

MEDITERRANEAN SAGE HYBRID *Salvia officinalis* × *S. ringens* AS GROWN UNDER REDUCED IRRIGATION IN A GREENHOUSE AND AN EXTENSIVE GREEN ROOF

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Abstract

Tolerance in xerothermic conditions of the new interspecific sage hybrid *S. officinalis* × *S. ringens* was examined aiming to its exploitation in extensive green roofs and xeriscaping. Two irrigation frequencies, a regular (every 2–3 days, when substrate moisture 17–23% v/v) and a reduced (every 3–5 days, when substrate moisture 7–13% v/v) were applied in a greenhouse (cultivation in 14 cm pots on peat: perlite, 2:1, v/v) and a green roof experiment (cultivation in 10 cm deep substrate mixture of grape-marc compost: perlite: pumice substrate 3:3:4, v/v, alongside with the parental species, *S. officinalis* and *S. ringens*). Both experiments started in April 2021 and lasted 3 and 5 months, respectively. In the greenhouse, most growth parameters of the hybrid, i.e., plant height, lateral shoot number and length and aboveground dry weight were not affected significantly by reduced irrigation, excepting root dry weight which was reduced. In the green roof, *S. officinalis* × *S. ringens* had higher plant height and aboveground dry weight than the parental species. Under reduced irrigation, all *Salvia* types had lower plant diameter, as well as aboveground and root dry weight compared to regular irrigation. *S. officinalis* × *S. ringens* hybrid, as well as its parental species *S. officinalis* and *S. ringens* grew satisfactorily under reduced irrigation, resulting to their recommendation for sustainable use in xeriscaping, including extensive green roofs.

Keywords: *drought resistance, Mediterranean sage, interspecific hybrid, Salvia officinalis, Salvia ringens*

Introduction

Mediterranean sages (*Salvia* spp. family Lamiaceae) are drought-resistant plants, part of the macchia shrubland, which could be ideal for use in xeriscaping, suitable for use in extensive type urban green roofs and valuable as bee-friendly plants. Aiming to introduce new drought-resistant species with interesting floricultural characteristics in the floriculture industry, interspecific crossbreeding was undertaken between sage species native to Greece (Papafotiou *et al.*, 2021). One of the crosses was between *S. officinalis*, used as the seed parent, and *S. ringens*, used as the pollen parent, resulting to the creation of a new interspecific hybrid *S. officinalis* × *S. ringens*.

S. officinalis L. is a strongly aromatic shrub, up to 60 cm tall, with greenish above and white felted beneath, oblong to elliptical leaves and violet-blue, pink or white flowers in May–July, that prefers garrigue, stony pastures, scrub, rocky places. It is widespread on the Apennines and eastern Adriatic coast, found in Northern Greece and the Ionian islands as well. It is one of the most important species of the genus *Salvia* worldwide, as it is cultivated in many varieties as medicinal, culinary and ornamental (Blamey and Grey-Wilson, 1993; Tutin *et al.*, 1972).

S. ringens Sibth. & Sm. is a hardy low, up to 30 cm tall, herbaceous plant, with dark green, pinnatisect or pinnate with 3–6 pairs of small lateral segments, appressed-hairy leaves and tall, branching flowering stems with large dark violet-blue flowers during late spring through summer. It is found in areas with macchia vegetation, forest glades and streams between 490 m and 1300 m, in South and Eastern parts of Balkan Peninsula, including North and Central Greece (Tutin *et al.*, 1972). It is resistant to low temperatures.

S. officinalis × *S. ringens* has inherited from *S. ringens* the segmented leaves and the long flowering stems. It is taller than *S. ringens* and produces many lateral shoots, while its segmented leaves are unique and particularly decorative, but they have very light aroma, as *S. ringens* does. Flowering stems are about 40 cm long with more flowers than those of *S. ringens*. Flower color is light violet-blue, resembling the flower color of *S. officinalis* (Papafotiou *et al.*, 2021).

The drought tolerance of *S. officinalis* has been thoroughly investigated (Raimondo *et al.*, 2015; Kemp *et al.*, 2019; Abreu and Munné-Bosch, 2008; Bettaieb *et al.*, 2011; Abate *et al.*, 2021), while it has been successfully tested for growth on extensive green roofs as well (Raimondo *et al.*, 2015; Papafotiou *et al.*, 2017; Kemp *et al.*, 2019; Kokkinou *et al.*, 2016). For *S. ringens* and their hybrid, it is the first time they were tested for sustainable exploitation as green roof plants.

