CHEMICAL ENGINEERING TRANSACTIONS

VOL. 58, 2017

A publication of AIDIC

The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Remigio Berruto, Pietro Catania, Mariangela Vallone

ISBN 978-88-95608-52-5; **ISSN** 2283-9216

DOI: 10.3303/CET1758117

Morpho-physiologic Traits in Two Sage Taxa Grown under Different Irrigation Regime

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Sage is an important aromatic crop, extensively cultivated worldwide. Drought stress affects yield and composition of secondary metabolites such as fatty acids, essential oils, antioxidants, changing the essential oil composition profile with respect to the ISO 9909 standard. Drought tolerance may differ among genotypes, so we compared the response of *Salvia officinalis* L. and *S. officinalis* cv. 'Maxima' grown under two different irrigation regimes to highlight differences in leaf growth, water potential, gas exchange and essential oil composition. Potted plants were grown in the greenhouse at 100% or 50% of field water capacity for three months. Monthly measurements of 3rd node leaf length, width and area were taken using ImageJ software. Midday leaf water potential was measured using a pressure chamber and leaf stomatal conductance, transpiration and net photosynthesis were measured using a portable infrared gas analyzer. Essential oil composition was determined by gas chromatography and mass spectrometry. Drought stress reduced leaf area in both taxa, but the effect was less pronounced in *S. officinalis* than in *S. officinalis* cv. 'Maxima' (-30%). Leaf water potential was slightly lower in the latter cultivar than in *S. officinalis* under water deficit. In irrigated *S. officinalis* cv. 'Maxima' plants transpiration and stomatal conductance rates were more than twice those of *S. officinalis*, while under water deficit the difference between the taxa was not significant. Interestingly, net photosynthesis in *S. officinalis* was about twice that measured in *S. officinalis* cv. 'Maxima', both in irrigated and in stressed plants. Furthermore, in *S. officinalis* water deficit resulted in a slight reduction of photosynthetic rate, while in *S. officinalis* cv. 'Maxima' the reduction was around 50%. Both taxa were affected by drought stress, responding with a reduction in leaf expansion and in transpiration, in order to reduce water loss. However, *S. officinalis* appeared to have a greater efficiency, maintaining higher levels of carbon assimilation.

1. Introduction

The genus *Salvia* (Lamiaceae, tribe Mentheae) is one of the largest and most important aromatic and medicinal genera of the Lamiaceae family and comprises about 900 species, widespread throughout the world (Walker et al., 2007), displaying a remarkable diversity in growth forms, secondary compounds, floral morphology and pollination biology. The essential oils produced by the leaves are acknowledged worldwide because of their beneficial uses: there are a great number of literature reports on analyses of essential oils from plants of this genus and morphological and genetic variations are also observed according to their geographical origin. As its Latin gender name Salvia means ''to cure'' and species name ''officinalis'' means medicinal, it is clear that sage has a historical reputation of promoting health and treating ailments (Russo et al., 2013). In Ancient Rome, it was even called the sacred plant (Durling et al., 2007; Kamatou et al., 2008).
Water deficit is an environmental stress experienced by many plants that strongly impairs production (Passioura 1996). Water stress causes large morphological or physiological changes across a range of temporal and spatial scales (Chaves et al. 2002) including reduced expansion of aerial organs, maintenance of root growth, decrease of transpiration and photosynthesis, accumulation of osmotica (Hummel et al., 2010). Drought tolerance may differ with genotype, and both environmental and genetic factors may influence essential oil composition, as reported for three of the many cultivars of sage (Böszörményi et al., 2009). The

Please cite this article as: Grisafi F., Oddo E., Maggio A., Panarisi A., Panarisi M., 2017, Morpho-physiologic traits in two sage taxa grown under different irrigation regime, Chemical Engineering Transactions, 58, 697-702 DOI: 10.3303/CET1758117

sage cultivar ‘Maxima’ is very decorative, strongly scented, with large silver-grey leaves and to the best of our knowledge, there are no published data on its response to stress and essential oil composition. The aim of this preliminary work was to compare growth, water relations, photosynthesis and main essential oil composition of *Salvia officinalis* L. and *S. officinalis* cv. ‘Maxima’ grown under two different irrigation regimes.

2. Materials and Methods

Plants of *Salvia officinalis* L. and *Salvia officinalis* cv. ‘Maxima’, obtained from cuttings and on average 25 cm high, were grown in 2 L pots in garden soil in a greenhouse of the Department of Agricultural and Forest Sciences, Palermo, Italy. Plants were watered with tap water twice a week to 100% (control, C) or 50% (moderate water deficit, MWD) of field water capacity for three months, from March to May 2016.

