

Article

Acclimatization of Mediterranean Native Sages (*Salvia* spp.) and Interspecific Hybrids in an Urban Green Roof under Regular and Reduced Irrigation

Maria Papafotiou ^{1,*} , Aikaterini N. Martini ¹, Lamprini Tassoula ¹, Eleftherios G. Stylias ², Anastasios Kalantzis ² and Eleftherios Dariotis ²

¹ Laboratory of Floriculture and Landscape Architecture, Department of Crop Science, School of Plant Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece; martini_agr@yahoo.com (A.N.M.); lamprinitasoula@gmail.com (L.T.)

² Kalantzis Plants, Agioi Saranta, 19007 Marathon, Greece; stylias_lefteris@hotmail.com (E.G.S.); anastasios@kalantzisplants.com (A.K.); eleftheriosdariotis@yahoo.com (E.D.)

* Correspondence: mpapaf@aua.gr; Tel.: +30-2105294555

Abstract: Native-to-Greece sage species, namely, *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens*, *S. tomentosa* and interspecific hybrids, were evaluated for their acclimatization in an extensive Mediterranean green roof during summer under regular and reduced irrigation (every 2–3 days with substrate moisture 16–22% v/v and 4–5 days with substrate moisture 7–11% v/v, respectively). A substrate (grape-marc compost:perlite:pumice, 3:3:4, v/v) that was 10 cm deep was used. Regardless of the irrigation frequency, *S. pomifera* ssp. *pomifera* × *S. ringens* and *S. officinalis* × *S. pomifera* ssp. *pomifera* showed the highest survival of all hybrids and species, along with satisfactory growth, while *S. fruticosa* showed the lowest survival. Reduced irrigation resulted in the reduction of aboveground and root biomass, with no damage to the photosynthetic apparatus. *S. fruticosa* showed the highest (53%) aboveground biomass reduction and *S. officinalis*, *S. officinalis* × *S. ringens* and *S. pomifera* ssp. *pomifera* × *S. ringens* showed the lowest (28, 23 and 3%, respectively), while *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. pomifera* ssp. *pomifera* × *S. ringens* showed the lowest reduction in root biomass (13 and 16%, respectively). With a reservation for *S. fruticosa*, Greek *Salvia* spp. and their interspecific hybrids studied in the present work are recommended for sustainable exploitation in extensive green roofs in arid regions and generally in xeriscaping.

Keywords: aboveground and root biomass; chlorophyll fluorescence; drought resistance; leaf stomatal resistance; *Salvia fruticosa*; *Salvia officinalis*; *Salvia pomifera* ssp. *pomifera*; *Salvia ringens*; *Salvia tomentosa*



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1. Introduction

Green roofs (GRs) are one type of green infrastructure that can be applied to city buildings and provide significant environmental, social and economical advantages to the urban environment that mitigate the adverse effects of urbanization and make cities more safe, sustainable and resilient to the climate crisis [1–4]. GRs not only contribute to biodiversity increase [5] but also constitute the missing link between the built and the natural environment, which is required for ecological living in cities. GRs are a sustainable alternative to conventional roofs that provide multiple ecosystem services, including improved stormwater management, CO₂ sequestration and reduction in the urban heat island effect [6,7]. Apart from ecosystem services, GRs could increase the aesthetic value of buildings and the city overall, contribute to the socialization of the multistory building residents, support the psychosomatic health of the elderly by volunteering in GR maintenance and provide environmental education [8].

Due to their benefits, GRs gain momentum, even in the semi-arid and arid regions [9], where water availability may be a limiting factor for the expansion of green roof technology [10]. One of the most critical steps in green roof installation in these areas is the selection of drought- and heat-tolerant plant species that can thrive under extreme microclimate conditions [9]. Apart from this, however, biodiversity, including concern for pollinator reduction worldwide [11] and the preservation of the local character, must be taken into account, which are factors that can be met by the use of native plants [12,13]. The adaptation of many native Mediterranean plants to drought stress and their floristic diversity leads them to be ideal for use in extensive GRs in the Mediterranean and other areas with similar climatic conditions [3,13–17].

Aromatic–medicinal plants are important sources for the development of new valuable products of interest to human and animal health, while they are often used as ornamental plants as well. The Mediterranean Basin hosts a great diversity of aromatic plants with medicinal and floricultural potential, a large part of which remains neglected and under-utilized despite the fact that such unique floristic elements could provide considerable and profitable value for local communities [18]. Overall, the successful implementation of sustainable exploitation of native, wild-growing and phylogenetic resources requires multidisciplinary research that covers fields and expertise ranging from artificial selection and breeding, propagation and cultivation to agronomical aspects [19].

Mediterranean sages (*Salvia* spp. family Lamiaceae) are drought-resistant plants that are part of the macchia shrubland; they could be ideal for use in xeriscaping, valuable as bee-friendly plants and suitable for use in extensive type urban green roofs. In order to introduce new drought-resistant species with interesting floricultural characteristics in the floriculture industry, interspecific crossbreeding was undertaken between five sage species that are native to Greece, i.e., *S. fruticosa* Mill., *S. officinalis* L., *S. pomifera* L. ssp. *pomifera*, *S. tomentosa* Mill. and *S. ringens* Sibth. & Sm. [20]. These species were chosen to incorporate a wide range of growth habits, flower color, time and duration of flowering, leaf aroma, and cold and drought resistance. From the hybrids developed, five, i.e., *S. fruticosa* × *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. tomentosa* and *S. pomifera* ssp. *Pomifera* × *S. ringens*, were chosen for their ornamental traits. The main ornamental traits of the above *Salvia* spp. and the interspecific hybrids were described by Papafotiou et al. [20], except for the hybrid *S. pomifera* ssp. *pomifera* × *S. ringens*. This hybrid differs from all the others, as it has a low height (about 30 cm) like *S. ringens*. Its canopy shape, leaf morphology and aroma are also like *S. ringens*, but its flowering stems are longer (about 90 cm), the longest of all species and hybrids, with larger and sparsely arranged light purple flowers. From the five sage species that were used in crossbreeding, only *S. officinalis* and *S. fruticosa* were tested previously for growth on extensive GRs [21–25]. Furthermore, the drought tolerance of *S. officinalis* was thoroughly investigated [22,24,26–30], with it being an important medicinal and aromatic crop, while drought-resistant varieties of this species were already produced to reduce the impact of drought on its productivity [31]. For four of the hybrids used in the present study, they were shown to respond better to water stress in greenhouse conditions compared to *S. fruticosa* [20].

The aim of the present study was to evaluate the acclimatization of the native-to-Greece *Salvia* spp., *S. fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. tomentosa*, and *S. ringens*, along with the interspecific hybrids *S. fruticosa* × *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. tomentosa* and *S. pomifera* ssp. *pomifera* × *S. ringens*, in an urban extensive green roof. Two irrigation frequencies, one considered adequate and one deficient, were applied during the hot and dry Eastern Mediterranean summer, and the acclimatization of the five sage species and five hybrids to drought was assessed on the basis of the plant survival rate, aboveground and root biomass, leaf stomatal resistance and photosystem efficiency. Apart from *S. fruticosa* and *S. officinalis*, all other species and hybrids were tested for sustainable exploitation as green roof plants for the first time.

