Dynamics of Yield Components and Essential Oil Production in a Commercial Hybrid Sage (*Salvia officinalis* × *Salvia fruticosa* cv. *Newe Ya'ar No. 4*)

Nativ Dudai,* Efraim Lewinsohn, Olga Larkov, Irena Katzir, Uzi Ravid, David Chaimovitsh, Diah Sa'adi, and Eli Putievsky

Aromatic, Medicinal and Spice Crops, Agricultural Research Organization, Newe Ya'ar Research Center, P.O. Box 1021, Ramat Yishay 30095, Israel

The fresh yields, the essential oil content, and the quality of a sage hybrid (*Salvia officinalis* × *Salvia fruticosa*, cv. *Newe Ya'ar No. 4*, Lamiaceae) as affected by development and harvest time were determined. Marked increases in plant height and in the number of nodes developed per plant together with a modest increase in leaf size were accompanied by dramatic increases (more than 20-fold) in the fresh yields throughout a 50-day growth period. No major changes in the essential oil content per fresh weight and its composition were detected throughout the growth period. In contrast, the compositions of the essential oils obtained from stems, as compared to leaves and leaf-primordia, had marked differences. Developmentally controlled changes in the extractives from individual leaf pairs from the same plant were also noted. In upper young leaves, the oxygenated diterpene manool and the sesquiterpene hydrocarbons α-humulene and *β*-caryophyllene constituted up to 20%, 8%, and 4% of the total extractives, respectively. In older leaves, the abundance of these components steadily dropped to roughly half their levels in young leaves. Conversely, the proportions of the monoterpenes, particularly the ketones camphor and α-thujone, steadily increased with leaf position. Minor changes in the levels of other extractives were also recorded. These studies imply independent regulatory patterns for di-, sesqui-, and monoterpenes in this sage hybrid, and suggest possible agrotechnical means to obtain preferred chemical compositions of its essential oil.

Keywords: *Salvia officinalis* × *fruticosa; Lamiaceae; monoterpenes; sesquiterpenes; manool; yield components; essential oils; fresh herbs*

INTRODUCTION

Sage (*Salvia officinalis* L.) is a common aromatic herb, worldwide used for culinary purposes, either fresh or dry, used also as a medicinal plant and as a source of essential oil (Boelens, 1997). The genus *Salvia* (Lamiaceae) includes more than 700 species, many of them collected from the wild and a few of them cultivated (Chalchat et al., 1998; Rivera et al., 1994; Tucker et al., 1995). *Salvia* spp. are either herbaceous or shrubby perennials and are also used as ornamentals and garden plants (Clebsch, 1997).

The essential oils and the aroma of *S. officinalis* are usually associated with high α- and *β*-thujone content (50−70%), 1,8-cineole (10−20%), and low camphor (less than 10%) levels (Boelens, 1997; Chalchat et al., 1998; Pino et al., 1997; Putievsky et al., 1986b). Most of the commercial high-quality dried sage imported into North America originates from the Dalmatian Coast of the former Yugoslavia. This material not only consists of dried *S. officinalis* gathered from the wild, but it is often mixed with dried three-lobed sage (*Salvia fruticosa*, formerly *Salvia triloba*) (Putievsky et al., 1986b). The essential oil of three-lobed sage normally has low α- and *β*-thujone levels (less than 5%), and the main components are 1,8-cineole (40−50%), camphor, (∼10%), and α- and *β*-pinene (∼10−20%) (Putievsky et al., 1986a; Putievsky and Ravid, 1987; Länger et al., 1996; Lawrence, 1988, 1998). The essential oil of *S. officinalis* inhibits fungal growth, an activity associated with its camphor content, but other components also possess medicinal, insecticidal, and fungicidal activity (Carta et al., 1996; Shimoni et al., 1993). The commercial cultivation of fresh market-quality *S. officinalis* in Israel is difficult, due to its slow growth rate, especially during the winter months (December to April), when European demand for Israeli fresh herbs is maximal. Conversely, *S. fruticosa* is an endemic species in Israel and is well suitable for cultivation in greenhouses in winter and early spring, and can also be grown outdoors (Putievsky et al., 1986a; Putievsky and Ravid, 1987). Since the morphology and flavor of *S. fruticosa* are markedly different from those of *S. officinalis*, we initiated a breeding program aimed at producing hybrid sage cultivars, suitable for Israeli intensive agriculture conditions, but resembling *S. officinalis* in morphology and flavor. During the past decade, we have developed a unique sage hybrid that is commercially grown in Israel. The hybrid was obtained by crossing high-quality *S. officinalis* clones collected from the Dalmatian Coast with a *S. fruticosa* selection from Mount Carmel (Israel) (Putievsky et al., 1990b). Followed by a selection program based on agronomical performance and quality of the essential oil, we developed a hybrid that has intermediate morphology, but its essential oil resembles that of *S. officinalis*. This hybrid was given the formal