Soluble fertilizer was dissolved in the irrigation water once every 3 weeks throughout the experiment.

The effect of the different water regimes on plant growth traits was followed monthly: from the beginning of the experiment, photographs of healthy, completely expanded young leaves (3^{rd} node from the top of the shoot) were taken, flattening leaves between two glass slides and including a section of graph paper as reference. Leaf length, width and area were measured on 12 leaves per treatment using ImageJ software.

At the end of the experiment, in May, physiological traits such as leaf water potential and gas exchange parameters were measured on three plants per treatment.

Leaf water potential (Ψ_L) was measured the day after irrigation on leaves collected at 11 AM using a pressure chamber (SKPM 1400, Skye Instruments). Non-destructive measurements of leaf stomatal conductance to water vapour (g_s), transpiration (E) and net photosynthesis (NP) were measured on 9 healthy, completely expanded 3^{rd} node leaves per treatment between 11 and 12 AM using a portable infrared gas analyzer (HCM-1000, Walz). All gas exchange data were expressed on a leaf area basis. All the measurements were conducted on sunny days in the greenhouse under ambient temperature and light conditions.

Air-dried leaf samples were crushed, then hydrodistilled for 3 h using a Clevenger-type apparatus. Oils were dried over anhydrous sodium sulphate and stored under N2 at -20°C. Samples were prepared with a GERSTEL MultiPurpose Sampler MPS equipped with a Cooled Agitator. Analysis was performed in a 7000C Triple Quadrupole GC/MS System, fitted with a fused silica Agilent HP-5 capillary column (10 m x 0.25 mm i.d.; 0.25 µm film thickness), coupled to a Agilent Mass Selective Detector; Diluted samples (1/100 v/v, in *n*-hexane) of 1 µL were injected at 250 °C automatically and in the splitless mode; transfer line temperature, 295 °C. MassHunter Workstation Software was used for chromatogram analysis.

Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. Further identification was made by comparison of their mass spectra with those stored in NIST 02 and Wiley 275 Libraries or with mass spectra from literature (Adams, 2012) and home-made library. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Results were plotted using Microsoft Office Excel 97-2003. Data are expressed as mean ± S.D. Data were analysed with one-way analysis of variance (ANOVA), using the software package SigmaPlot 12 (Systat Software, Inc., San Jose, USA). Fisher’s LSD test was used to compare means when ANOVA was significant ($P < 0.05$).

3. Results and Discussion

Plant growth was measured on the basis of leaf length, width and leaf area. In March, at the beginning of the experiment, 3^{rd} node leaf area was on average 10 cm^2 for *S. officinalis* and 18 cm^2 for S. *officinalis* cv. ‘Maxima’. Leaf area decreased during the experiments in both species, independently from irrigation regime (Figure 1). At the end of the experiment, leaves of the 3^{rd} node of S. *officinalis* had a 50% decrease in leaf area, while this decrease was slightly larger for S. *officinalis* var. ‘Maxima’ (55%). When comparing control plants and MWD plants, after one month, leaf area was reduced about 30 % in S. *officinalis*, and 12 % in S. *officinalis* var. ‘Maxima’. At the end of the experiment, leaf area was not significantly different among treatments in S. *officinalis*, while S. *officinalis* var. ‘Maxima’ under 50% water irrigation had a 30% reduction in leaf area. Correspondingly, leaf length was reduced about 50% from the beginning to the end of the experiment in both taxa and all treatments, while leaf width was not reduced as much in S. *officinalis* cv. ‘Maxima’ as in S. *officinalis* (data not shown).

Exposure of plants to extreme stress conditions such as drought will initiate a diverse set of physiological, morphological, and developmental changes in order to survive (Gomes et al., 2010; Ozkur et al., 2009) and the response of plants to drought varies greatly depending on genotype and stress severity (Verslues et al., 2006). The depressive effect of drought on plant growth we observed under moderate water stress is in agreement with other reports for *Salvia officinalis*, with reductions in shoot length, leaf area, and leaf number (Bettaieb et al., 2009, Mansori et al., 2016).

The leaf water potential values measured at the end of the experimental period showed small though not significant differences, probably due to the low number of replicates. However, on average, C plants reached less negative Ψ_L values than MWD plants in both taxa (Figure 2).

