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## RESEARCH REPORT

# Effect of Planting Density and Harvest Date on Yield and Chemical Composition of Sage Oil

Roberta Piccaglia,\* Mauro Marotti and Vittorio Dellacecca

Dipartimento di Agronomia - Università di Bologna

Via Filippo Re, 6-8 - 40126 - Bologna, Italy

### Abstract

The yield and composition of sage (*Salvia officinalis* L.) oil cultivated in northern Italy and subjected to different growing conditions (planting densities and harvest managements) were evaluated. Sage harvested in spring at flowering stage (1st cut) gave the highest yields of fresh and dried matter. A harvesting regimen with three successive harvests produced the highest biomass yield. Twenty-nine compounds were identified in the oil. The oil of sage harvested at flowering stage greatly differed in composition from the oil of sage cut at vegetative stage. Very important differences were observed also between the oil composition of sage harvested at vegetative stage in spring and in the autumn. The highest content of thujones was found in the oils of sage harvested in autumn.

### Key Word Index

*Salvia officinalis*, Lamiaceae, growing conditions, oil yield, herbage yield, essential oil composition,  $\beta$ -pinene, 1,8-cineole,  $\alpha$ -thujone.

### Introduction

Sage (*Salvia officinalis* L.), which belongs to the Lamiaceae family, is a typical aromatic plant of the Mediterranean area and has been used since the ancient times for its numerous properties. It occurs wild or can be cultivated and is generally harvested at full flowering stage to isolate its oil (*Aetheroleum salviae*) and at the vegetative stage to obtain dried herb (*Salviae folium*) (1).

Sage is mainly used in the food industry as a flavoring and preservative, while the oil is sometimes used in the manufacture of perfumes and cosmetics. In folk medicine it has been known for its antispasmodic, astringent and tonic effects and is an authorized drug in most pharmacopoeias.

More recently, it has been reported that sage oil and particularly some of its main components such as thujone, camphor and 1,8-cineole, possess antimicrobial, antioxidant and possible anticancer properties (2-4).

The biological activity and the uses of the oil depend on its chemical composition which is pre-determined by the genotype, and greatly affected by environmental factors and agronomic techniques (5,6).

\*Address for correspondence

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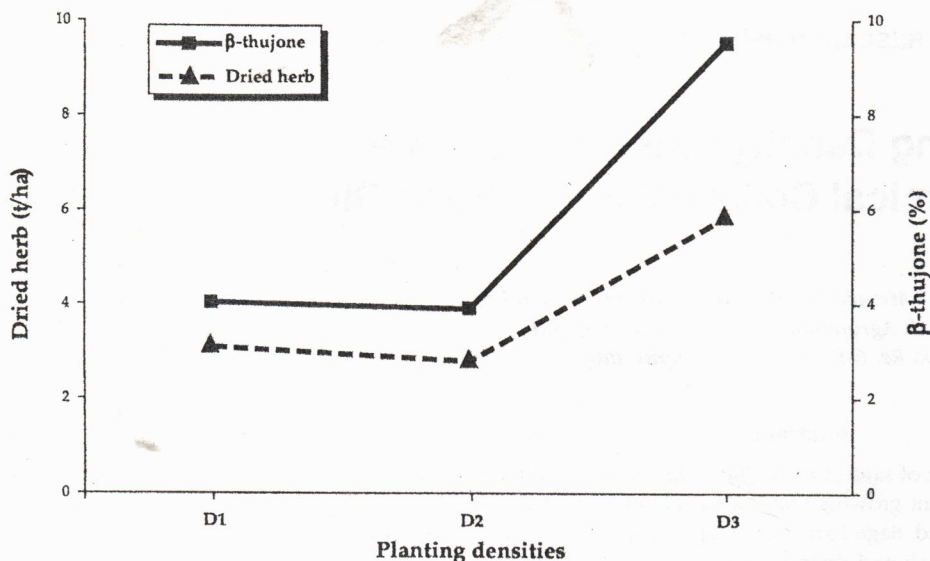


Figure 1. Effects of planting densities on dried herbage yield (means of autumn vegetative harvests) and  $\beta$ -thujone content (springtime vegetative harvest)

The aim of this work was to study the yield and composition of the oil of sage which has been subjected to different growing conditions and harvesting regimens.

