was very slow and no reduction in the depth of the substrate was observed during the two-year experimental period. In combination with the partial change of the foliage, which constantly enriched the substrate with organic matter (Moran *et al.*, 2005), led to no problem in plant growth.

The high percentage of pumice participation in the growth substrates, probably favored plant growth, as well, as researchers recommend it as a substrate material for extensive green roofs, at a rate of 45% (Eksi and Rowe, 2019), and even at a rate of 70% (Fassman and Simckock, 2012). Pumice at participation rates of 20%,

30% and 40% was also considered suitable for growing turfgrass (Nydrioti *et al.*, 2009; Ntoulas *et al.*, 2012), as well as the xerophyte plant *Dianthus fruticosus* (Nektarios *et al.*, 2011).

#### *Water use efficiency*

Plant species resistant to deficient irrigation are able to maintain WUE, preventing water loss in various ways (Yu *et al.*, 2020). Otherwise WUE decreases alarmingly, leading to reduced plant biomass (Farooq *et al.*, 2009). In *A. halimus* and *L. cretica* WUE was higher under sparse irrigation compared to normal irrigation (Table 3). Similarly, in a cotton plantation half the amount of irrigation resulted in higher WUE values than in full irrigation (Howell *et al.*, 2004). *A. halimus*' higher WUE values compared to all other species were probably due to its C4 photosynthesis pathway versus C3 of the other species (Ghannoum *et al.*, 2011). In the other three species, *C. cneorum*, *O. dictamnus* and *S. athena*, WUE was not affected by irrigation frequency (Table 3). *C. cneorum* was the only species that its WUE was affected by the type of substrate, being higher in the soil amended one (Table 3). In *C. cneorum*, *O. dictamnus* and *S. athena* the combination of soil substrate with normal irrigation lead to WUE values similar to the combination of soilless substrate and sparse irrigation, while in *A. halimus* and *L. cretica* the later combination presented higher values of WUE. This is an important result as it shows that a lightweight substrate combined with reduced water supply secures high WUE in an urban green roof when the appropriate plant species are incorporated. According to Bresta *et al.* (2011) WUE is improving while water stress is increasing, up to a threshold. High WUE values were presented by *A. halimus* (Table 3) and this was expected as it is a C4 plant that easily adapts to dry climates (Le Houerou, 1992). Something also expected were the low WUE values that were presented by *L. cretica* for its irrigation program contained sparse and regular irrigation applications with the shortest interval compared to the other plant species. International standards for green roofs' irrigation, such as the German F.L.L. (2010), refer to the climatic conditions prevailing in the northern countries. Therefore, many researchers argue that when green roofs are located in arid areas, irrigation programs should be implemented depending on the conditions, especially during summer (William *et al.*, 2016; Getter and Rowe, 2006). In environments where the thermal island effect is enhanced, even sparse irrigation may be able to contribute to its reduction (Dunnnett and Kingsbury, 2010). Therefore, in cases where there is the possibility of irrigation, it is important to take into account the above environmental parameter.

#### *Total chlorophyll concentration ( $Chl_{tot}$ )*

In most plant species, the sparsely irrigated plants and those cultured on soilless substrate showed lower  $Chl_{tot}$  values compared to those under normal irrigation and soil substrate during the hot dry season (Table 4), indicating water stress (Gheek Batra *et al.*, 2014).

#### *Leaf thickness*

Sparse irrigation caused the increase of leaf thickness during the summer period in all plant species (Table 5). Increasing the thickness of the leaves under water stress is a xerophytic characteristic that might permit relatively efficient photosynthesis with rationalized water losses (Turner, 1994). The range of the difference of the leaf thickness between sparse and normal irrigation frequency in plant species was 68.8  $\mu\text{m}$ .