* To whom correspondence should be addressed. Phone: 972-4-953-9510. Fax: 972-4-983-6936. E-mail: tavlinim@netvision.net.il.

10.1021/jf9901587 CCC: $18.00
Published on Web 09/30/1999

cultivar name of *Newe-Ya'ar No. 4*. This cultivar can be grown in greenhouses and produces more than 3 kg/m^2 high-quality fresh yield during the winter, while other sage selections cultivated under similar conditions typically produce less than 1 kg/m^2. *Newe-Ya'ar No. 4* sage has become the leading sage cultivar exported in winter from Israel to the European Union.

MATERIALS AND METHODS

Plant Material. Rooted cuttings of *Newe Ya'ar No. 4* sage were planted on June 1, 1997, at the Newe Ya'ar Research Center in Israel. They were grown in a medium that consisted of volcanic tuff, placed in 1 m × 1.2 m × 0.24 m containers, in a plastic I.R type (Plastofil, Genigar, Israel) tube house (9.6 m wide, 34 m length, and 3.5 m height at the highest place in the center), drop irrigated, and fertilized through the irrigation system with 0.2% N/P/K (5:3:8) twice a day. For each treatment, 24 plants were grown in 4 rows, established in each container (20 cm distance from each plant). In preparation for the experiments, and to ensure uniformity, plants were harvested twice—in October 1996 and the beginning of July 1997. Starting late July 1997, two containers were randomly harvested (25 cm above the ground) to allow for the different harvest times. On Oct 11, 1997, all plants were harvested. The plant material from each harvest interval was mixed and then divided into four samples, each examined for morphological characters, and analyzed for essential oil content as described below. Plants did not flower throughout the experiments.

Preparation of the Dry Herb. Samples consisting of shoots and leaves, about 500 g, were dried in a steady oven for 48 h at 40 °C. Leaves were manually separated from the shoots and weighed. The dry herb consisted of dry leaf material (Putievsky et al., 1986a,b, 1990b).

Essential Oil Determination. *Hydrodistillation*. Samples of at least 250 g of fresh plant material were hydrodistilled for 1.5 h in a modified Clevenger apparatus. The essential oil was cooled, separated from the cohobated water (Dudai et al., 1992), pooled, and analyzed by GC−MS (see below).

Extraction with Methyl tert-Butyl Ether (MTBE). We have previously demonstrated the usefulness of extraction of mono- and diterpenes with MTBE (Lewinsohn et al., 1993). Individual leaf pairs (about 1 gr FW) from the same branches were extracted with 10 mL of MTBE for 2 h with gentle shaking at room temperature. The extract was cleaned by passing it through a small (Pasteur pipet) column containing anhydrous Na_2SO_4 and silicic acid (Silicagel 60, 230−400 mesh, Merck), to dry the sample and remove high molecular weight polar substances that interfere with the GC analyses.

GC−MS Analyses. Samples consisting of 1.0 μL of diluted essential oils (1:10000 in hexane) or undiluted MTBE extracts were analyzed on a HP-GCD apparatus equipped with a HP5 (30 m × 0.25 mm) fused-silica capillary column. Helium (1 mL/min) was used as a carrier gas. The injector temperature was 270 °C. Oven conditions: 70 °C for 2 min, from 70 to 200 °C at a rate of 4 °C/min, and kept at 200 °C for 10 min. Identification of the main components was done by co-injection of authentic standards and comparison of the EI-MS obtained from authentic standards and complemented with computerized libraries.