### Experimental

**Plant Material:** The sage plants were grown from seed in nursery bed and transplanted in the experimental field in spring 1993. Three planting densities 4(D1), 6(D2), 8(D3) plants/m<sup>2</sup> having plants in rows 80, 60, 40 cm apart respectively were adopted. The sage was cut in 1994 using two harvest managements: A) three successive cuts: April 23 at vegetative stage (A1), June 9 at flowering stage (A2) and October 26 at vegetative stage (A3); B) two successive cuts: May 26 at flowering stage (B1) and October 26 at vegetative stage (B2). At the vegetative stage the tops of the plants were harvested, whereas at the flowering stage the whole plant was harvested. The trial was performed in northern Italy (Montombraro, Modena) at 630 m above sea level on a lay-like and muddy soil. The experimental design was a split-plot with three replications.

**Oil Isolation:** Fresh plant material (3 kg) was hydrodistilled in a Clevenger-type apparatus for 1 h. The oil was dried over anhydrous sodium sulfate and stored in a dark glass bottle at 4°C until analysis.

**GC and GC/MS Analysis:** An oil solution (1.0%) in ethanol was injected into a Carlo Erba HRGC 5160 Mega gas chromatograph equipped with a FID detector and an Hitachi-2000 integrator adopting the following conditions: injection system on column; carrier gas helium at flow rate of 1 mL/min; detector temperature 250°C column SPB-5 (Supelco) 30 m x 0.32 mm, film thickness 0.25  $\mu$ m; oven temperature from 70°-150°C at 5°C/min, holding the initial temperature for 18 min and the final one for 8 min, then from 150°C to 200°C at 50°C/min holding the final temperature for 10 min.

Mass spectral analyses were run on a Finnigan Mat Ion Trap detector model 800 set at 70 eV and equipped with a software version 3.0. Quantitative data were obtained using ethylene glycol monobutyl ether as internal standard. Each analytical result was the average of two GC analyses.

### Results and Discussion

**Effects of Planting Densities:** The planting densities did not show considerable effects on yield and oil composition. Only two significant effects determined by the highest density (D3) were observed

Table I. Sage yield (t/ha) and oil content (percentage)<sup>a</sup>

Material	Harvest regimen						
	A1	A2	A3	Total or	B1	B2	Total or
	April 23 Vegetative	June 9 Flowering	October 26 Vegetative	mean of A	May 26 Flowering	October 26 Vegetative	mean of B
Fresh	11.2	12.6	10.8	34.6**	20.5**	10.9	31.4
Dried	2.3	3.1	3.9	9.3**	4.6**	3.9	8.4
Oil content	0.1	0.1	0.1	0.1	0.1	0.2	0.2

<sup>a</sup>Means of three planting densities; oil content on fresh matter basis

\*\*Significant at  $P \leq 0.01$  for B1 vs A1 and A2 and total yield of A vs total yield of B

(Figure 1). This density affected the mean yield of dried herbage from the two harvests performed in October at the vegetative stage (A3 and B2) which resulted in higher yields than those obtained at the other crop densities (5.8-2.8 and 3.1 t/ha, respectively) over the same time period. No significant effect was observed at this density, on the yields from earlier harvests of sage both at vegetative and flowering stage. Also the oil content was not significantly affected by planting densities.

With respect to the oil composition, only the  $\beta$ -thujone content of sage harvested in spring at the vegetative stage (A1) was found significantly higher at the highest planting density compared with those of vegetative sage grown at lower densities (9.52 - 3.89 and 4.02%, respectively). Probably at the highest density, the availability of light could have been reduced resulting in an accumulation of the ketone, the production of which seemed to be favored by cold temperature and poor light conditions (6).

**Effects of Harvest Managements:** The effects of harvest managements on yields and oil content are reported in Table I. The highest yield of fresh and dried material (20.5 and 4.6 t/ha, respectively) was obtained at the 1st harvest at the flowering stage.

The sage yield of the two harvests of October (A3 and B2) was very similar for fresh and dried matter values (10.8 - 3.9 and 10.9 - 3.9 t/ha, respectively) in spite of the fact that one was obtained from a third cut and the other from a second cut. The harvest management A on the whole, resulted in fresh and dried material yields significantly higher than those of the B one (34.6, 9.3 and 31.4, 8.4 t/ha respectively). The oil yield was not affected by the harvesting regimen.

The oil composition of sage from different harvests is reported in Table II along with the quantitative data. Twenty-nine compounds were identified in the oil which was characterized by a predominance of monoterpenes the total amount of which ranged from 76% to 89%.