Aiming to evaluate tolerance in xerothermic conditions, which would facilitate exploitation in extensive green roofs and xeriscaping, the new interspecific sage hybrid *S. officinalis* × *S. ringens* was grown under two irrigation frequencies, one considered adequate and one deficient, during the hot and dry Eastern Mediterranean summer, in a greenhouse and, alongside with its parental species (*S. officinalis* and *S. ringens*), in an extensive green roof.

Materials and methods

Aiming to test growth and drought resistance of the new interspecific sage hybrid *S. officinalis* × *S. ringens* in a greenhouse, in early April 2021, four-week-old rooted cuttings were transplanted singly in plastic pots, 14 cm in diameter, which contained 1 L of peat-perlite mixture 2:1 (v/v). Climatic conditions during the experiment are shown in Figure 1 a-b. Plants were fertilized monthly with 2 g/L water soluble fertilizer (20-20-20 plus, HUMOFERT, Metamorfosi, Greece). In each pot, 100 ml of fertilizer was applied. Two irrigation frequencies were applied, i.e., (i) when the moisture content of the substrate was 20–23% v/v (normal irrigation) and (ii) when the moisture content of the substrate was 8–13% v/v (sparse irrigation). In the first month of cultivation, the plants under normal irrigation were irrigated every 3–4 days and under sparse irrigation every 5 days, while in the following months every 2 and 3–4 days, respectively.

Aiming to evaluate drought tolerance of the hybrid *S. officinalis* × *S. ringens*, alongside with its parental species *S. officinalis* and *S. ringens*, in an extensive green roof, in early April 2021 as well, rooted cuttings about 8 weeks old were planted in plastic containers, 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. Climatic conditions during this experiment are shown in Figure 1 c-e. Irrigation frequencies, like the greenhouse experiment, were applied i.e., a regular (when substrate moisture 17–23% v/v) and a reduced (when substrate moisture 7–13% v/v). In the

first month of cultivation, the plants were irrigated normally every 3 days and sparsely every 5 days, while in the following months every 2 and 4 days, respectively. 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).

Plant growth was evaluated monthly, but in the present study, the final results were presented after three in the greenhouse and five months in the green roof experiment.

The completely randomized design was used. The significance of the results was tested by either one- or two- or three-way analysis of variance (ANOVA) and the means of the treatments were compared by Student's t test at $p < 0.05$ (JMP 11.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