RESULTS AND DISCUSSION

The fresh yields, quantity, and quality of the essential oil in *Newe Ya'ar No. 4* sage (*Salvia officinalis* × *fruticosa* cv. *Newe Ya'ar No. 4*) as affected by harvest time and development are shown in Tables 1 and 2. Total fresh yields steadily increased and reached a maximum of 3.2 kg/m^2 when plants were harvested 73 days after the previous harvest (Table 1). These increases in yields were accompanied by increases in plant height (almost 3-fold) and the number of nodes per branch (2-fold) and a modest increase in leaf size (Table 2). In general, the ratio leaf/total yield remained steady during the same period (about 60%). These observations indicate that the increases in yields are due to actual addition of leaves, and not solely to node elongation. The yield reaches a plateau around 73 days and then diminishes, when yield increases due to continued growth are offset by leaf senescence and drop.

Table 1. Yields and Yield Components of *Newe Ya'ar* Sage (*S. officinalis* × *fruticosa* cv. *Newe Ya'ar No. 4*) Harvested at Different Time Intervals[a]

harvest time from previous harvest (days)	fresh yield (kg/m^2)	total dry herb yield (g/m^2)	essential oil	
			% in fresh matter	yield mL/m^2
24	0.14 ± 0.03	16.8 ± 3.8	0.33 ± 0.01	0.5 ± 0.1
31	0.64 ± 0.21	70.4 ± 23.1	0.40 ± 0.03	2.6 ± 1.0
38	1.31 ± 0.09	142.0 ±8.6	0.41 ± 0.03	5.3 ± 0.1
45	1.54 ± 0.11	191.0 ± 12.4	0.42 ± 0.02	6.4 ± 0.2
52	1.68 ± 0.03	206.6 ± 2.4	0.45 ± 0.05	7.6 ± 0.9
59	2.30 ± 0.35	276.4 ± 42.0	0.47 ± 0.04	10.6 ± 0.8
66	2.67 ± 0.15	326.1 ± 17.8	0.49 ± 0.01	13.0 ± 0.8
73	3.19 ± 0.01	379.6 ± 1.1	0.43 ± 0.03	13.7 ± 1.0

[a] Means and standard errors of four determinations are given.

Table 2. Morphological Characteristics of *Newe Ya'ar* Sage (*S. officinalis* × *fruticosa* cv. *Newe Ya'ar No. 4*) Harvested at Different Time Intervals[a]

harvest time from previous harvest (days)	plant height (cm)	maximal leaf size		no. of nodes per branch
		length (mm)	width (mm)	
24	29 ± 1	70 ± 5	32 ± 2	5.3 ± 0.8
31	38 ± 3	72 ± 1	28 ± 1	5.5 ± 0.6
38	51 ± 1	78 ± 5	30 ± 2	7.3 ± 1.0
45	55 ± 1	88 ± 7	26 ± 2	8.8 ± 0.3
52	66 ± 9	89 ± 4	29 ± 1	9.3 ± 0.3
59	75 ± 3	99 ± 4	25 ± 2	9.5 ± 0.6
66	83 ± 2	95 ± 9	28 ± 2	10.3 ± 0.5
73	84 ± 2	92 ± 3	30 ± 2	10.3 ± 0.5

[a] Means and standard errors of four determinations are given.

The dry herb yields obtained were roughly 12 ± 1% of the total fresh weight (Table 1), and about 60% of the total dry matter (not shown). Dry herb yields increased steadily during the growth period, reaching 38 g/m^2. However, in the longest harvest interval, clear signs of leaf senescence were noted, and leaves turned yellow and dropped.

