GROWTH OF MEDITERRANEAN SAGE SPECIES AND INTERSPECIFIC HYBRIDS UNDER LIMITED IRRIGATION IN A GREEN ROOF

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Abstract

Mediterranean sages (Salvia spp.) could be ideal for use in xeriscaping, as bee-friendly plants or in green roofs. Salvia fruticosa, S. officinalis, S. pomifera ssp. pomifera, S. ringens and S. tomentosa, along with five new interspecific hybrids of them, were evaluated for their growth under regular (every 2-3 days when substrate moisture 17-23% v/v) and limited irrigation (every 4–5 days when substrate moisture 7–13% v/v), in a Mediterranean extensive green roof. A substrate (grape-marc compost: perlite: pumice, 3:3:4, v/v), with 10 cm depth, was used. S. pomifera ssp. pomifera \times S. ringens and S. officinalis \times S. pomifera ssp. pomifera survived at the highest percentage. Limited irrigation resulted in the reduction of aboveground and root biomass of all sage types, but to varying degrees depending on sage type. S. officinalis, S. officinalis \times S. ringens and S. pomifera ssp. pomifera \times S. ringens showed the lowest aboveground biomass reduction, in contrast to S. fruticosa that showed the highest, while S. officinalis, S. ringens, S. officinalis × S. pomifera ssp. pomifera and S. pomifera ssp. *pomifera* \times S. *ringens* showed the lowest reduction in root biomass. All studied sage types, with a reservation for S. fruticosa, grew satisfactorily under limited irrigation, being suitable for sustainable exploitation in xeriscaping, including extensive green roofs in arid regions. Especially the hybrids of S. officinalis or S. ringens or S. pomifera ssp. pomifera were found to be even more resistant to limited irrigation than their parental species.

Keywords: Salvia fruticosa, Salvia officinalis, Salvia pomifera ssp. pomifera, Salvia ringens, Salvia tomentosa

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 (Caneva *et al.*, 2015; Shafique *et al.*, 2018). One of the most critical steps in green roof installation in these areas is the selection of droughtand heat-tolerant plant species that can thrive under extreme microclimate conditions (Savi *et al.*, 2016). 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 (Papafotiou *et al.*, 2013; Caneva *et al.*, 2015; Ondoño *et al.*, 2015; Tassoula *et al.*, 2021).

Mediterranean sages (*Salvia* spp.), as members of the macchia vegetation, are droughtresistant plants and could be ideal for use in xeriscaping, as bee-friendly plants or in green roofs. In order to introduce new drought-resistant species with interesting floricultural characteristics in the floriculture industry, interspecific crossbreeding was undertaken between five native to Greece sage species and five hybrids, 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 (Papafotiou *et al.*, 2021). S. officinalis and S. fruticosa have been tested previously for growth on extensive GRs (Raimondo *et al.*, 2015; Papafotiou *et al.*, 2015; Kemp *et al.*, 2019), while the drought tolerance of S. officinalis has thoroughly been investigated (Raimondo *et al.*, 2015; Kemp *et al.*, 2019; Abate *et al.*, 2021). Besides, four of the studied hybrids used were found to respond better to water stress in greenhouse conditions compared to S. fruticosa (Papafotiou *et al.*, 2021).

In the present study, the species *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*, along with five new interspecific hybrids of them, were evaluated for their growth under regular and limited irrigation, in a Mediterranean extensive green roof.

Materials and methods

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*, about 8 weeks old, were planted on early 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 and six containers per treatment were used. The containers were placed on a second-floor flat roof 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.



Figure 1. Climatic conditions, i.e., temperature (a), daily rainfall (b) and average daily wind speed (c) in the green roof, during the 5-month experimental period (from April to September 2021).

Two irrigation frequencies were applied, a regular (normal, when substrate moisture 17–23% v/v) and a limited one (sparse, 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 L h⁻¹, irrigation period: 60 min). The experiment lasted for 5 months, until September 2021, and plant survival and growth were evaluated. The completely randomized design was used. The significance of the results was tested by either one- or two- 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

Results and Discussion

Inc., Cary, NC, 2013, USA).

Five months after planting in an extensive green roof, *S. fruticosa* presented the lowest survival rate, regardless of irrigation frequency (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. ringens* was the species with the highest survival rate under normal irrigation, but under sparse irrigation, its survival rate was significantly reduced. However, all three *S. ringens* hybrids showed a high survival rate under sparse irrigation. The hybrid *S. officinalis* × *S. pomifera* ssp. *pomifera* was the only *Salvia* type that showed 100% survival at both irrigation frequencies (Figure 2).