2. Materials and Methods

2.1. Plant Material, Substrate and Experimental Setup

Rooted cuttings of five sage species native to Greece, i.e., *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. tomentosa* and *S. ringens*, along with five interspecific hybrids of them, i.e., *S. fruticosa* × *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. tomentosa* and *S. pomifera* ssp. *pomifera* × *S. ringens*, that were about 8 weeks old were planted on 4 April 2021 in plastic containers that were 40 cm (width) × 60 cm (length) × 22 cm (depth) in size. Each container had a green roof infrastructure fitted, i.e., moisture retention and protection of the insulation mat FLW-500, a drainage layer Diadrain-25H and a filter sheet VLF-150 (Landco Ltd., Diadem Green Roof Systems, Athens, Greece). Two plants of the same type per container with six containers per treatment were used. The containers were arranged following a completely randomized design on a second-floor flat roof (12 m approximate height) at the Agricultural University of Athens (37°59' N, 23° 42' E). The substrate used was grape marc compost:perlite:pumice (3:3:4, v/v) and had a 10 cm depth. This is a lightweight substrate that is adequate for extensive green roofs according to FLL guidelines for green roofs [32], which was tested in a previous study on the use of Mediterranean xerophytes in extensive green roofs [13]. The characteristics of the grape marc compost (Anagnostou- Soils, Compost & Substrates, Athens, Greece) were the following: pH in extract (1:5) 8.8, ash (550 °C) 45.5 g/100 g, EC 3050 µS/cm, total nitrogen (N) 2.6 g/100 g, ammoniacal nitrogen 1451 mg/Kg, C/N ratio 10.5, soluble P₂O₅ in inorganic acids (total) 0.9 g/100 g, K (total potassium) 2.1 g/100 g, Na_α (total sodium) 0.2 g/100 g, Ca (total calcium) 10.9 g/100 g, Mg (total magnesium) 1.1 g/100 g, Fe (DTPA extractable) 77 mg/Kg, Mn (DTPA extractable) 89 mg/Kg, Zn (DTPA extractable) 37 mg/Kg, Cu (DTPA extractable) 2.6 mg/Kg and B (DTPA extractable) 24 mg/Kg. The chemical properties of the pumice (dimensions 0–3 mm, Anagnostou- Soils, Compost & Substrates, Athens, Greece) were: SiO₂ 71.91%, Al₂O₃ 12.66%, Fe₂O₃ 1.13%, CaO 1.46%, MgO 0.32%, SO₃ 0.03%, K₂O 4.30%, NaO 3.45% and others 0.21%. Perlite (particles diameter 1–5 mm, Perterra, NORDIA S.A., Athens, Greece) had a bulk density 80 K/m³ ± 15%, soluble Cl[−] < 0.01%, sulfates soluble in acids SO₃ < 0.01%, total S < 0.01% and heavy metals below the limits permitted by law.

A factorial experiment with two factors, i.e., *Salvia* type (10 species and hybrids) and irrigation frequency (normal, sparse), was utilized. Therefore, 20 treatments were applied (10 plant types × 2 irrigation frequencies), and in each treatment, six containers were used, with two plants of the same type per container. The number of repetitions (*n*) is shown in each data table.

2.2. Irrigation Scheduling

For the first 10 days after planting, the plants were watered every second day for the plants to overcome transplant stress. On 14 April 2021, the plants were irrigated and then exposed to a preliminary drought experiment in order to determine the number of days that the plants could withstand without irrigation. Moisture (% v/v) of the substrate was recorded daily (three measurements from each container at 1900 to 2000 HR) using a handheld moisture meter (HH2; Delta-T devices, Cambridge, UK), with a soil moisture dielectric sensor (WET-2; Delta-T devices) inserted from the surface that measured 65 mm in depth and 45 mm in width. Most plants showed wilting symptoms 5 days after irrigation. On this day, the mean substrate moisture measured was 7–11% v/v. Therefore, this was decided to be the “sparse” irrigation frequency. The “normal” irrigation frequency was decided to be when the moisture of the substrate was 16–22% v/v and this was measured on day 3. Substrate moisture tests were carried out regularly and based on these, on 5 June 2021, the irrigation frequency was reset to every 2 days (normal) and 4 days (sparse) in order to have substrate moisture before an irrigation event similar to the previous experimental period. This irrigation schedule was kept until the end of the experiment.

Automatic drip irrigation on the substrate surface was applied before sunrise by two drippers placed at equal distances from the center of the container and the plants

(dripper supply $4 \text{ L}\cdot\text{h}^{-1}$, irrigation period: 60 min, adequate to allow water to drain off the container).

2.3. Meteorological Data

The ambient average, maximum and minimum daily air temperature; daily rainfall; and average daily wind speed during the experimental period (April to September 2021) are presented in Figure 1 (<http://meteosearch.meteo.gr/>, 23 February 2022 date of access). Regarding insolation, the monthly total radiation from April to September varied from 0.24 (September) to 0.32–0.37 KW m^{-2} (June to August). These data were recorded from a meteorological cage in the proximity of the experimental roof, however small differences between these data and the actual temperature and wind speed on the roof may have been present.