During the first 66 days after harvest, the essential oil content per fresh weight steadily increased from 0.33% in total fresh matter, up to 0.49% (Table 1). This was followed by a moderate decrease in the essential oil content when plants were harvested at day 73 (Table 1), and is consistent with other observations in *Salvia* and *Mentha* spp. (Piccaglia et al., 1997; Putievsky et al., 1986b, 1988, 1992; Tucker 1995). The high (27-fold) increases in the essential oil yields recorded (Table 1) are caused by both increases in plant yields and increases in essential oil content per fresh weight. It is possible that some of the essential oil is lost due to leaf damage, senescence, and loss, or it is catabolized (Funk et al., 1992; Burbott and Loomis, 1969). However, it seems that monoterpene turnover is minimal under normal physiological conditions in *S. officinalis* and in other terpene-rich plants (Gershenzon et al., 1993).

Newe Ya'ar No. 4 sage is an artificial hybrid between a *S. fruticosa* accession first collected in Mount Carmel, Israel (male parent), and a female *S. officinalis* that originated in the Dalmatian Coast of Croatia (accession no. 32/6). The *S. fruticosa* accession from Mount Carmel

Table 3. Content and Composition of the Essential Oils Obtained by Hydrodistillation from Different Tissues of *Newe Ya'a* Sage (*S. officinalis* × *fruticosa* cv. *Newe Ya'ar No. 4*)

component (% in the essential oil)	stem	leaves (mature)	leaves (young)	leaf primordia in main branch	leaf primordia in secondary branches	upper shoots	lower shoots
tricyclene		0.12	0.07			0.09	
α-thujene		0.32	0.21	0.12		0.28	0.24
α-pinene	0.37	2.77	2.13	1.53	0.28	2.41	1.93
camphene	0.76	5.53	3.80	2.73	0.64	4.90	3.92
sabinene	0.48	0.35	0.43	0.47	0.09	0.40	0.32
β-pinene	0.56	2.43	2.43	2.40	0.67	2.31	1.96
myrcene	0.36	2.87	2.73	2.02	0.73	2.38	2.29
α-terpinene	0.10	0.25	0.13	0.09		0.24	0.24
limonene	0.38	1.76	0.98	0.82	0.60	1.38	1.42
1,8-cineole	4.40	13.67	17.15	15.52	10.92	13.73	12.46
γ-terpinene	0.39	0.52	0.33	0.25	0.11	1.38	0.55
terpinolene	0.05	0.63	0.39	0.31	0.09	0.53	0.42
α-thujone	34.75	22.20	20.90	21.19	31.29	24.05	25.55
β-thujone	7.97	5.04	4.59	5.05	5.34	5.50	6.03
camphor	13.80	28.19	19.89	17.35	22.16	25.50	24.30
borneol	1.19	1.22	1.71	1.95	0.77	1.35	1.85
terpinen-4-ol	0.81	0.48	0.24	0.23	0.06	0.51	0.69
α-terpineol		0.18	0.30	0.31		0.16	0.17
bornyl acetate	3.86	1.55	1.76	1.97	2.42	0.56	1.06
β-caryophyllene	2.13	2.43	4.24	5.44	0.40	2.39	2.01
α-humulene	5.19	3.17	4.96	6.59	6.34	3.42	3.86
viridiflorol	6.53	2.11	5.68	7.50	6.37	2.96	3.4
manool	9.87	1.12	3.24	4.51	5.70	2.07	3.61
essential oil content (% FW)	0.07	0.53	1.25	1.20	0.93	0.45	0.24