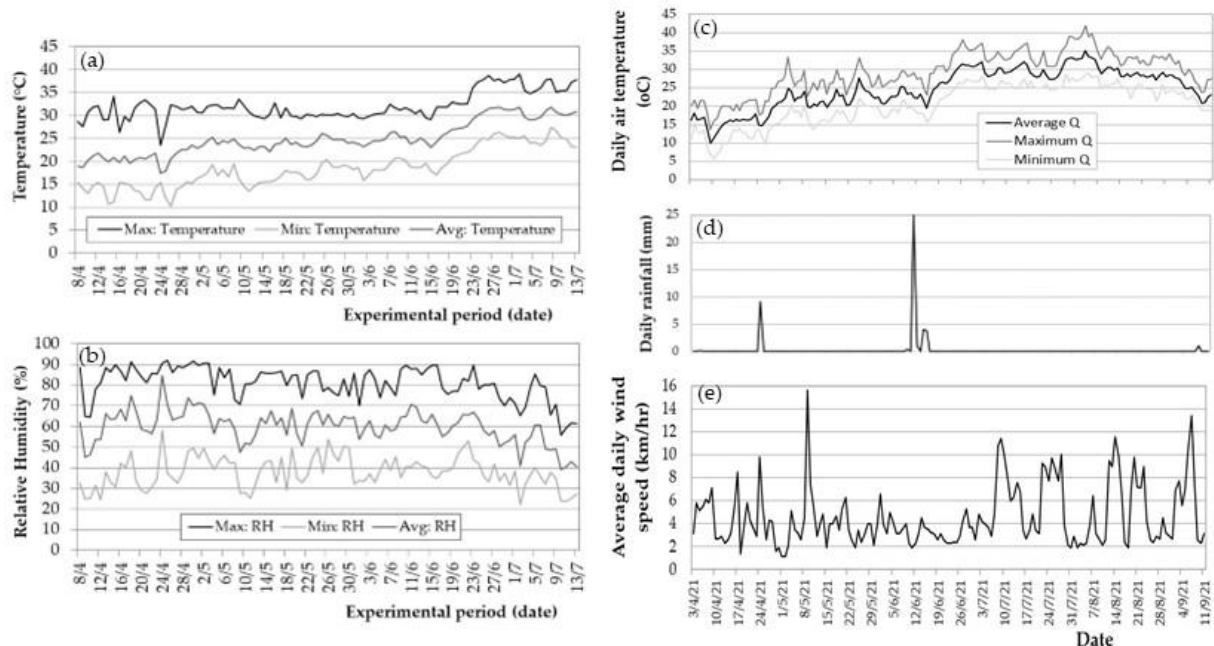


Figure 1. Climatic conditions, i.e., temperature (a) and relative humidity (b) inside the glass greenhouse where the experiment was conducted, during the 3-month experimental period (from April 2021 until July 2021), as well as temperature (c), daily rainfall (d) and average daily wind speed (e) in the green roof, during the 5-month experimental period (from April to September 2021).

Results and Discussion

In the greenhouse, all plants survived and most growth parameters of the hybrid were not affected significantly by reduced irrigation, excepting root dry weight which was reduced (Table 1, Figure 2).

In the green roof, *Salvia* types didn't differ in their survival percentage, which was ranging between 66.7% and 100% (Table 2). *S. officinalis* \times *S. ringens* had higher plant height and aboveground dry weight than the parental species (Table 2, Figure 3). Under reduced irrigation, all *Salvia* types had lower plant diameter, as well as aboveground and root dry weight compared to regular irrigation (Table 2, Figure 3). Nevertheless, their growth was satisfactory (Figure 3).

Growth parameters of *S. officinalis* \times *S. ringens* hybrid in the greenhouse were less affected by reduced irrigation than those in the green roof, as only root dry weight was reduced in the first experiment (Tables 1 & 2, Figures 2 & 3). Water deficiency has been reported to reduce plant height, as well as above ground and leaf fresh and dry weight in *S. fruticosa*

(Chrysargyris *et al.*, 2016] and *S. officinalis* (Sabry *et al.*, 2016; Soltanbeigi *et al.*, 2021). Decreases in plant height are common under drought stress (Wang *et al.*, 2003), due to the lack of water which leads to clogging of vascular tissue and reduction of cell elongation (Abdalla and El-Khoshiban, 2007).

The vigorous canopy growth of *S. officinalis* × *S. ringens* hybrid, which seemed to have been inherited from its pollen parent *S. ringens*, in combination with its rich root system, which seemed to have been inherited from its seed parent *S. officinalis*, probably contributed to its better adaptation in the green roof under both irrigation frequencies (Table 2, Figure 3). This is reinforced by the fact that the root system is the main plant organ for adaptation to drought stress conditions (Abobatta, 2019; Zhou *et al.*, 2018).

Table 1. Growth of the new interspecific hybrid *S. officinalis* × *S. ringens* for three months (April–July 2021) in a greenhouse under normal and reduced irrigation (data per plant).

Irrigation frequency	Plant height (cm)	Lateral shoot number	Lateral shoot mean length (cm)	Lateral shoot total length (cm)	Above ground d.w. (g)	Root d.w. (g)	Root d.w./Above ground d.w.
Normal	27.5 a [†]	9.0 a	10.1 a	87.4 a	12.9 a	3.4 a	0.3 a
Reduced	27.0 a	7.6 a	10.6 a	75.0 a	12.1 a	2.4 b	0.2 b
Significance §							
<i>F</i> _{one-way ANOVA}	NS	NS	NS	NS	NS	**	*

[†] Mean values ($n = 10$) in each column followed by the same lowercase letter (a-b) 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.