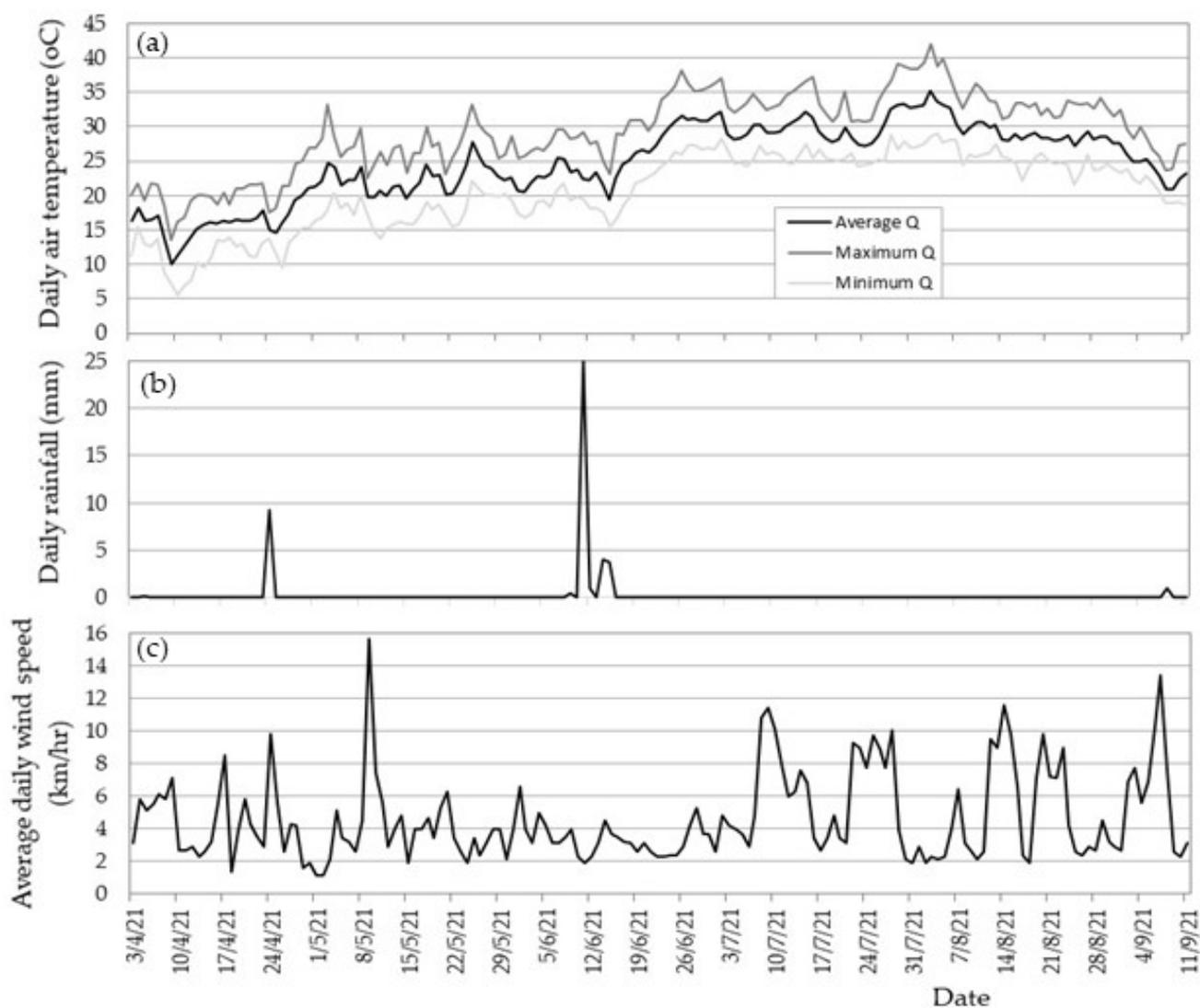


Figure 1. Average, maximum and minimum daily air temperature (a), daily rainfall (b) and average daily wind speed (c) during the experimental period from April to September 2021 (23 February 2022, 11:00 p.m., meteosearch.meteo.gr/data/athens/2021-04.txt; meteosearch.meteo.gr/data/athens/2021-05.txt; meteosearch.meteo.gr/data/athens/2021-06.txt; meteosearch.meteo.gr/data/athens/2021-07.txt; meteosearch.meteo.gr/data/athens/2021-08.txt; meteosearch.meteo.gr/data/athens/2021-09.txt (accessed on 23 February 2022)).

2.4. Plant Growth and Physiological Parameters Evaluation

In August (21st and 22nd), the day before and one day after irrigation, the leaf stomatal resistance (R_{leaf}) and the maximum quantum yield of photosystem II (Φ_{PSII_0}) were recorded.

R_{leaf} was measured with an AP4 Porometer (Delta-T devices) in two fully developed young leaves of each plant (the average value was recorded, i.e., $n = 12$) from 11.00–13.00 HR, as defined by the daily fluctuation of R_{leaf} .

The Φ_{PSII_0} was measured before sunrise with a Photosynthesis Yield Analyzer (MINI-PAM, Portable Fluorometer, Walz, Effeltrich, Germany). One measurement per plant was taken, as described by Tassoula et al. [13], in eight randomly selected plants of each treatment ($n = 8$). The intensity of the measuring light of the MINI-PAM was set once so that the chlorophyll fluorescence yield base levels (F_0) were within the limits set by the manufacturer and held constant thereafter. Maximum fluorescence yield (F_m) was recorded by applying a saturation pulse of $12,000 \text{ mmol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for a 0.8 s duration and Φ_{PSII_0} was calculated as $(F_m - F_0)/F_m$.

At the end of the experiment (10 September 2021), it was not possible to separate the root system of each plant in the container; therefore, in the statistical analyses, the average values of aboveground and root biomass of the two plants of each container were used. The aboveground part of each plant was collected, its fresh weight was measured, placed in an oven at 70°C for 7 days to dry and the dry weight was measured. In addition, the root systems of the plants per container were excised from the substrate, rinsed under running tap water in a sieve and their fresh and dry weights were measured.

2.5. Statistical Analysis

The significance of the results was tested using one- and two-way analysis of variance (ANOVA) (F test, discrete variables followed the normal distribution). The treatment means were compared using Student's t -test at $p \leq 0.05$ (JMP 13.0 software, SAS Institute Inc., Cary, NC, USA, 2013).

3. Results

3.1. Plant Growth and Survival

Five months (early April to early September 2021) after planting in an extensive green roof, the survival rates of *Salvia* spp. and their hybrids were affected by both the plant genotype and the frequency of irrigation (Figure 2). Plant losses occurred sporadically during the June–September period, with most losses in July and August (monthly data not shown), which were the hottest, driest and most windy months (Figure 1a–c). *S. fruticosa* presented a low survival rate (42–50%), regardless of irrigation frequency, while its hybrid with *S. ringens*, although under normal irrigation, also showed a low survival rate; in sparse irrigation, its survival rate was among the highest (92%). *S. ringens* was the species with the highest survival rate (100%) under normal irrigation, but under sparse irrigation, its survival rate was significantly reduced to 67%. However, all three *S. ringens* hybrids showed a high survival rate under sparse irrigation (83–100%). The hybrids *S. pomifera* ssp. *pomifera* \times *S. ringens* and *S. officinalis* \times *S. pomifera* ssp. *pomifera* were the only *Salvia* types that showed 100% survival under sparse irrigation (Figure 2).

The experimental factors (*Salvia* type and irrigation frequency) significantly affected the aboveground fresh and dry weights and the root dry weight, as well as the ratio of root/aboveground dry weight of the plants (Table 1). Sparse irrigation resulted in lower aboveground and root biomasses and root/aboveground dry weight ratio compared to normal irrigation. As shown in Figure 1b, during the experimental period, there were only three rain incidents, one in April and two close to each other on 12 and 14 June; therefore, the irrigation treatments were not affected by rain incidents. Concerning plant type, the highest aboveground biomass was recorded for the *S. officinalis* \times *S. ringens* hybrid with no statistical difference from *S. tomentosa*. The same hybrid also showed one of the highest values of root biomass, along with *S. officinalis* \times *S. pomifera* ssp. *pomifera* and *S. officinalis*,

while the latter showed the highest of all species' and hybrids' root/aboveground dry weight ratio as well (Table 1, Figure 3).

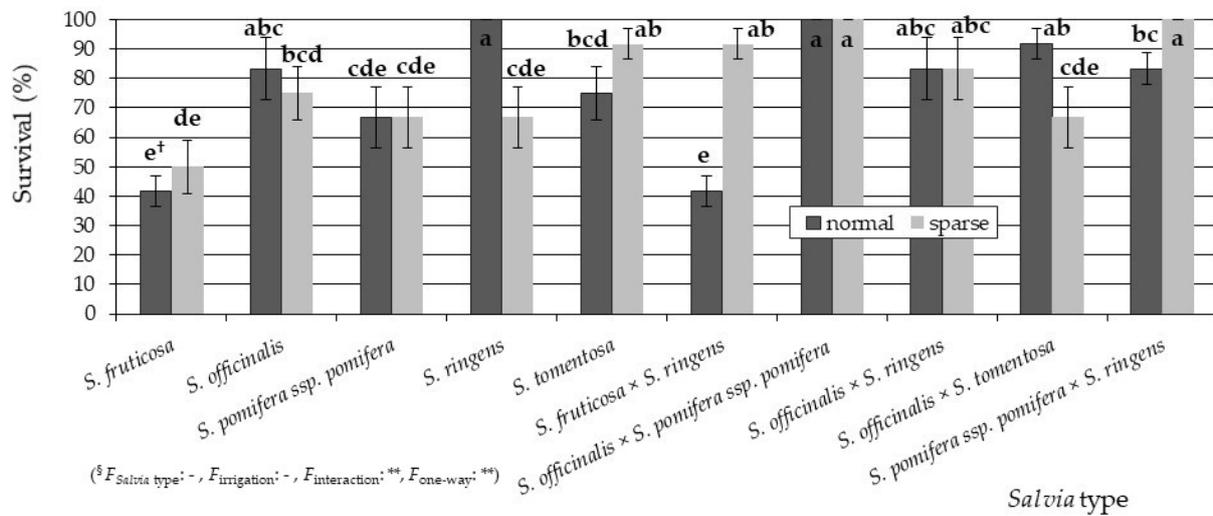


Figure 2. Survival (%) of *Salvia* species and interspecific hybrids five months after establishment in an urban Mediterranean green roof under normal and sparse irrigation during the hot and dry season ($n = 6$). [†] Mean comparison with Student's t -test at $p \leq 0.05$; means followed by the same letter (a–e) were not significantly different at $p \leq 0.05$. [§] NS or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$, respectively.

