Organ- and Season-Dependent Variation in the Essential Oil Composition of *Salvia officinalis* L. Cultivated at Two Different Sites

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More than 50 compounds were identified in essential oils from stems and leaves of *Salvia officinalis* L. plants harvested in July, in Arouca, in northern Portugal. About 40 of those compounds were also present in flower essential oils, collected from the same plants. α-Thujone was the major compound, representing about 55, 30, and 18% of the essential oils from stems, leaves, and flowers, respectively. Significant percentage variations in the main compound classes of the essential oils from shoots sampled over the year were recorded at two different sites in northern Portugal. From December to April, oxygenated monoterpenes (MO) decreased from ∼67−72% to values of 42−43% of the essential oils. During the same time interval, the percentage of monoterpene hydrocarbons (MH) rose from 8−11% to 17−22%. At both sites, sesquiterpene hydrocarbons (SH) rose from ∼7% in February to 19−22% in April, decreasing thereafter to ∼9% in July. Oxygenated sesquiterpenes (SO) increased from a minimum of ∼5% in July to a maximum of 8−11% in February, decreasing thereafter. The compounds that mostly accounted for the essential oil composition variation were α-pinene, *β*-pinene, and camphene, as MH; α-thujone and camphor, as MO; α-humulene and *β*-caryophyllene, as SH; and viridiflorol, as SO.

Keywords: *Salvia officinalis; sage; essential oils*

INTRODUCTION

The great demand for natural products, such as essential oils, allows the implementation of aromatic and medicinal plant cultures as one reasonable alternative to traditional food plant cultures. Native wild and cultured aromatic and medicinal plant species of different families, including Lamiaceae, are being investigated in Portugal to meet the needs of the essential oil and extract industries and the need of finding new agrarian cultures as alternatives to the surplus traditional ones. *Salvia officinalis* L., a type of sage plant, is one targeted species. Reports on the essential oil composition of this species have been published by several authors (*1*−*4*). Some authors have reported variations in the sage essential oil composition depending on soil mineral fertilization (*5*), light intensity (*6*), organ age (*7*), climate conditions (*8*), season (*9*, *10*), and organ, season, and culture site (*11*). However most of these types of studies concern the variations of a few major sage essential oil constituents, overlooking what happens with the high number of minor ones. The percentage composition of the essential oil provides probably the most important parameter for the characterization of the respective plants. We believe that the knowledge of the way in which the essential oil composition of the exploited sage plants varies over the year will be important in making decisions on the most appropriate organ type and timing of the respective biomass feedstock harvest. The aims of the research here reported were the determination of the essential oil composition from leaves, stems, and flowers of sage plants and the determination of compositional variation profiles of the respective aerial parts taken as a whole in two experimental fields established in localities where the agrarian exploitation of these cultures is intended to begin.

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MATERIALS AND METHODS

Plant Material. *S. officinalis* L. cultivation was started by sowing in a greenhouse (20−30 °C) in February 1997. At the beginning of April, plantlets were transferred to soil in experimental fields from DRAEDM at Arcos de Valdevez (Arcos) and at Arouca. Aerial parts from 8−12 plants of *S. officinalis* L. were randomly collected in each of the two experimental fields on July 27, October 6, and December 3, 1998, and on February 9, April 6, and July 20, 1999. As routine procedure, the distal parts 20−25 cm in length of leafy shoots were gathered and chopped into pieces of ∼5 cm immediately before hydrodistillation.

Leaves, stems, and flowers from one subsample of plant material collected in Arouca on July 27, 1998, were separated, before chopping, and processed independently in the hydrodistillation and essential oil analysis. As anthesis occurred from May to July, in an asynchronous way, the flower sample included flowers of different ages within that time interval. Voucher specimens are maintained in the experimental field of Arouca under the control of the DRAEDM from the Portuguese Agriculture Office.

Hydrodistillation and Analysis Procedure. Subsamples of ∼5 g of fresh biomass of *S. officinalis* plants were submitted to hydrodistillation in a Clevenger type apparatus over 1 h, using volumes of 1.0 mL of *n*-hexane, containing 5α-cholestane (1 mg/mL), for retention of the hydrodistillate components. All samples were analyzed by GC and GC-MS. GC analyses were performed using a Perkin-Elmer Autosystem gas chromatograph equipped with a fused silica DB5 column (30 m long ×

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0.25 mm i.d., 0.25 μm film thickness composed by 5% phenyl methylpolysiloxane, J&W Scientific). The temperature program included a ramp from 60 to 285 °C at 3 °C min^{-1} for the column, 300 °C for the injector, and 320 °C for the FID. H_2 was used as carrier gas at a flow rate of 1.49 mL/min under a column head pressure of 12.5 psi. Injections were performed in a split/splitless injector with the splitter opened at the 1:13 split ratio. Three replicates of each sample were processed in the same way.