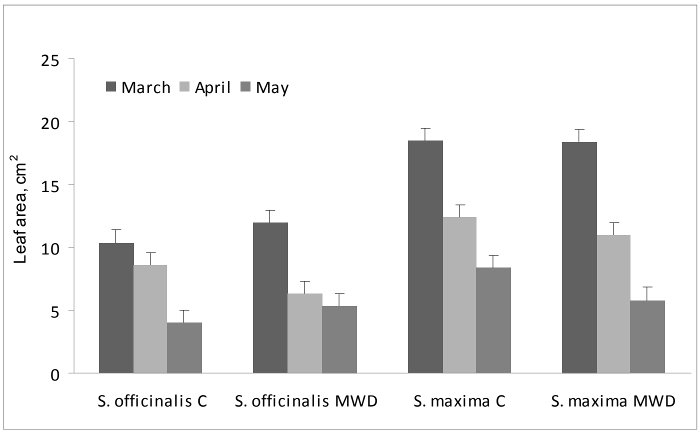

Figure 1. Changes in leaf area of 3rd node leaves in plants of S. officinalis and S. officinalis cv. 'Maxima' irrigated at 100% (C) and 50% (MWD) of field capacity. Data are reported as mean ± SD (n=12).

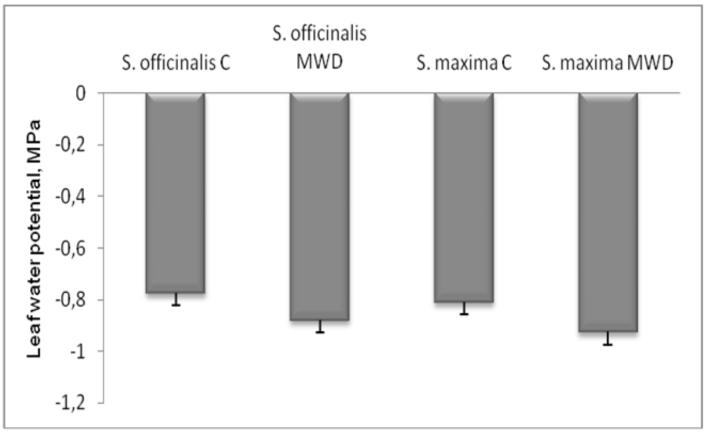

Figure 2. Leaf water potential measured at the end of the experiment (May) at 11 AM on samples of S. officinalis and S. officinalis cv. 'Maxima' irrigated at 100% (C) and 50% (MWD) of field capacity. Data are reported as mean ± SD (n=3).

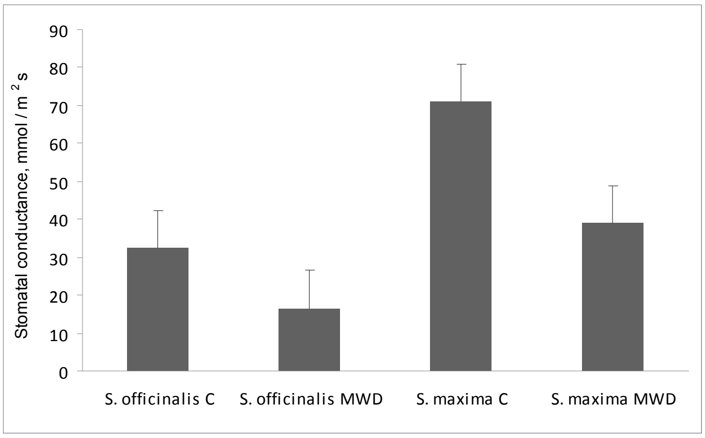

Figure 3: Leaf stomatal conductance measured at the end of the experiment (May) between 11 and 12 AM on samples of S. officinalis and S. officinalis cv. 'Maxima' irrigated at 100% (C) and 50% (MWD) of field capacity. Data are reported as mean ± SD (n=9).