contains more than 50% 1,8-cineole, with lower levels of camphor (13%), α-pinene (9.5%), and other mono- and sesquiterpenes (Putievsky et al., 1986a, 1990b). Conversely, the female parent (*S. officinalis* 32/6) contains high levels of α-thujone (60%), 1,8-cineole (13.5%), β-thujone (9.5%), and no camphor (Putievsky et al., 1986a, 1990b). *Newe Ya'ar No. 4* sage has an essential oil that contains components of both parents: α-thujone (28%), camphor (23%), 1,8-cineole (14%), and lower levels of α- and β- pinene, limonene, and other mono- and sesquiterpenes (Putievsky et al., 1986a, 1990b). A comparison of the essential oils obtained by extraction and steam distillation of *S. officinalis* and *S. fruticosa* was carried by Länger et al. (1996). They found that the essential oil of *S. fruticosa* is dominated by 1,8-cineole (60−80% of steam-distilled oil), while *S. officinalis* is characterized by a summation of α-thujone, β-thujone, and camphor as a constant parameter (58−81% of the steam-distilled oil). Manool, usually found in small quantities (0.1−5.9%) in *S. officinalis* essential oils (Lawrence, 1988, 1998) was recently found in relatively high amount (14.7%) in *S. officinalis* originating from Cuba (Pino et al., 1997). The diterpene manool, similarly to sclareol (found in *S. sclarea* and in other plants), serves as a chemical precursor in the manufacture of Ambrox, a compound found in ambergris (formerly extracted from the alimentary tract of sperm whales and of commercial importance in the tobacco and perfume industries (Ohloff, 1982).

The compositions of the essential oil of *Newe Ya'ar No. 4* sage plants harvested at different harvest regimes were not significantly different (not shown). Moreover, only minor differences were found in the composition of the essential oil obtained from the top parts of the shoots (including leaves) as compared with the bottom parts (Table 3). Intensive agricultural practices require that plants are harvested at a minimal height to increase yields. Nevertheless, sufficient photosynthetic tissue should remain in the plants after harvest to allow for a fast recovery and vigorous growth toward subsequent harvests (Putievsky et al., 1986b, 1990a, 1992). It is therefore important to determine whether the quality of the top parts of the crop is similar to that of the bottom parts, to allow for higher harvesting practices, if needed. We thus compared the essential oil obtained from the upper parts (shoots and leaves) of *Newe Ya'ar No. 4* sage to that obtained from the bottom parts and found minimal differences in the essential oil composition (Table 3). Furthermore, the essential oil content is slightly higher in the top tissues as compared with the bottom parts, ensuring a high-quality product, in both the top and bottom parts. In any case, these properties of *Newe Ya'ar No. 4* sage allow for a relatively high harvest, ensuring a fast recovery, and shortening the time till the next harvest. This pattern is similar to the one found for other aromatic plants grown under cultivation, including *S. officinalis* (Putievsky et al., 1986b), sweet basil (Werker et al., 1985), oregano (Putievsky et al., 1988), and geranium (Putievsky et al., 1990a). Interestingly, the leaf weight increased rapidly and linearly with development, followed by a slight decrease in the three older leaf pairs (not shown).

The essential oil in sage, similarly to other Lamiaceae, is accumulated in glandular trichomes located on the surface of the leaves (Croteau et al., 1981). In *Salvia* spp., glandular trichomes are normally present in both leaves and stems (Croteau et al., 1981; Werker et al., 1985). Different plant tissues, including stems, branches, and young and old leaves, were separately analyzed to determine similarities and differences in the composition of the essential oil derived from each tissue. The results are shown in Table 3.

The essential oil of mature leaves is mainly comprised of monoterpenes constituting up to 90% of the essential oil. The major components are the monoterpene ketones camphor, accounting for 28% of the essential oil, and α-thujone, accounting for 22%. The monoterpene ether 1,8-cineole is also a major component (14%). Other components that range from 1% to 5% of the oil were also detected, including oxygenated and unsaturated mono- and sesquiterpenes and manool, a diterpene (Figure 1). Sesqui- and diterpenes constitute less than 10% of the essential oil. Low levels of bornyl acetate (0.5−4%) were also noted (Table 3).

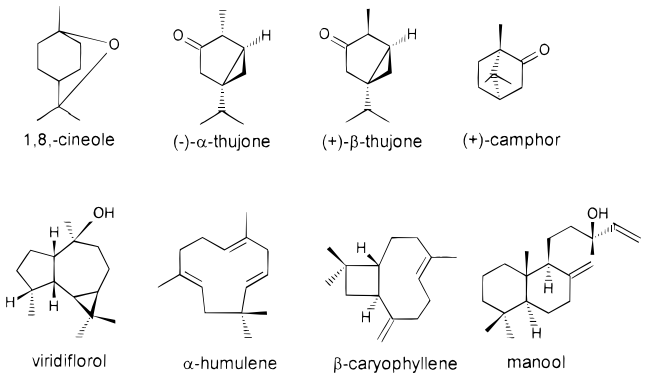

Figure 1. Principal mono-, sesqui-, and diterpene components found in the essential oil and MTBE extracts of *Newe Ya'ar No. 4* sage.