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 $p \le 0.05$; means followed by the same letter were not significantly different at $p \le 0.05$. [§] NS or **, non-significant at $p \le 0.05$ or significant at $p \le 0.05$, respectively.

The experimental factors (*Salvia* type and irrigation frequency) significantly affected the aboveground and the root dry weight (Figure 3). Sparse irrigation resulted in lower aboveground and root biomasses compared to normal irrigation. Concerning *Salvia* type, the highest aboveground biomass was recorded for the *S. officinalis* × *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* × *S. pomifera* ssp. *pomifera* and *S. officinalis* (Figures 3 and 4).

Under normal irrigation, the species S. fruticosa, S. tomentosa and S. pomifera ssp. pomifera and the hybrids S. officinalis \times S. pomifera ssp. pomifera and S. officinalis \times S. ringens developed the highest aboveground biomass compared to all other species and hybrids, followed by S. ringens, S. fruticosa \times S. ringens and S. officinalis \times S. tomentosa (Figures 3 and 4). As for the root biomass, this was highest in S. fruticosa, S. tomentosa and S. officinalis and the hybrids S. officinalis \times S. pomifera ssp. pomifera and S. officinalis \times S. ringens (Figures 3 and 4).



Figure 3. Aboveground (a) and root (b) dry weight (d.w.) 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, as well as reduction percentage (%) of aboveground or root d.w. under sparse irrigation is presented.

Limited irrigation resulted in the reduction of aboveground and root biomass of all sage types, but in a different degree depending on sage type. *S. officinalis*, *S. officinalis* \times *S. ringens* and *S. pomifera* ssp. *pomifera* \times *S. ringens* showed the lowest aboveground biomass reduction, in

contrast to *S. fruticosa* that showed the highest, while *S. officinalis*, *S. ringens*, *S. officinalis* \times *S. pomifera* ssp. *pomifera* and *S. pomifera* ssp. *pomifera* \times *S. ringens* showed the lowest reduction in root biomass (Figure 3).

The survival and growth 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 irrigation frequency (Figure 2, 3). Regarding hybrid parents, S. officinalis is considered suitable for use in green roofs (Raimondo et al., 2015; Papafotiou et al., 2017), as supported by the present work as well. In general, the hybrids acclimatized more efficiently to the green roof conditions compared to their parents and the hybrids of S. pomifera ssp. pomifera showed the highest survival of all species and hybrids at both irrigation frequencies. 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 (Alarcón et al., 2006), 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 (Zhou et al., 2018), which optimizes water uptake (Chaves et al., 2003). 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 (Figure 3).



Figure 4. 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 and sparse irrigation frequencies.

Conclusions

All studied sage types, with a reservation for *S. fruticosa*, grew satisfactorily under limited irrigation, being suitable for sustainable exploitation in xeriscaping, including extensive green roofs in arid regions. Especially the hybrids of *S. officinalis* or *S. ringens* or *S. pomifera* ssp. *pomifera* were found even more resistant in limited irrigation than their parental species.