The most negative Ψ_L was reached by *S. officinalis* var. 'Maxima', -0.923 MPa. The $\Delta\Psi_L$ between C and MWD plants belonging to the same taxa was slightly larger in *S. officinalis* var. 'Maxima' (0.115 MPa) than in *S. officinalis* (0.104 MPa). Measured values were in all cases above the level of turgor loss point reported in the literature for *S. officinalis* saplings, -1.26 MPa (Savi et al., 2017). Though leaf water potential values were similar in both taxa, gas exchange traits differed greatly, especially when comparing C treatments. *S. officinalis* var. 'Maxima' C showed the highest levels of stomatal conductance (Figure 3). When comparing the taxa, g_s values of *S. officinalis* var. 'Maxima' were twice those of *S. officinalis*, both under 100% and 50% irrigation. The reduced irrigation regime led to a 50% reduction in g_s in *S. officinalis* MDW, and a slightly smaller reduction in *S. officinalis* var. 'Maxima' MDW (45%), indicating stomatal closure as an early response to stress, which aided in maintaining relatively constant Ψ_L. A similar pattern was recorded for leaf transpiration (Figure 4), with *S. officinalis* var. 'Maxima' C showing transpiration rates 2.5 times greater than *S. officinalis* C. However, differences in E among *S. officinalis* C, *S. officinalis* MWD and *S. officinalis* var. 'Maxima' MWD were not significant. Stomatal conductance is often used as a proxy for photosynthetic capacity, based on the assumption that higher g_s allows a greater uptake of CO_2 into the leaves. In this study, however, higher g_s did not correspond with higher photosynthetic rates: in *S. officinalis* NP was almost twice that of *S. officinalis* var. 'Maxima' under both irrigation regimes (Figure 5).

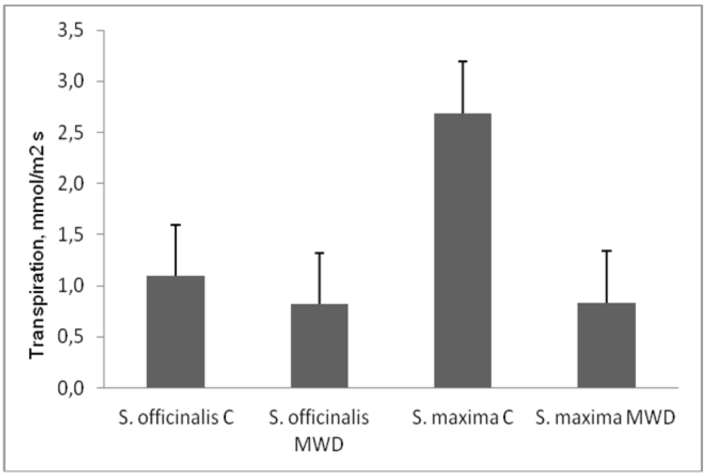

Figure 4: Leaf transpiration measured at the end of the experiment (May) between 11 and 12 AM on samples of S. officinalis and S. officinalis cv. 'Maxima' irrigated at 100% (C) and 50% (MWD) of field capacity. Data are reported as mean ± SD (n=9).

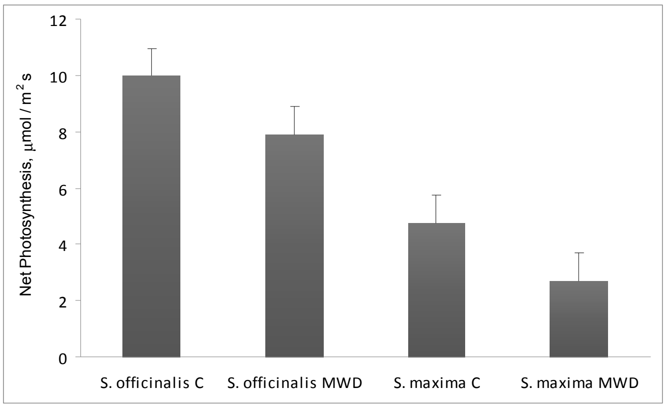

Figure 5: Net photosynthesis measured at the end of the experiment (May) between 11 and 12 AM on samples of S. officinalis and S. officinalis cv. 'Maxima' irrigated at 100% (C) and 50% (MWD) of field capacity. Data are reported as mean ± SD (n=9).

In both taxa the reduced irrigation regime led to a decrease in NP rates. This reduction was small but significant (-20%) in *S. officinalis* MWD compared to *S. officinalis* C, while it was quite more consistent in *S. officinalis* var. 'Maxima' MWD compared to *S. officinalis* var. 'Maxima' C (-40%).During the experiment, all plants were exposed to some degree of water deficit, as the irrigation schedule did not guarantee a constant replenishment of water lost by evapotranspiration especially in the month of May. However, the effects of the two different watering regimes were evident both from the morpho-physiological data and from the appearance of the whole plants (Figure 6), where leaves of the MWD plants were deeply wilted and folded, particularly in *S. officinalis* cv. 'Maxima'. Leaf wilting can act as a defence mechanism against excessive water loss and excessive radiation (Pérez-Estrada et al., 2000, Savi et al., 2016).