Under normal irrigation, the species *S. fruticosa*, *S. tomentosa* and *S. pomifera ssp. pomifera* and the hybrids *S. officinalis* × *S. pomifera ssp. pomifera* and *S. officinalis* × *S. ringens* developed the highest aboveground biomass compared to all other species and hybrids, followed by *S. ringens*, *S. fruticosa* × *S. ringens* and *S. officinalis* × *S. tomentosa* (Table 2, Figure 3). As for the root biomass, this was highest in *S. fruticosa*, *S. tomentosa* and *S. officinalis* and the hybrids *S. officinalis* × *S. pomifera ssp. pomifera* and *S. officinalis* × *S. ringens*, while *S. officinalis* presented the highest ratio of root/aboveground biomass of all (Table 2).

Table 1. The effects of experimental factors, i.e., *Salvia* type (*S. fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens*, *S. tomentosa*, *S. fruticosa* × *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. tomentosa*, *S. pomifera* ssp. *pomifera* × *S. ringens*) and irrigation frequency (normal, sparse) on aboveground and root system growth parameters of sage species and interspecific hybrids after five months of growth (April–September 2021) in an urban Mediterranean green roof.

2-Way Anova	Aboveground d.w. (g)	Root d.w. (g)	Root d.w./ Aboveground d.w.	Aboveground f.w. (g)	Root f.w. (g)	Root f.w./ Aboveground f.w.
<i>S. fruticosa</i>	74 cd	48.1 cd †	0.6 cd	209.0 bc	188.0	0.8
<i>S. officinalis</i>	60.3 e	85.4 a	1.4 a	155.5 e	268.5	1.7
<i>S. pomifera</i> ssp. <i>pomifera</i>	87.9 b	39.6 d	0.4 d	216.8 bcd	142.5	0.7
<i>S. ringens</i>	71.6 cde	52.9 cd	0.7 bc	204.8 bcde	175.8	0.9
<i>S. tomentosa</i>	90.7 ab	65.5 bc	0.7 bc	215.3 bcd	216.1	1.0
<i>S. fruticosa</i> × <i>S. ringens</i>	69.3 cde	43.2 d	0.6 cd	167.2 de	167.7	1.0
<i>S. officinalis</i> × <i>S. pomifera</i> ssp. <i>pomifera</i>	86.3 b	70.1 ab	0.8 b	222.3 ab	287.8	1.3
<i>S. officinalis</i> × <i>S. ringens</i>	103.5 a	74.2 ab	0.7 bc	267.7 a	266.6	1.0
<i>S. officinalis</i> × <i>S. tomentosa</i>	68.8 de	47.0 d	0.7 bc	175.2 de	162.2	0.9
<i>S. pomifera</i> ssp. <i>pomifera</i> × <i>S. ringens</i>	71.8 cde	44.9 d	0.6 cd	183.4 cde	150.6	0.8
Normal	93.6 a	71.4 a	0.8 a	239.1 a	252.1	1.1
Sparse	63.2 b	42.8 b	0.7 b	164.3 b	152.9	0.9
Significance §						
F_{Salvia} type	**	**	**	**	-	-
$F_{irrigation}$	**	**	**	**	-	-
F_{Salvia} type × irrigation	NS	NS	NS	NS	*	**

† Mean comparison in columns within each factor with Student's *t*-test at $p \leq 0.05$; means followed by the same letter (a–e) were not significantly different at $p \leq 0.05$. § NS or * or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.

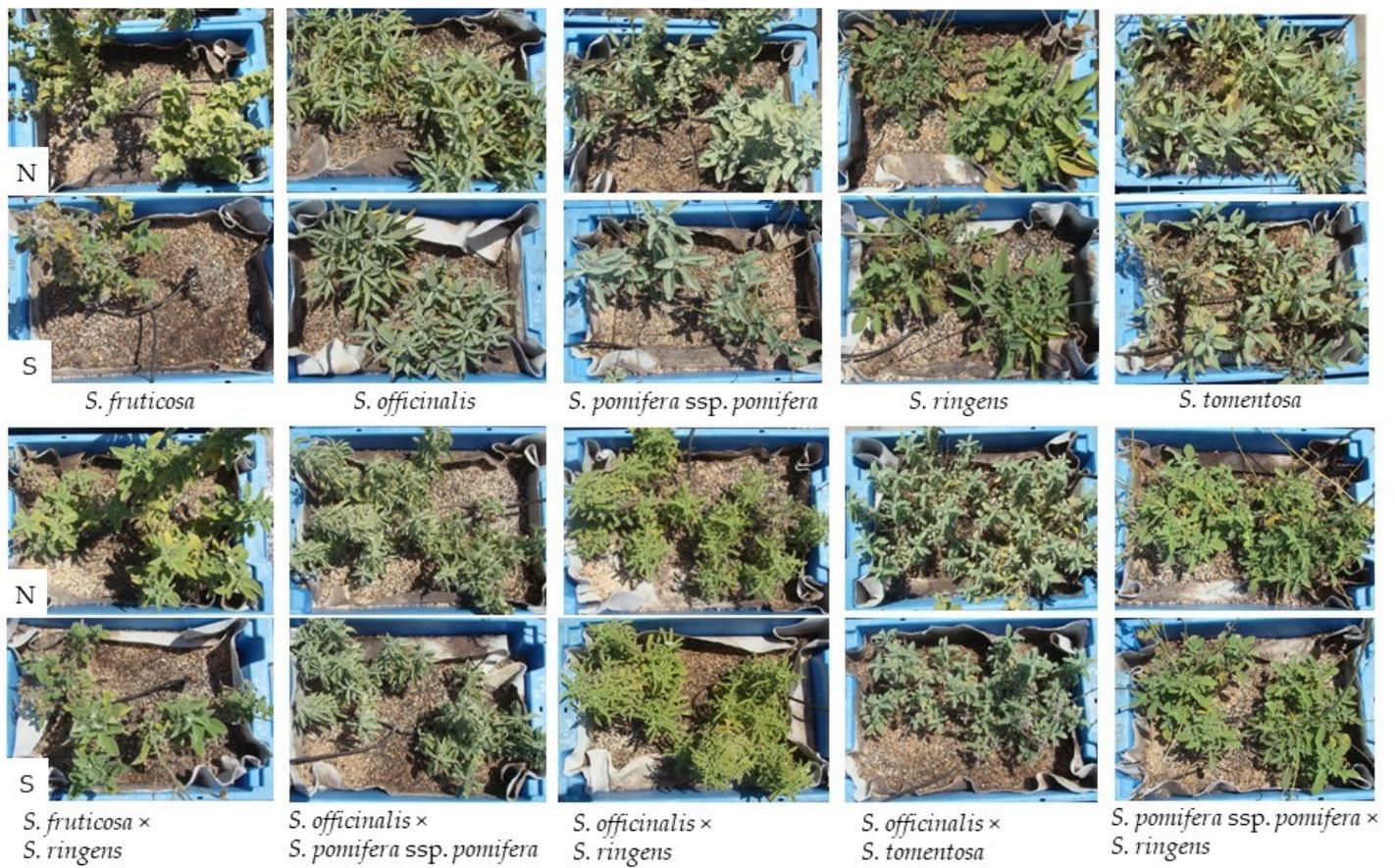


Figure 3. Typical aboveground growth of *Salvia* species and interspecific hybrids after five months of growth (April–September 2021) in an urban Mediterranean green roof under normal (N) and sparse (S) irrigation frequencies.