The composition of the essential oils obtained from senescent and mechanically damaged leaves present in the lower parts of the plants was similar to that of intact leaves (not shown), indicating that the presence of damaged and senescent leaves after harvest does not significantly affect the essential oil quality.

In the essential oil obtained from leaf primordia and young (up to 31 × 12 mm) leaves, the major component was α-thujone (21%, Table 3). Lower levels of camphor (17−20%) were found in young as compared to mature leaves (28%). 1,8-Cineole was also prominent (15−17%) in young leaves. Interestingly, the levels of the sesquiterpenes viridiflorol, α-humulene, and *β*-caryophyllene and the diterpene manool were substantially higher than those levels found in mature leaves, and accounted for up to 20% of the essential oil.

In stems, α-thujone was most prominent, and its level was almost 35%, camphor was only 14%, and the levels of the sesquiterpenes reached up to 24%. The essential oil derived from leaf primordia of the secondary branches differed somewhat from that derived from leaf primordia on primary branches (Table 3). The reason for these differences is unknown.

Concluding, the essential oil found in different tissues of *Newe Ya'ar No. 4* sage varies in its composition; therefore, one can direct the production of certain specialty essential oil compositions by carefully choosing the tissues from which it is prepared.

To further refine and better study the above findings, MTBE extracts of individual leaf pairs throughout single *Newe Ya'ar No. 4* sage branches were prepared. The results are described in Figure 2. The major component of the extracts derived from the first leaf pair (top) contained 23% of the diterpene manool. This compound was detected in the essential oils at much lower levels (1−3%, Table 3) probably due to its lower volatility, as compared to other essential oil components. Manool levels strongly decreased with development, accounting for only 10−13% in older leaves (Figure 2). Similarly, the levels of the sesquiterpenes α-humulene, *β*-caryophyllene, and viridiflorol steadily decreased with leaf age from 4−8% initially to 2−3% in older leaves (Figure 2). Conversely, the levels of the monoterpene ketones camphor and α-thujone steadily increased with leaf age, from 12% in the first leaf pair, up to 17−20% in older leaves (Figure 2). In fact, lower abundance monoterpenes, such as camphene, *β*-thujone, myrcene, borneol, and limonene, had increased percentages in older leaves, while the relative levels of other monoterpenes such as 1,8-cineole did not significantly change

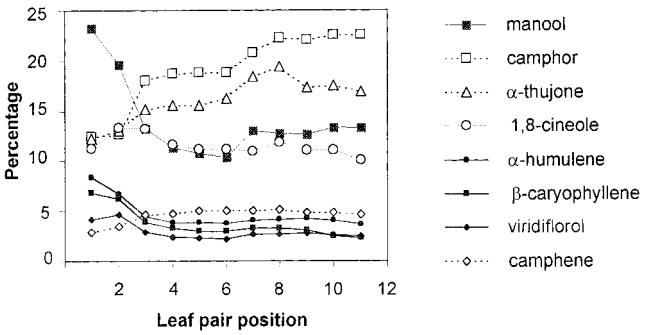

Figure 2. Effect of leaf position on the composition of the major essential oil components in *S. officinalis* × *fruticosa* cv. *Newe Ya'ar No.* 4. MTBE extracts derived from separate leaf pairs from an individual branch were obtained as described in the Materials and Methods. Similar results were obtained from other branches. Open symbols and broken lines represent monoterpene components; closed symbols and solid lines represent di- and sesquiterpenes.

with development (Figure 2). Differences in the essential oil composition of plant tissues at different developmental stages are well-known (Werker et al., 1985, 1993). Moreover, differences in the composition of the essential oil present in individual glandular trichomes in *Mentha* have also been reported (Voirin and Bayet, 1996). The biogenesis of the diastereoisomeric thujones in *Salvia* has been investigated and reviewed (Croteau, 1992).