Figure 2. Typical above ground and root system of the new interspecific hybrid *S. officinalis* × *S. ringens*, after having grown for three months in greenhouse conditions (A: without and B: with 25 g/L attapulgate/normal irrigation, C: without and D: with 25 g/L attapulgate/sparse irrigation). Size bar = 10.0 cm.

Conclusions

Under reduced irrigation, growth of all *Salvia* types was diminished compared to regular irrigation. However, *S. officinalis* × *S. ringens* hybrid, as well as its parental species *S. officinalis* and *S. ringens* grew satisfactorily under reduced irrigation, resulting to their recommendation for sustainable use in xeriscaping, including extensive green roofs.

Acknowledgements

Table 2. Comparative evaluation of the growth of two Greek sage species (*S. officinalis* and *S. ringens*) and their interspecific hybrid (*S. officinalis* × *S. ringens*) after five months of growth (April–September 2021) in an urban Mediterranean green roof under normal and reduced irrigation.

Data	Per container		Per plant		Per container (2 plants)		
	Irrigation Frequency	Survival (%)	Foliage height (cm)	Plant diameter (cm)	Above ground d.w. (g)	Root d.w. (g)	Root d.w./Above ground d.w.
<i>S. officinalis</i>	Normal	83.3 a [†]	23.9 b	29.2 ab	70.3 c	95.9 a	1.4 a
	Reduced	75.0 a	20.8 c	25.4 c	50.4 d	74.9 ab	1.5 a
<i>S. ringens</i>	Normal	100.0 a	18.1 c	29.8 a	86.6 b	61.5 b	0.7 b
	Reduced	66.7 a	14.1 d	25.5 bc	56.6 cd	46.5 b	0.8 b
<i>S. officin.</i> × <i>S. ringens</i>	Normal	83.3 a	25.6 ab	27.0 abc	116.9 a	91.3 a	0.8 b
	Reduced	83.3 a	28.5 a	23.9 c	90.0 b	57.1 b	0.6 b
Significance [§]							
$F_{Salvia\ type}$		NS	-	NS	**	*	**
$F_{irrigation\ frequency}$		NS	-	**	**	**	NS
$F_{interaction}$		NS	**	NS	NS	NS	NS
$F_{one-way\ ANOVA}$		NS	**	**	**	**	**

[†] Mean values ($n = 8-12$) in each column using Student's t test; means followed by the same letter are not significantly different at $p \leq 0.05$

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§ 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 growth of aboveground and root system of *S. officinalis* (a), *S. ringens* (b), and *S. officinalis* × *S. ringens* (c), after five months cultivation (April - September 2021) in an extensive green roof, under normal (N) and sparse (S) irrigation.

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MEDITERRANEAN SAGE HYBRID *Salvia officinalis* × *S. ringens* AS GROWN UNDER REDUCED IRRIGATION IN A GREENHOUSE AND AN EXTENSIVE GREEN ROOF

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Introduction

The new Mediterranean sage hybrid *Salvia officinalis* × *S. ringens* was produced through interspecific crossbreeding between *S. officinalis* and *S. ringens* native to Greece (Papafotiou *et al.*, 2021). Aiming to evaluate tolerance in xerothermic conditions, which would facilitate exploitation in extensive green roofs and xeriscaping, it was grown under two irrigation frequencies, one considered adequate and one deficient, during the hot and dry Eastern Mediterranean summer, in a greenhouse and, alongside with its parental species, in an extensive green roof.

Table 1. Growth of the new interspecific hybrid *S. officinalis* × *S. ringens* for three months (April–July 2021) in a greenhouse under normal and reduced irrigation (data per plant).

Irrigation frequency	Plant height (cm)	Lateral shoot number	Lateral shoot mean length (cm)	Lateral shoot total length (cm)	Above ground d.w. (g)	Root d.w. (g)	Root d.w./Above ground d.w.
Normal	27.5 a [†]	9.0 a	10.1 a	87.4 a	12.9 a	3.4 a	0.3 a
Sparse	27.0 a	7.6 a	10.6 a	75.0 a	12.1 a	2.4 b	0.2 b
Significance §							
F _{one-way ANOVA}	NS	NS	NS	NS	NS	**	*

[†] Mean values (n = 10) in each column followed by the same lowercase letter (a-b) 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.

Results and Discussion

In the greenhouse, all plants survived and most growth parameters of the hybrid were not affected significantly by sparse irrigation, excepting root dry weight which was reduced (Table 1, Figure 1).