Table 2. Comparative evaluation of the growth of Greek sage species and interspecific hybrids after five months of growth (April–September 2021) in an urban Mediterranean green roof under normal and sparse irrigation.

<i>Salvia</i> Species	Irrigation Frequency	Aboveground d.w. (g)	Root d.w. (g)	Root d.w./Aboveground d.w.	Aboveground f.w. (g)	Root f.w. (g)	Root f.w./Aboveground f.w.
<i>S. fruticosa</i>	Normal	100.8 ± 2.7 ab [†]	74.5 ± 11.4 ab	0.7 ± 0.1 bc	276.9 ± 8.7 ab	301.5 ± 29.8 ab	1.1 ± 0.1 cd
	Sparse	47.3 ± 1.5 e	21.8 ± 1.8 f	0.5 ± 0.0 bcd	141.1 ± 10.9 ef	74.5 ± 5.0 f	0.5 ± 0.0 f
<i>S. officinalis</i>	Normal	70.3 ± 5.9 cd	95.9 ± 11.2 a	1.4 ± 0.1 a	174.0 ± 21.2 def	294.2 ± 31.2 ab	1.7 ± 0.1 a
	Sparse	50.4 ± 6.6 e	74.9 ± 10.7 ab	1.5 ± 0.1 a	137.0 ± 17.4 f	242.9 ± 32.5 bc	1.8 ± 0.1 a
<i>S. pomifera</i> ssp. <i>pomifera</i>	Normal	107.5 ± 8.5 a	51.5 ± 6.5 cde	0.5 ± 0.1 bcd	256.9 ± 22.2 ab	179.9 ± 20.3 cde	0.7 ± 0.1 def
	Sparse	68.4 ± 4.6 cd	27.7 ± 1.3 f	0.4 ± 0.0 d	176.6 ± 9.9 def	105.1 ± 5.7 ef	0.6 ± 0.0 ef
<i>S. ringens</i>	Normal	86.6 ± 6.0 bc	61.6 ± 8.0 bc	0.7 ± 0.1 bc	244.3 ± 18.4 bc	201.5 ± 23.9 cde	0.8 ± 0.1 de
	Sparse	56.6 ± 6.0 de	44.1 ± 6.3 de	0.8 ± 0.0 b	165.2 ± 14.4 ef	148.2 ± 34.0 de	0.9 ± 0.2 d
<i>S. tomentosa</i>	Normal	109.4 ± 7.7 a	93.1 ± 27.7 a	0.8 ± 0.2 b	274.1 ± 29.5 ab	295.5 ± 68.4 ab	1.0 ± 0.1 cd
	Sparse	72.0 ± 5.9 cd	38.0 ± 9.2 ef	0.5 ± 0.1 bcd	156.4 ± 17.3 ef	136.6 ± 27.7 ef	0.9 ± 0.1 d
<i>S. fruticosa</i> × <i>S. ringens</i>	Normal	83.2 ± 3.0 bc	60.7 ± 2.9 bc	0.7 ± 0.1 bc	195.3 ± 0.3 de	233.7 ± 5.7 bc	1.2 ± 0.0 bc
	Sparse	55.4 ± 2.1 e	25.7 ± 6.0 f	0.5 ± 0.1 bcd	139.1 ± 11.0 f	101.7 ± 20.8 ef	0.7 ± 0.1 def
<i>S. officinalis</i> × <i>S. pomifera</i> ssp. <i>pomifera</i>	Normal	103.6 ± 7.6 ab	74.8 ± 5.8 ab	0.7 ± 0.0 bc	262.9 ± 18.1 ab	315.0 ± 19.4 ab	1.2 ± 0.0 bc
	Sparse	69.1 ± 4.6 cd	65.3 ± 7.3 bc	0.9 ± 0.1 b	181.8 ± 11.5 def	260.6 ± 26.0 abc	1.4 ± 0.1 b
<i>S. officinalis</i> × <i>S. ringens</i>	Normal	116.9 ± 6.5 a	91.3 ± 9.5 a	0.8 ± 0.1 b	301.2 ± 16.4 a	322.6 ± 30.4 a	1.1 ± 0.1 cd
	Sparse	90.0 ± 2.6 b	57.1 ± 3.0 bc	0.6 ± 0.0 bc	234.2 ± 5.8 bc	210.6 ± 6.3 cd	0.9 ± 0.0 d
<i>S. officinalis</i> × <i>S. tomentosa</i>	Normal	85.1 ± 4.4 bc	61.8 ± 6.0 bc	0.7 ± 0.1 bc	212.2 ± 9.9 cd	214.0 ± 17.1 cd	1.0 ± 0.1 cd
	Sparse	52.5 ± 4.9 e	32.2 ± 4.4 ef	0.6 ± 0.1 cd	138.2 ± 9.7 f	110.4 ± 11.7 ef	0.8 ± 0.1 de
<i>S. pomifera</i> ssp. <i>pomifera</i> × <i>S. ringens</i>	Normal	72.9 ± 6.7 cd	48.9 ± 7.5 cde	0.7 ± 0.1 bc	193.4 ± 16.6 de	162.6 ± 23.0 cde	0.8 ± 0.1 de
	Sparse	70.8 ± 2.3 cd	40.9 ± 7.6 def	0.6 ± 0.1 bc	173.4 ± 5.3 def	138.5 ± 21.2 ef	0.8 ± 0.1 de
Significance §							
<i>F</i> _{one-way ANOVA}		**	**	**	**	**	*

[†] Mean values ($n = 6$) (\pm SE) in each column followed by the same lowercase letter (a–f) did not differ significantly at $p \leq 0.05$ using Student's *t*-test. § NS or * or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.

Under sparse irrigation, the hybrid *S. officinalis* × *S. ringens* presented the highest aboveground biomass of all hybrids and species and the highest root biomass, similar to *S. officinalis* and the hybrid *S. officinalis* × *S. pomifera* ssp. *pomifera*, while *S. officinalis* presented the highest root/aboveground biomass ratio (Table 2, Figure 3).

Low irrigation frequency reduced both the aboveground and root system biomasses to varying degrees between species and hybrids (Figure 4). Regarding species, the highest percentage of aboveground dry matter reduction using sparse irrigation was observed in *S. fruticosa* and the lowest in *S. officinalis*; the former showed the highest aboveground biomass reduction of all species and hybrids. As for the hybrids, *S. officinalis* × *S. ringens* and *S. pomifera* ssp. *Pomifera* × *S. ringens* showed the lowest reduction in aboveground dry matter, especially the latter, whose aboveground biomass was almost unaffected. The highest percentage of root dry matter reduction using sparse irrigation was observed in *S. fruticosa* and the lowest in species *S. officinalis* and *S. ringens* and in hybrids *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. pomifera* ssp. *pomifera* × *S. ringens* (Figure 4).