The biosynthetic pathways to camphor and other monoterpenes found in sage have been investigated to a great extent (Croteau et al., 1981, 1987). The camphor content in *S. officinalis* leaves correlated with glandular trichome density, and based on $^{14}CO_2$ incorporation studies, and in the measurement of key rate-limiting step biosynthetic enzymes, it was concluded that camphor is synthesized and accumulated most rapidly in young expanding *S. officinalis* leaves (Croteau et al., 1981).

In conclusion, the stage of plant harvest and the tissue used might affect the quality and quantity of the essential oil obtained. The above observations are of great importance for growers and breeders aimed at producing high-quality sage for the fresh and dry markets, and also as a source of essential oils.

ACKNOWLEDGMENT

We thank Izhak Michaelovich for growing the plants and technical help. Contribution No. 108/99 from the Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel.

LITERATURE CITED

Boelens, M. H. Chemical and sensory evaluation of three sage oils. *Perfum. Flavor.* **1997**, *22*, 19−40.

Burbott, A. J.; Loomis. Evidence for metabolic turnover of monoterpenes in peppermint. *Plant Physiol.* **1969**, *44*, 173−179.

Carta,-C.; Moretti,-M. D. L.; Peana,-A. T. Activity of the oil of *Salvia officinalis* L. against *Botrytis cinerea*. *J. Essent. Oil Res.* **1996**, *8*, 399−404.

Chalchat, J. C.; Michet, A.; Pasquier, B. Study of clones of *Salvia officinalis* L. Yields and chemical composition of essential oil. *Flavour Fragrance J.* **1998**, *13*, 68−70.

Clebsch, B. *A book of salvias:sages for every garden*; Timber Press: Portland, OR, 1997; 221 p.

Croteau, R. Biosynthesis and catabolism of monoterpenoids. *Chem. Rev.* **1987**, *87*, 929−954.

Croteau, R. The biosynthesis of thujane monoterpenes. In *Recent Advances in Flavor and Fragrance Chemistry*; Hopp, R., Mori, K., Eds.; VCH: Weinheim, Germany, 1992; pp 263−273.

Croteau, R.; Felton, M.; Karp, F.; Kjonaas, R. Relationship of camphor biosynthesis to leaf development in sage (*Salvia officinalis*). *Plant Physiol.* **1981**, *67*, 820−824.

Dudai, N.; Putievsky, E.; Ravid, U.; Palevitch, D.; Halevy, A. H. Monoterpene content in *Origanum syriacum* L. as affected by environmental conditions and flowering. *Physiol. Plant* **1992**, *84*, 453−459.

Funk, C.; Koepp, A. E.; Croteau, R. Catabolism of camphor in tissue cultures and leaf disks of common sage (*Salvia officinalis*). *Arch. Biochem. Biophys.* **1992**, *294*, 306−313.

Gershenzon, J.; Murtagh, G. J.; Croteau, R. Absence of rapid terpene turnover in several diverse species of terpene-accumulating plants. *Oecologia* **1993**, *96*, 583−592.

Länger, R.; Mechtler, C.; Jurenitseh, I. Composition of the essential oils of commercial samples of *Salvia officinalis* L. and *S. fruticosa* Miller: a comparison of the oils obtained by extraction and steam distillation. *Phytochem. Anal.* **1996**, *7*, 289−293.

Lawrence, B. M. Sage oil. *Perfum. Flavor.* **1988**, *13* (3), 53−56.

Lawrence, B. M. Sage oil. *Perfum. Flavor.* **1998**, *23* (2), 47−52.

Lewinsohn, E.; Savage, T. J.: Gijzen, M.; Croteau, R. Simultaneous analysis of monoterpenes and diterpenoids of conifer oleoresin. *Phytochem. Anal.* **1993**, *4*, 220−225.

Ohloff, G. The fragrance of ambergris. In *Fragrance Chemistry: The Science of Sense and Smell*; Theimer, E. T., Ed.; Academic Press: New York, 1982; pp 535−573.