In the green roof, *Salvia* types did not differ in their survival range (Table 2). *S. officinalis* × *S. ringens* developed higher plant height and aboveground dry weight than the parental species (Table 2, Figure 2). Under sparse irrigation, all *Salvia* types had lower plant diameter, as well as aboveground and root dry weight compared to normal irrigation (Table 2, Figure 2). Nevertheless, their growth was satisfactory (Figure 2).

The vigorous canopy growth of *S. officinalis* × *S. ringens* hybrid, which seemed to have been inherited from its pollen parent *S. ringens*, in combination with its rich root system, which seemed to have been inherited from its seed parent *S. officinalis*, probably contributed to its better adaptation in the green roof under both irrigation frequencies (Table 2, Figure 3). This is reinforced by the fact that the root system is the main plant organ for adaptation to drought stress conditions (Abobatta, 2019; Zhou *et al.*, 2018).

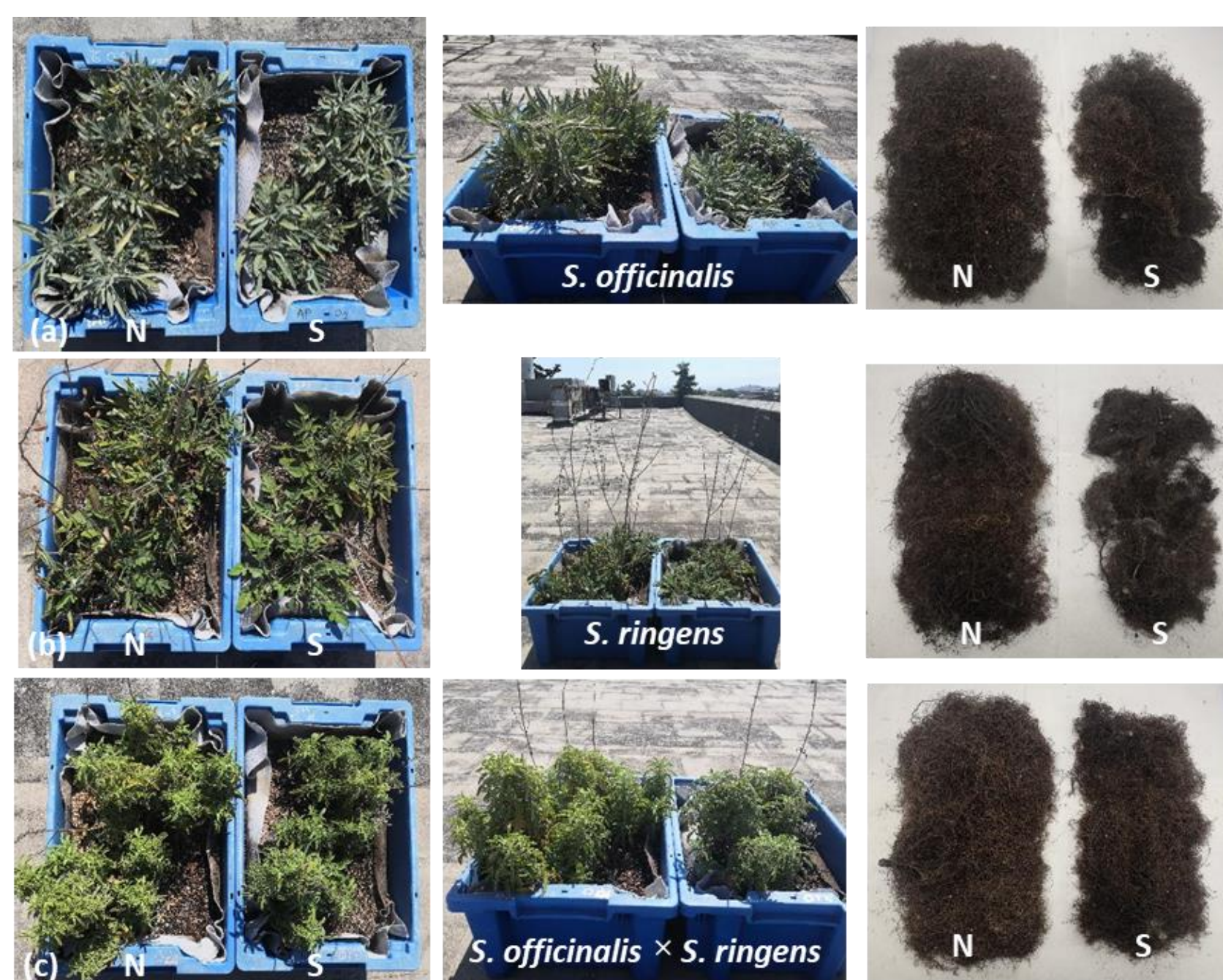


Figure 2. Typical growth of aboveground and root system of *S. officinalis* (a), *S. ringens* (b), and *S. officinalis* × *S. ringens* (c), after five months cultivation (April - September 2021) in an extensive green roof, under normal (N) and sparse (S) irrigation.



Figure 1. Typical above ground and root system of the new interspecific hybrid *S. officinalis* × *S. ringens*, after three months of growth in greenhouse conditions, under normal (N) and sparse (S) irrigation.

Materials and Methods

In a greenhouse experiment, 4-week-old rooted cuttings of the new interspecific sage hybrid *S. officinalis* × *S. ringens*, were grown singly in 14 cm pots, on a peat-perlite mixture 2:1 (v/v). Plants were fertilized monthly with 2 g/L water soluble fertilizer 20-20-20 (100 ml fertilizer/ pot).

In an extensive green roof experiment, 8-week-old rooted cuttings of the hybrid *S. officinalis* × *S. ringens*, alongside with its parental species *S. officinalis* and *S. ringens*, were cultivated in plastic containers with a green roof infrastructure fitted, on a second-floor flat roof at the Agricultural University of