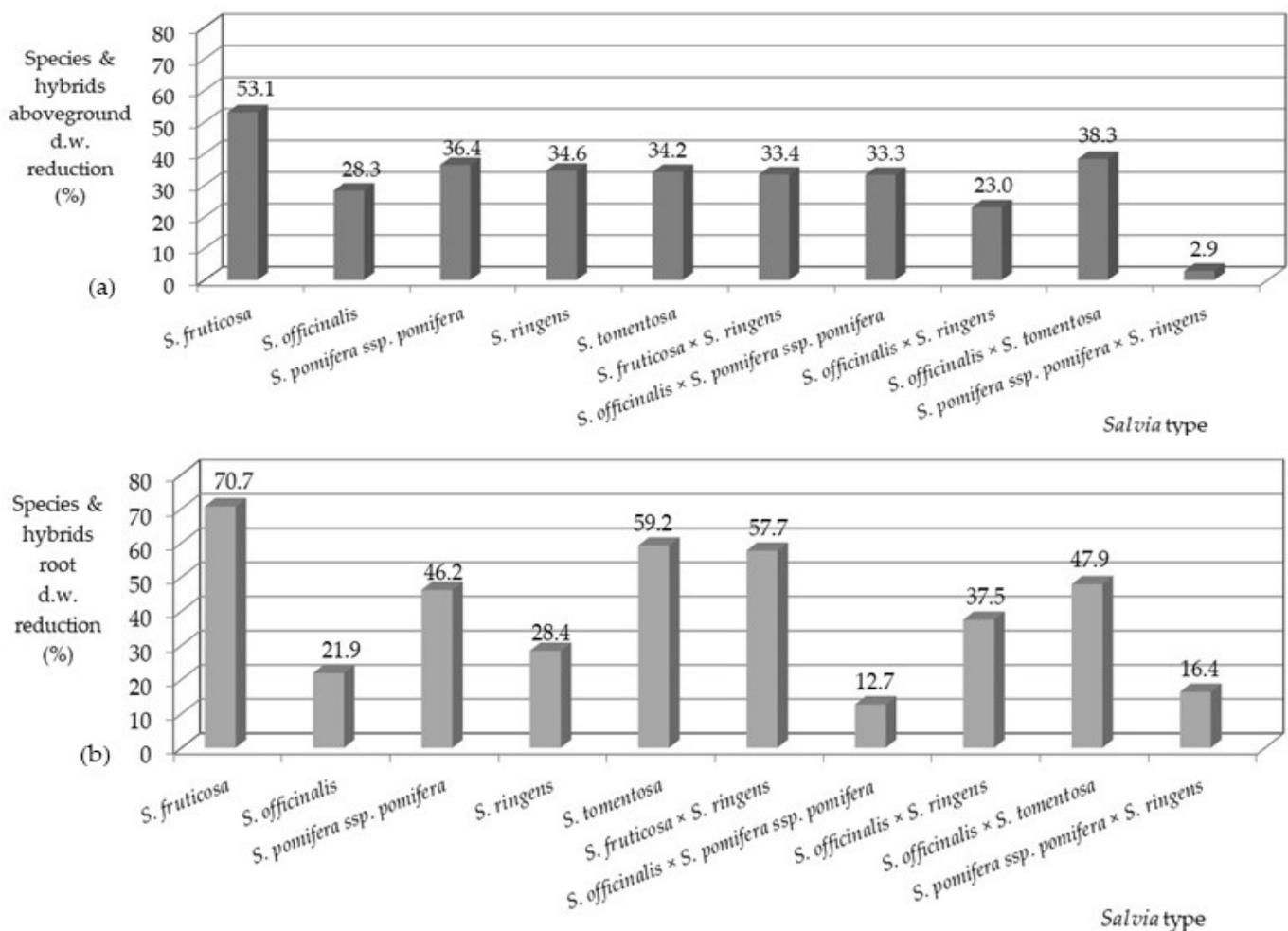


Figure 4. Reduction (%) in the aboveground (a) and root (b) dry weights of *Salvia* species and their interspecific hybrids in sparse irrigation compared to normal irrigation after five months of growth (April–September 2021) in an urban Mediterranean green roof.

S. officinalis had the highest root/aboveground fresh and dry weight ratios under both irrigation frequencies compared to all other species and hybrids, which did not change using sparse irrigation, while *S. fruticosa* and *S. fruticosa* × *S. ringens* were the only ones to have a significant reduction in the root/aboveground fresh weight ratio under sparse irrigation (Table 2).

3.2. Physiological Parameters

The day before irrigation, all species and hybrids had increased R_{leaf} compared to R_{leaf} one day after irrigation, and under sparse irrigation, the R_{leaf} was significantly increased compared to normal irrigation in *S. pomifera* ssp. *pomifera* and *S. ringens* and in all hybrids, except *S. pomifera* ssp. *pomifera* × *S. ringens*, indicating water stress (Figure 5).