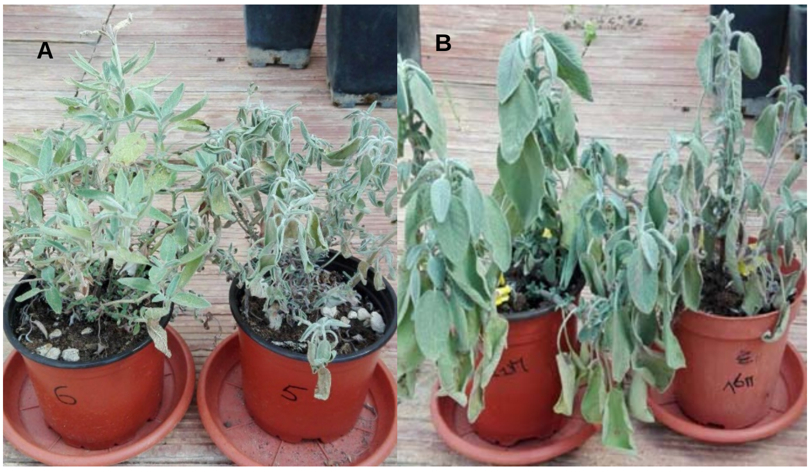

Figure 6: Appearance of representative samples after three months of irrigation supplied twice a week at 100% and 50% of field capacity. (A) S. officinalis, (B) S. officinalis cv. 'Maxima'. Pictures were taken immediately before one of the scheduled irrigations.

Analysis of essential oils showed the presence of about a hundred components, of which the most abundant are presented in Table 1. There were qualitative and quantitative differences between *S. officinalis* and *S. officinalis* cv. 'Maxima' under 100% irrigation regime. Mild water deficit did not affect the qualitative and quantitative composition of oils in *S. officinalis*, while in *S. officinalis* cv. 'Maxima' there were greater differences between the two treatments. Comparing *S. officinalis* and *S. officinalis* cv. 'Maxima' MDW, a similar qualitative composition was found, but noteworthy quantitative differences.

Table 1: Main essential oil composition of plants of S. officinalis and S. officinalis cv. 'Maxima' irrigated at 100% (C) and 50% (MWD) of field capacity.

RT	$RT_{corrected}$	KI	Compound	S. officinalis C Area Sum %	S. officinalis MWD Area Sum %	S. Maxima C Area Sum %	S. Maxima MWD Area Sum %
12.70	10.64	933	α-thujene	3.81	0.84	3.32	4.51
13.50	11.44	946	α-pinene	5.76	6.28	0.14	4.03
14.00	11.94	954	camphene	-	-	7.27	0.12
15.45	13.39	974	sabinene	2.65	1.58	0.02	8.19
18.80	16.74	1020	α-terpinene	0.54	0.97	9.14	0.68
19.20	17.14	1026	p-cymene	11.88	12.32	0.18	9.82
24.49	22.44	1097	linalool	3.23	3.98	0.03	7.41
25.29	23.24	1105	trans-sabinene hydrate	7.29	6.54	0.09	3.24
27.09	25.04	1121	cis-pinene hydrate	10.44	9.81	0.85	9.94
28.69	26.64	1134	camphor	8.61	8.33	0.31	3.43
45.39	43.33	1305	undecanal	4.04	4.63	0.74	1.73
51.19	49.13	1400	1,7-di-epi-a-cedrene	1.40	1.39	2.85	1.01
55.54	53.48	1477	γ-muurolene	2.22	2.35	0.28	8.42
58.29	56.23	1525	eugenyl acetate	2.81	3.52	0.32	-
78.93	76.87	1935	methyl hexadecanoate	5.10	6.74	-	1.90

4. Conclusions

Our results indicated that water deficit caused a significant reduction of plant growth. Both *Salvia officinalis* and *Salvia officinalis* cv. 'Maxima' are affected by drought stress, responding with a significant reduction in leaf expansion and in transpiration, in order to reduce water loss. However, *S. officinalis* exhibited a greater photosynthetic efficiency, maintaining higher levels of carbon assimilation also under mild water deficit. Essential oil composition differed between the two genotypes and was influenced by drought stress more in *Salvia officinalis* cv. 'Maxima' than in *Salvia officinalis*.

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