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References

- Abate E., Nardini A., Petruzzellis F., Trifilò P. (2021). Too dry to survive: Leaf hydraulic failure in two *Salvia* species can be predicted on the basis of water content. Plant Physiology and Biochemistry, vol. 166, pp. 215–224.
- Alarcón J.J., Morales M.A., Ferrández T., Sánchez-Blanco M.J. (2006). Effects of water and salt stresses on growth, water relations and gas exchange in *Rosmarinus officinalis*. Journal of Horticultural Science and Biotechnology, vol. 81, pp. 845–853.
- Caneva G., Kumbaric A., Savo V., Casalini R. (2015). Ecological approach in selecting extensive green roof plants: A data-set of Mediterranean plants. Plant Biosystems, vol. 149, pp. 374–383.
- Chaves M.M., Maroco J.P., Pereira J.S. (2003). Understanding plant responses to drought— From genes to the whole plant. Functional Plant Biology, vol. 30, pp. 239–264.
- Kemp S., Hadley P., Blanuša T. (2019). The influence of plant type on green roof rainfall retention. Urban Ecosystems, vol. 22, pp. 355–366.
- Ondoño S., Martínez-Sánchez J.J., Moreno J.L. (2015). Evaluating the growth of several Mediterranean endemic species in artificial substrates: Are these species suitable for their future use in green roofs? Ecological Engineering, vol. 81, pp. 405–417.
- Papafotiou M., Pergialioti N., Tassoula L., Massas I., Kargas G. (2013). Growth of native aromatic xerophytes in an extensive Mediterranean green roof as affected by substrate type and depth and irrigation frequency. HortScience, vol. 48, pp. 1327–1333.
- Papafotiou M., Martini A.N., Papanikolaou E., Stylias E.G., Kalantzis A. 2021. Hybrids development between Greek *Salvia* species and their drought resistance evaluation along with *Salvia fruticosa*, under attapulgite-amended substrate. Agronomy, vol. 11, 2401.
- Raimondo F., Trifilò P., Lo Gullo M.A., Andri S., Savi T., Nardini A. (2015). Plant performance on Mediterranean green roofs: Interaction of species-specific hydraulic strategies and substrate water relations. AoB Plants, vol. 7, plv007.
- Papafotiou M., Koutri A., Massas I. (2017). Heavy metal concentration in sage plants cultivated on an urban green roof or roadside location as affected by substrate type and fertilization. Acta Horticulturae, vol. 1189, pp. 439–442.
- Savi T., Dal Borgo A., Love V.L., Andri S., Tretiach M., Nardini A. (2016). Drought versus heat: What's the major constraint on Mediterranean green roof plants? Science of the Total Environment, vol. 566, pp. 753–760.
- Shafique M., Kim R., Rafiq M. (2018). Green roof benefits, opportunities and challenges–A review. Renewable and Sustainable Energy Reviews, vol. 90, pp. 757–773.
- Tassoula L., Papafotiou M., Liakopoulos G., Kargas G. (2021). Water use efficiency, growth and anatomic-physiological parameters of Mediterranean xerophytes as affected by substrate and irrigation on a green roof. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, vol. 49, 12283.
- Zhou G., Zhou X., Nie Y., Bai S.H., Zhou L., Shao J., Cheng W., Wang J., Hu F., Fu Y. (2018). Drought-induced changes in root biomass largely result from altered root morphological traits: Evidence from a synthesis of global field trials. Plant Cell Environment, vol. 41, pp. 2589–2599.

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Introduction

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Materials and Methods

Rooted cuttings of the five sage species native to Greece, along with the five interspecific hybrids of them, mentioned in the introduction, about 8 weeks old, were planted on early April 2021 in plastic containers with a green roof infrastructure fitted. Two plants of the same type per container and six containers per treatment were used. The containers were placed 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. Two irrigation frequencies were applied, a normal (every 2–3 days when substrate moisture was 17–23% v/v) and a sparse (every 4–5 days when substrate moisture was 7–13% v/v). The experiment lasted for 5 months, until September 2021, and plant survival and growth were evaluated. The completely randomized design was used. The significance of the results was tested by either one- or two- way analysis of variance (ANOVA) and the means of the treatments were compared by Student's *t* test at $p \leq 0.05$ (JMP 11.0 software, SAS Institute Inc., Cary, NC, 2013, USA).



Results and Discussion

Five months after planting in an extensive green roof, *S. fruticosa* presented the lowest survival rate. *S. ringens* was the species with the highest survival rate under normal irrigation, but under sparse irrigation, its survival rate was significantly reduced. However, all three *S. ringens* hybrids showed a high survival rate under sparse irrigation. The hybrid *S. officinalis* × *S. pomifera* ssp. *pomifera* was the only *Salvia* type that showed 100% survival (Figure 1a).

The experimental factors (*Salvia* type and irrigation frequency) significantly affected the aboveground and the root dry weight. Sparse irrigation resulted in lower aboveground and root biomasses compared to normal irrigation. The *S. officinalis* × *S. ringen*s hybrid showed the highest aboveground biomass and one of the highest values of root biomass, along with *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. officinalis* (Figures 1b and 1c, Figure 2).

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 (Figure 1b, Figure 2). 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* ssp. *pomifera* and *S. officinalis* × *S. tomentosa* and *S. ringens* (Figure 1c).

Limited irrigation resulted in the reduction of aboveground and root biomass of all sage types, but in a different degree depending on sage type. *S. officinalis*, *S. officinalis* × *S. ringens* and *S. pomifera* ssp. *pomifera* × *S. ringens* showed the lowest aboveground biomass reduction, in contrast to *S. fruticosa* that showed the highest, while *S. officinalis*, *S. ringens*, *S. officinalis* × *S. pomifera* and *S. pomifera* ssp. *pomifera* and *S. pomifera* ssp. *pomifera* and *S. pomifera* ssp. *pomifera* (Figures 1b and 1c).

Figure 1. Survival (%) (a), aboveground (b) and root (c) dry weight (d.w.) 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. Reduction percentage (%) of aboveground or root dr.wt. under sparse irrigation is also presented .

† Mean comparison with Student's *t*-test at $p \le 0.05$; means followed by the same letter were not significantly different at $p \le 0.05$.

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Literature Cited

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