Piccaglia, R.; Marotti, M.; Dellececca, V. Effect of planting density and harvest date on yield and chemical composition of sage oil. *J. Essent. Oil Res.* **1997**, *9*, 187−191.

Pino, J. A.; Estarrón, M.; Fuentes, V. Essential oil of sage (*Salvia officinalis* L.) grown in Cuba. *J. Essent. Oil Res.* **1997**, *9*, 221−222.

Putievsky, E.; Ravid, U. Selection and cultivation of *Salvia fruticosa* Mill. (syn. *S. triloba*) from wild populations in Israel. In *Aromatic and Medicinal Plants*; Proceedings of the EUCARPIA International Symposium on Conservation of Genetic Resources of Aromatic and Medicinal Plants, Oeiras, Portugal, 1984; Mota, M., Baeta, J., Eds.; 1987; pp 87−94. Estação Agronomica Nacional, Oeiras, Portugal.

Putievsky, E.; Ravid, U.; Dudai, N. The effect of water stress on yield components and essential oil of *Pelargonium graveolens* (L.). *J. Essent. Oil Res.* **1990a**, *2*, 111−114.

Putievsky, E.; Ravid, U.; Diwan-Rinzler, N.; Zohary, D. Genetic affinities and essential oil composition of *Salvia officinalis* L.; *S. fruticosa* Mill.; *S. tomentosa* Mill. and their hybrids. *Flavour Fragrance J.* **1990b**, *5*, 121−123.

Putievsky, E.; Ravid, U.; Dudai, N. The essential oil and yield components from various plant parts of *Salvia fruticosa*. *J. Nat. Prod.* **1986a**, *49*, 1015−1017.

Putievsky, E.; Ravid, U.; Dudai, N. The influence of season and harvest frequency on essential oil and herbal yields from a pure clone of sage (*Salvia officinalis*) grown under cultivated conditions. *J. Nat. Prod.* **1986b**, *49*, 326−329.

Putievsky, E.; Ravid, U.; Dudai, N. Phenological and seasonal influences on essential oil of a cultivated clone of *Origanum vulgare* L. *J. Sci. Food Agric.* **1988**, *43*, 225−228.

Putievsky, E.; Ravid, U.; Senderovich, D. Morphological observations and essential oil of sage (*Salvia officinalis* L.) under cultivation. *J. Essent. Oil Res.* **1992**, *4*, 291−293.

Rivera, D.; Obon, C.; Cano, F. The botany, history and traditional uses of three-lobed sage (*Salvia fruticosa* Miller) (Labiatae). *Econ. Bot.* **1994**, *48*, 190−195.

Shimoni, M.; Putievsky, E.; Ravid,-U.; Reuveni, R. Antifungal activity of volatile fractions of essential oils from four aromatic wild plants in Israel. *J. Chem. Ecol.* **1993**, *19*, 1129−1133.

Tucker, A.O.; Maciarello, M. J.; Clebsch, B. B. Volatile leaf oils of cultivars of *Salvia clevelandii* (Gray) Greene X *S. leucophylla* Greene. *J. Essent. Oil Res.* **1995**, *7*, 101−104.

Voirin, B.; Bayet, C. Developmental changes in the monoterpene composition of *Mentha* x *piperita* leaves from individual peltate trichomes. *Phytochemistry* **1996**, *43*, 573−580.

Werker, E.; Ravid, U.; Putievsky, E. Structure of glandular hairs and identification of the main components of their secreted material in some species of the Labiatae. *Isr. J. Bot.* **1985**, *34*, 31−45.

Werker, E.; Putievsky, E.; Ravid, U.; Dudai, N. and Katzir, I. Glandular hairs and essential oil in developing leaves of *Ocimum basilicum* L. (Lamiaceae). *Ann. Bot.* **1993**, *71*, 43−50.

Received for review February 16, 1999. Revised manuscript received June 15, 1999. Accepted July 21, 1999. This work was supported by a grant from the Chief Scientist, Ministry of Agriculture.

JF9901587