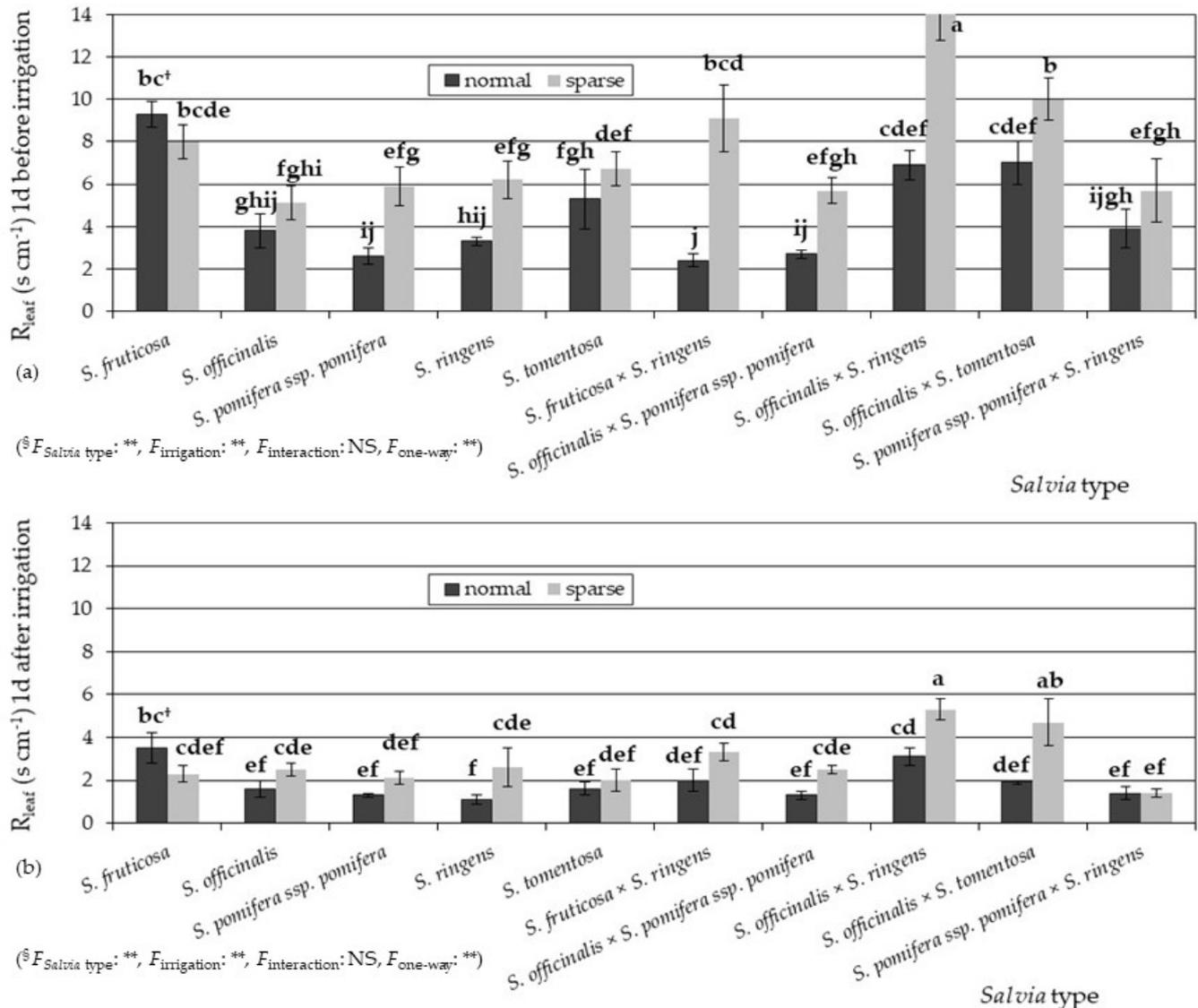


Figure 5. Effect of *Salvia* type and irrigation frequency on plants' R_{leaf} ($s\ cm^{-1}$) the day before (a) and one day after (b) an irrigation event in August 2021. [†] Mean values ($n = 12$) in each figure followed by the same lowercase letter (a–j) did not differ significantly at $p \leq 0.05$ using Student's *t*-test. [§] NS or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.01$, respectively.

All species and hybrids showed Φ_{PSII_0} values above 0.8 under both irrigation frequencies, indicating normal operation of the photosynthetic apparatus (Figure 6). Before irrigation, there were no differences in Φ_{PSII_0} values between plants under normal and sparse irrigation (Figure 6a). Interestingly, one day after an irrigation event, the plants under the sparse irrigation regime showed increased Φ_{PSII_0} values (significant in the two-way ANOVA). *S. pomifera* ssp. *pomifera*, *S. tomentosa* and *S. officinalis* × *S. pomifera* ssp. *pomifera* had higher Φ_{PSII_0} values under sparse irrigation compared to those under normal irrigation (Figure 6b).

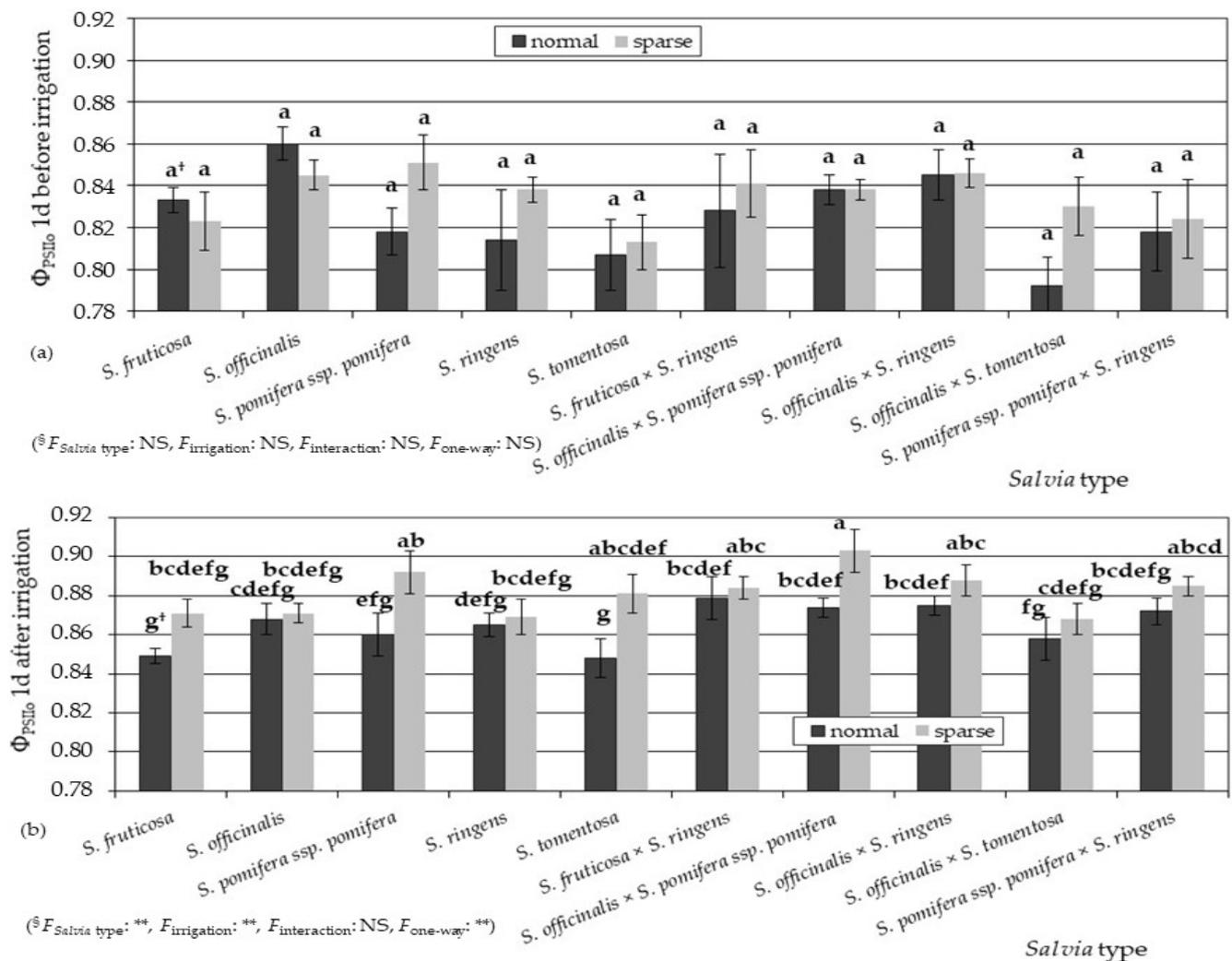


Figure 6. Effect of *Salvia* type and irrigation frequency on plants' Φ_{PSIIo} the day before (a) and one day after (b) an irrigation event in August 2021. [†] Mean values ($n = 8$) in each figure followed by the same lowercase letter (a–g) did not differ significantly at $p \leq 0.05$ by Student's *t*-test. [§] NS or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.01$, respectively.

4. Discussion

The evaluation of native species with minimal water requirements under diverse growth conditions is considered a crucial stage in developing plants that are suitable for sustainable green roofs in semi-arid Mediterranean regions [9]. Apart from addressing the problem of limited water availability in such regions, biodiversity, including concern for pollinator reduction worldwide [11], and the preservation of the local character can be met via the use of native plants in landscaping and green roofs in particular [12,13].

Greek flora and especially macchia vegetation is rich in species with potential use as green roof plants and *Salvia* spp. are among them. *S. officinalis* and *S. fruticosa* were tested previously with promising results, especially *S. officinalis*, in terms of growth in extensive green roofs [21–25], while *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa* were exploited for the first time as landscape plants in the present study. Interspecific hybrids between these native-to-Greece *Salvia* spp. that were developed to serve the horticulture industry's need for new ornamental species were shown to grow successfully under limited water supply [20] and were also tested in this work as green roof plants.

The growth and survival of all five sage species and five hybrids in an extensive green roof during the hot and dry season of the Eastern Mediterranean were affected by both the plant genotype and the frequency of irrigation. *S. fruticosa* showed the lowest survival rate

of all sage types under both normal and sparse irrigation. This response appeared to have been inherited in the *S. fruticosa* × *S. ringens* hybrid when grown under normal irrigation but not under sparse irrigation. *S. fruticosa* × *S. ringens* resembles *S. fruticosa* in height and branching (tall with few lateral shoots), but it has intensely hairy segmented leaves like *S. ringens* [20]. Thus, leaf morphology may be one of the reasons the hybrid had a higher survival rate under reduced irrigation compared to *S. fruticosa*.