Important variations in quantitative composition were observed in the oils obtained from sage harvested in different seasons and at different development stages. In fact the comparison between sage tops of April (A1) and those of October (A3 and B2) revealed a significant decrease of sesquiterpenes and an increase of 1,8-cineole, thujones and camphor. These findings, which are in good agreement with those reported by other authors (7,8), could be a further indication of the possible formation of ketones from sesquiterpenes during plant development. However, these changes could equally be occurring simultaneously and be completely unrelated. The shorter length of day-light and colder temperature typical of the autumnal period could also have contributed to stimulate thujones and camphor accumulation as previously reported (7).

The oils obtained from flowering sage both of May (B1) and June (A2) were characterized by high contents of monoterpene hydrocarbons and 1,8-cineole, and by relatively low amounts of  $\alpha$ - and  $\beta$ -thujone. This composition greatly differed from those of sage harvested at vegetative stage both in April (A1) and October (A3 and B2).

All the oils obtained from the different harvesting regimens can be considered to be of commercial interest (9) because of their high  $\alpha$ - and  $\beta$ -thujone content (>30%) and low camphor amount (<20%)

Table II. Percentage composition of sage oil<sup>a</sup>

Material	Harvest regimen						
	A1	A2	A3	B1	B2	A1 vs A2	A1 vs A3
	April 23 Vegetative	June 9 Flowering	October 26 Vegetative	May 26 Flowering	October 26 Vegetative	and vs B1	and vs B2
cis-salvene	0.14	0.34	0.41	0.36	0.34	-	-
trans-salvene	0.02	0.09	0.11	0.11	0.08	-	-
α-thujene	0.10	0.27	0.63	0.27	0.16	-	-
α-pinene	0.56	3.21	2.92	2.41	2.07	**	**
camphene	0.63	1.72	2.79	1.41	2.15	**	**
sabinene	0.22	0.26	0.30	0.32	0.22	-	-
β-pinene	2.82	10.62	2.38	12.87	2.06	**	n.s.
myrcene	0.78	0.83	1.14	0.72	0.98	-	-
α-terpinene	0.11	0.23	0.17	0.24	0.12	-	-
p-cymene	0.22	0.23	0.42	0.27	0.48	-	-
limonene	0.59	0.91	1.18	0.61	0.96	-	-
1,8-cineole	4.93	16.59	9.15	17.74	9.14	**	**
(Z)-β-ocimene	0.84	0.95	0.13	1.05	0.12	-	-
(E)-β-ocimene	0.15	0.24	0.02	0.22	0.02	-	-
γ-terpinene	0.44	0.43	0.38	0.45	0.31	-	-
terpinolene	0.11	0.27	0.23	0.16	0.17	-	-
linalool	0.18	0.35	0.21	0.25	0.16	-	-
α-thujone	29.66	24.55	44.71	25.86	48.93	n.s.	**
β-thujone	5.81	5.18	9.27	5.32	8.86	n.s.	**
camphor	3.40	3.62	8.97	1.75	10.09	***	**
borneol	1.31	4.04	1.14	2.98	1.09	**	n.s.
terpinen-4-ol	0.33	0.41	0.37	0.41	0.39	-	-
bornyl acetate	0.25	0.64	0.27	0.16	0.20	-	-
β-caryophyllene	8.96	6.77	1.66	7.19	1.82	n.s.	**
aromadendrene	0.33	0.15	0.08	0.31	0.07	-	-
α-humulene	13.97	6.54	2.78	5.95	2.65	**	**
allo-aromadendrene	0.33	0.11	0.04	0.13	0.04	-	-
caryophyllene oxide	0.69	0.28	0.27	0.32	0.19	-	-
viridiflorol	11.88	3.04	1.96	4.63	1.79	**	**

<sup>a</sup>Means of three planting densities; \*\*Significant at  $P \leq 0.01$ ; n.s.= not significant; - = not processed; \*\*\*Significant only vs B1

and, in particular, the oils from autumn harvested sage which possessed a thujone content of 54-58%. It is possible that further studies for subsequent years could reveal some environmental effects on oil yield and composition.

### Note Added by Editor

The results obtained are based on the evaluation of herbage and oil yield and compositional changes of sage grown at different planting densities and harvesting regimens of the first year of crop growth.

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