Athens. The substrate used was grape marc compost: perlite: pumice (3: 3: 4, v/v) and had a 10 cm depth. Both experiments started in April 2021 and lasted 3 and 5 months, respectively. Two irrigation frequencies, a regular (every 2–3 days, when substrate moisture 17–23% v/v) and a sparse (every 3–5 days, when substrate moisture 7–13% v/v) were applied.

Table 2. Comparative evaluation of the growth of two Greek sage species (*S. officinalis* and *S. ringens*) and their interspecific hybrid (*S. officinalis* × *S. ringens*) after five months of growth (April–September 2021) in an urban Mediterranean green roof under normal and sparse irrigation.

Data	Per container		Per plant		Per container (2 plants)		
	Irrigation Frequency	Survival (%)	Foliage height (cm)	Plant diameter (cm)	Above ground dr.wt. (g)	Root dr.wt. (g)	Root dr.wt./Above ground dr.wt.
<i>S. officinalis</i>	Normal	83.3 a [†]	23.9 b	29.2 ab	70.3 c	95.9 a	1.4 a
	Sparse	75.0 a	20.8 c	25.4 c	50.4 d	74.9 ab	1.5 a
<i>S. ringens</i>	Normal	100.0 a	18.1 c	29.8 a	86.6 b	61.5 b	0.7 b
	Sparse	66.7 a	14.1 d	25.5 bc	56.6 cd	46.5 b	0.8 b
<i>S. officinalis</i> × <i>S. ringens</i>	Normal	83.3 a	25.6 ab	27.0 abc	116.9 a	91.3 a	0.8 b
	Sparse	83.3 a	28.5 a	23.9 c	90.0 b	57.1 b	0.6 b
Significance §							
F _{Salvia type}		NS	-	NS	**	*	**
F _{irrigation frequency}		NS	-	**	**	**	NS
F _{interaction}		NS	**	NS	NS	NS	NS
F _{one-way ANOVA}		NS	**	**	**	**	**

[†] Mean values (n = 8-12) in each column using Student's *t* test; means followed by the same letter are 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.

Conclusions

S. officinalis × *S. ringens* hybrid grew very satisfactorily in a green house under sparse irrigation.

In a green roof, growth of *S. officinalis* × *S. ringens* hybrid and its parental species was diminished under sparse irrigation. However, all three sage types grew satisfactorily even under sparse irrigation, and thus are recommended for sustainable use in xeriscaping, including extensive green roofs.

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<https://www.salvia-breed-gr.com/el/>

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