#### *Leaf stomatal resistance ( $R_{leaf}$ )*

$R_{leaf}$  depends on the plant species and is proportional to the stage of development and directly related to climatic factors and water availability. One d before an irrigation event, the highest  $R_{leaf}$  values in all five species were caused by treatments involving sparse irrigation, regardless of the type of substrate, and one d after an irrigation event the  $R_{leaf}$  values decreased significantly in all species, thus confirming that plants under sparse irrigation were water stressed (Figure 3). It has been shown previously the ability of many plant species to adapt normally to arid conditions and to return to normal within 48 hours after re-irrigation (Souza *et al.*, 2010). *A. halimus* recorded high  $R_{leaf}$  values both before and after irrigation (Figure 3), which was to be expected since it

had the longest period between two irrigation events and a large above ground growth. For a number of xerophytic aromatic and medicinal plants, as *Ballota acetabulosa*, *Helichrysum orientale*, *Melissa officinalis*, *Rosmarinus officinalis* and *Salvia fruticosa*, has been found to have the highest  $R_{leaf}$  values when irrigated sparingly (Kokkinou *et al.*, 2016). Many annual or perennial species that appear capable of growing under water scarcity follow a water-saving strategy, also referred to as “drought avoidance”. According to this strategy, plants minimize loss and maximize uptake of water. Functional acclimation, such as stricter stomatal closure, and anatomical characteristics such as thick cell walls of epidermal cells, thick cuticular layer, glandular exudation of resinous mixtures or pubescence, further restrict transpiration by increasing resistance to water vapor diffusion and minimizing thermal energy input by reflecting more light (Chaves *et al.*, 2003; Karabourniotis *et al.*, 2020a). For example, an important anatomical feature in *A. halimus* is the glandular hairs on the surface of its leaves (Mozafar and Goodin, 1970), which are superficially covered by a waxy material and are attached to epidermal cells (Smaoui *et al.*, 2011). In fact, all plant species in the present research, namely *C. cneorum* (Figure 5A), *O. dictamnus* (Figure 5B), *S. athena* (Figure 5C-F), *A. halimus* (Figure 5D) and *L. cretica* (Figure 5E), appear intensely sericeous in their lamina, having short and sometimes longer hairs. Non-glandular hairs, as a kind of mechanical, optical and chemical barrier, protect the leaves from insects or pathogens, as well as from ultraviolet radiation that increases leaf temperature (Karabourniotis and Fasseas, 1996; Stavrianiakou *et al.*, 2010; Karabourniotis, 2020a). The role of essential oils in plants is to help them interact with the environment, while protecting them from herbivores and pathogens (Hallahan, 2000). In *S. athena* (Figure 5C-F), the peltate glands are made up of 8-12 secretory cells, the density of which varies from season to season. These play a protective role against excess sunlight, judged by the observation that they increase after winter (Bozambalidis, 2008; Figueiredo *et al.*, 2008). In addition, *C. cneorum* (Figure 5A), *A. halimus* (Figure 5D), and *L. cretica* (Figure 5E), contain carbon cellular inclusions in their leaves, which may play a protective role (Karabourniotis *et al.*, 2020b). Researchers claim that leaf crystals have various roles, such as storage and regulation of calcium cations (Franceschi and Horner, 1980), plant protection (Ward *et al.*, 1997), inactivation of heavy metals (Zindler-Frank, 1991), osmoregulation (Raven and Smith, 1976), tissue support, etc. In addition, Tooulakou *et al.* (2016) demonstrated that the calcium oxalate crystals in the leaves could function as a biochemical reservoir that collects non-atmospheric carbon, especially during the night, and during the day, crystal degradation provides additional carbon for photosynthetic assimilation, especially in drought conditions. This new photosynthetic pathway called “alarm photosynthesis” seems to provide a number of adaptive benefits, such as water economy, reduction of carbon losses in the atmosphere and protection of photosystem II, roles that justify the presence of carbon cellular inclusions in plants (Karabourniotis *et al.*, 2020b).