In general, the hybrids acclimatized more efficiently to the green roof conditions compared to their parents and the hybrids of *S. pomifera* ssp. *pomifera*, with the latter being either a seed parent to *S. ringens* or a pollen parent to *S. officinalis*, showed the highest survival of all species and hybrids at both irrigation frequencies. Regarding hybrid parents, *S. officinalis* is considered suitable for use in green roofs [21–23], as supported by the present work as well, whereas *S. fruticosa* showed fast water consumption and irrigation requirements of at least 50% of the daily pan evaporation [25], where this partly explained the low survival rate of this species in the present work. Furthermore, all hybrids used in the present study, especially *S. officinalis* × *S. pomifera* and *S. officinalis* × *S. tomentosa*, survived drought stress better than *S. fruticosa* in greenhouse conditions [20].

Significant differences were observed in the growth of *Salvia* hybrids in the green roof, which seemed to have been determined by the characteristics inherited from each of their parents, as described by Papafotiou et al. [20]. For instance, the hybrids *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. officinalis* × *S. ringens* produced the greatest aboveground and root biomasses of all the hybrids. The high value of their aboveground biomass seemed to have been inherited from their pollen parent, while that of the root from their seed parent *S. officinalis*. The vigorous canopy growth, in combination with the rich root system of these two hybrids, probably contributed to their higher survival rate. This is reinforced by the fact that the root system is the main plant organ for adaptation to drought stress conditions [33,34], while large biomass allocation into the root system likely allows for the higher accumulation of reserves for sustaining post-drought recovery [29].

All species and hybrids, except for *S. pomifera* ssp. *pomifera* × *S. ringens*, showed a reduction in aboveground plant biomass under water stress, which is an avoidance mechanism caused by a dehydration process [35], leading to water loss reduction. In several plant species, under drought conditions, the root biomass is reduced less than the aboveground biomass, resulting in a higher root/aboveground ratio [34], which optimizes water uptake [36]. In the present work, all species and hybrids, except *S. officinalis*, *S. ringens* and *S. officinalis* × *S. pomifera* ssp. *pomifera*, showed a greater reduction in root biomass than aboveground biomass under sparse irrigation. Thus, *S. officinalis*, *S. ringens* and *S. officinalis* × *S. pomifera* ssp. *pomifera* were the only ones to show an increase (not statistically significant) in the root/aboveground biomass ratio, but this was not associated with a higher survival rate compared to the other species. The root system was found to play a key role in plant drought resistance in *S. officinalis* [30], a result that was also supported by our research since *S. officinalis* showed the largest root/aboveground fresh and dry matter under both normal and sparse irrigation.

Moreover, *S. officinalis* had the lowest reduction rate of all species in both aboveground and root dry matter under sparse irrigation. The opposite was observed in *S. fruticosa* and its hybrid *S. fruticosa* × *S. ringens*, whose aboveground and root dry matter, particularly that of the root, was much reduced under sparse irrigation. This led to a lower root/aboveground biomass ratio under deficit irrigation that was statistically significant in the case of fresh matter and observed only in these two plant types. However, although the *S. fruticosa* × *S. ringens* hybrid showed high plant mortality at the end of the hot and dry season under normal irrigation, similar to *S. fruticosa*, under sparse irrigation, in contrast to *S. fruticosa*, its plant mortality was among the lowest. Plant mortality in a green roof with low substrate depth (extensive type green roof) is affected by both drought and heat and is possibly affected to a larger extent by substrate temperature than drought per se [9]. The important role of temperature is further supported by a preliminary experiment (data not presented) we conducted the previous year (2020) with these five species of *Salvia* in the

same green roof. In that year, *S. fruticosa* survived at much higher rates (83–91%) similar to those of the other species, probably because the maximum air temperatures in 2020 in the period June–August were 3–4 °C lower than in 2021.

Of all sages tested in the present study, *S. officinalis*, which is widely used worldwide as a medicinal and ornamental plant, was studied in terms of its drought resistance by many researchers [26–28,37]. Unlike other Mediterranean xerophytes, such as *Origanum dictamnus*, which are characterized by defoliation during the hot and dry season [13], which is a mechanism of adaptation to drought, sages did not show leaf loss under water stress in the present study. It was reported for several plant species that when exhibiting over 50% leaf desiccation in a green roof during drought, they were unable to survive [38]. In the present study, sage plants, regardless of genotype, that did not survive the end of summer did not show significant defoliation.

Chlorophyll *a* fluorescence is widely used in studying plant response and adaptation to stressful environments [39]. A slight decrease in Φ_{PSII_0} values often accompanies mild water stress conditions, despite the significant reduction in the gas exchange parameters, while severe water stress causes pronounced effects on the Φ_{PSII_0} parameter [40]. R_{leaf} values one day before irrigation were increased, indicating water stress and Φ_{PSII_0} values were reduced, possibly due to limited CO₂ supply at the carboxylation centers. However, the values of Φ_{PSII_0} under water stress indicated that the Φ_{PSII} photochemistry was functional. In addition, the recovery of Φ_{PSII_0} values after irrigation at optimal levels indicated that no permanent photoinhibition had developed. This was consistent with previous findings for other Mediterranean xerophytes grown in extensive green roofs [13,41]. In the driest period (August), sage plants of all species and hybrids under both irrigation frequencies bore several light green-yellow leaves; however, the Φ_{PSII_0} values were above 0.7, even in these leaves, indicating that there was no critical damage to the photosynthetic apparatus.

Prior to irrigation, there were no differences in Φ_{PSII_0} values between plants under normal and sparse irrigation. However, the day after irrigation, the plants under sparse irrigation had higher Φ_{PSII_0} values compared to those under normal irrigation. In August, after 4 months in the green roof, plants under sparse irrigation were smaller and possibly better acclimatized to green roof conditions compared to those under normal irrigation; it was argued that water stress can change the gene expression, morphology and plant physiology and improve photosynthesis [42,43].

Native-to-Greece *Salvia* spp. and even more so the interspecific hybrids, with a reservation for *S. fruticosa*, were shown to be suitable for use in extensive green roofs in the Eastern Mediterranean, even under deficit irrigation. In addition, as non-invasive species, they are recommended for sustainable exploitation in green roofs and xeriscaping in other regions with a semi-arid/arid climate as well.

5. Conclusions

The acclimatization of five native-to-Greece sage species, namely, *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*, and five interspecific hybrids in a Mediterranean extensive green roof was affected by both the plant genotype and the frequency of irrigation.

The hybrids *S. pomifera* ssp. *Pomifera* × *S. ringens* and *S. officinalis* × *S. pomifera* ssp. *pomifera* showed the highest survival rate of all *Salvia* spp. and hybrids tested, along with satisfactory growth both under regular and reduced irrigation frequency.

S. fruticosa showed the lowest plant survival rate under both normal and sparse irrigation and the *S. fruticosa* × *S. ringens* hybrid showed a low survival rate under normal irrigation.

All hybrids, except *S. officinalis* × *S. tomentosa*, grew more efficiently in a green roof under water deficiency compared to their parental species.

Greek *Salvia* spp. and their interspecific hybrids, with a reservation for *S. fruticosa*, are recommended for sustainable exploitation in green roofs in regions with a Mediterranean climate and generally in xeriscaping.

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