#### *Maximum quantum yield of PSII photochemistry ( $\Phi_{PSIIo}$ )*

According to the PSII photochemical parameters, one day before an irrigation event, when the plants were facing the maximum water limitation as  $R_{leaf}$  values revealed (Figure 3), photochemical efficiency of PSII photochemistry ( $\Phi_{PSIIo}$ ) values never fell to critical levels, ruling out irreversible damage to the photosynthetic machinery (Figure 4). Both  $\Phi_{PSIIo}$  and  $R_{leaf}$  show significant response to the increase of water availability as they change notably after each irrigation event compared to the day before. So, they show promising to be used as sensitive water stress probes in similar applications. This conclusion is further supported by the observation that the day after every irrigation event,  $\Phi_{PSIIo}$  values were fully recovered (Figure 4). It has been shown in the past that plants subjected to water stress may recover after irrigation, as in the case of *Eucalyptus globulus* (Correia *et al.*, 2013). In addition, a drought-tolerant chrysanthemum variety had better photosynthesis during water stress than a drought-sensitive one (Sun *et al.*, 2013). On the other hand, the water stress brought about a significant reduction of  $\Phi_{PSIIo}$  in the succulent *Sedum alba* (Rowe *et al.*, 2014). Limiting factors for photosynthesis during water stress vary depending on the plant species (Galmes *et al.*, 2007), the intensity of previous water stresses (Flexas *et al.*, 2009), light and temperature (Galle *et al.*, 2009), plant age (Varone *et al.*, 2012) and the application of successive stress/recovery cycles (Galle *et al.*, 2011). Values of  $\Phi_{PSIIo}$  indicating

healthy plants are within an empirical range of 0.78-0.84 (Maxwell and Johnson, 2000). In none of the five plant species did the  $\Phi_{PSII_0}$  value fall below 0.70, indicating that PSII was affected albeit not irreversibly.  $\Phi_{PSII_0}$  was at its lowest levels in May, possibly because the plants emerged from a long period without adequate rain events (Figure 2) and no-irrigation. In winter also, the  $\Phi_{PSII_0}$  in *C. cneorum*, *O. dictamnus* and *S. athoa* had the lowest values, while in *A. halimus* and *L. cretica* the values measured were at the highest range (Figure 4). This could be due to the prevailing temperatures that were lower in February 2013 ( $T_{max}$  13,2,  $T_{min}$  5,7,  $T_{average}$  9,3) compared to January 2014 ( $T_{max}$  17,4,  $T_{min}$  9,4,  $T_{average}$  13,4).

A decisive factor for the results of the research was the mat for moisture retention and protection of insulation, especially during periods of water stress, which probably improved irrigation efficiency as shown by the results of other experiments (Rowe *et al.*, 2014). It is very likely that when the roots of the plants were elongated, they were pumping water from the infrastructure and therefore the moisture of the substrate did not affect the growth of the plants, which confirms the positives of the infrastructure especially during the period of water stress (Savi *et al.*, 2013).

## Conclusions

Extensive type green roofs are very important for arid and semi-arid regions such as Eastern Mediterranean, especially if applied on a large scale in urban environment and join green networks in order to improve the microclimate (Getter and Rowe, 2006). More research and experimental data are needed to determine as many parameters as possible for substrates and plant species that can survive in the xerothermic urban conditions, with minimal irrigation. The Mediterranean xerophytes *C. cneorum* L., *O. dictamnus* L., *S. athoa* L., *A. halimus* L. and *L. cretica* L. showed satisfactory growth, while cultivated under sparse irrigation and on soilless (light) substrate. By monitoring their particular moisture requirements and adjusting the duration between irrigations, all five are suitable plant species for use on extensive green roofs in the Mediterranean region and other areas with similar to the Mediterranean climate.

## Authors' Contributions

Conceptualization: MP and LT; Data curation: LT; Formal analysis: LT; Investigation: LT and MP; Methodology: MP, GK and GL; Resources: MP, GK and GL; Supervision: MP; Writing - original draft: LT and MP. All authors read and approved the final manuscript.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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