Percentage values from the listed compounds correspond to the values given in the GC report without correction factors. For determination of the absolute specific content of each essential oil compound, correction factors relative to the internal standard, 5α-cholestane, were used that accounted for the differential responses of the FID and for the column inlet discrimination of the essential oil compounds due to the injector split ratio. Considering that the determination of individual correction factors is impracticable, due to either the high number of compounds or their absence in the market, compounds belonging to the same group [monoterpene hydrocarbons (MH), oxygenated monoterpenes (MO), monoterpene esters (ME), sesquiterpene hydrocarbons (MH), oxygenated sesquiterpenes (SO), sesquiterpene esters (SE), and oxygenated diterpenes (DO)] were assumed to have the same quantitative GC correction factor. Three replicates of mixtures at equal amounts of 5α-cholestane and limonene (MH), camphor (MO), bornyl acetate (ME), *trans*-caryophyllene (SH), *trans*,*trans*-farnesol (SO), *trans*,*trans*-farnesyl acetate (SE), and phytol (DO) were prepared, and each was injected three times. The average values, corrected for the purity grade of each reference compound, gave the following quantitative GC correction values: 0.741 (MH), 1.014 (MO), 1.071 (ME), 0.747 (SH), 1.018 (SO), 1.263 (SE), and 0.794 (DO). A correction factor of 1 was assumed for compounds that did not belong to any of these groups. The sum of the specific contents of all individual essential oil compounds was assumed as a parameter for the determination of the total specific essential oil yield.

GC-MS analysis was performed with a Perkin-Elmer 8500 gas chromatograph equipped with a fused silica DB5, the same as that for GC, connected to a Finnigan MAT ion trap detector (ITD; software version 4.1) operating in EI mode at 70 eV. Injector, interface, and ion source temperatures were 300, 260, and 220 °C, respectively. The oven temperature program and injection conditions were as above-described for GC. He_2 was used as carrier gas with a column head pressure of 12.5 psi.

Compounds were identified by comparing their mass spectra with those in computer libraries and by comparison of their GC retention times and mass spectra with those of reference compounds. The respective retention indices were determined relative to *n*-alkanes of a complete series, from *n*-octane to *n*-tetratriacontane, eluted in the same conditions as the essential oil samples.

RESULTS AND DISCUSSION

The essential oils from the aerial parts of the *S. officinalis* plant cultivar used in this study were composed of >60 compounds, 55 of which were identified. Table 1 shows the composition of the essential oils, obtained by hydrodistillation, from leaves, stems, and flowers of plants of this species cultivated at Arouca. As can be seen, a lower diversity of compounds was found in essential oil from flowers, which lacked two monoterpene hydrocarbons, four oxygenated monoterpenes, two sesquiterpene hydrocarbons, four monoterpene esters, and one oxygenated sesquiterpene, which were identified in the essential oils from vegetative aerial parts. Tetradecane, α-muurolene, γ-cadinene, and the putative *trans*-pinene hydrate, identified in the essential oil from flowers, were absent in leaves and stems of this *S. officinalis* cultivar. In any case either the compounds that were restricted to the flowers or those that were restricted to the vegetative parts represented a low percentage of the respective essential oils. The identified compounds corresponded to 99.4, 99.0, and 99.8% of the essential oils from leaves, stems, and flowers, respectively. As reviewed before (*4*), there are many reports on essential oils of *S. officinalis*. To our knowledge, however, the compound lists shown in Table 1 more completely describe the composition of the essential oils from leaves, stems, and flowers from this species. Although some authors (*11*) had performed studies on essential oils from separated flowering parts, leaves, and stems from *S. officinalis*, they did not report the respective compositions. According to the same authors, *S. officinalis* flowering parts had the lowest percentage of total thujones (16%) and the highest percentage of β-pinene (27.1%), contrary to what occurred with stems, which showed the highest percentage of total thujones (37%) and the lowest percentage of β-pinene (7.6%). In coherence to those results we found that the percentages of total thujones in sage essential oils were 21.2% in flowers, 29.4% in leaves, and 61.8% in stems, whereas β-pinene, in the essential oils from the same organs, represented 17.0, 2.2, and 1.8% respectively. Our results showed that α-thujone was the main essential oil compound in the vegetative parts, representing 25.5 and 55.1% of the essential oils from sage leaves and stems, respectively. In flowers, α-thujone represented 17.7%, a percentage similar to those of 1,8-cineole (17.3%) and β-pinene (17.0%) (Table 1). Oxygen-containing monoterpenes (MO) constituted the major compound group in essential oils from leaves, stems, and flowering parts followed by monoterpene hydrocarbons (MH), sesquiterpene hydrocarbons (SH), and oxygen-containing sesquiterpenes (SO) (Table 1). The flower essential oil had the lowest MO/MH and SO/SH ratios (1.74:1 and 0.35:1, respectively) relative to leaves (MO/MH = 6.43:1; SO/SH = 0.87:1) and stems (MO/MH = 8.83:1; SO/SH = 0.87:1).

Chemotypes of *S. officinalis* with either α-thujone, β-thujone, camphor, or 1,8-cineole as the main constituent or based on their α/β-thujone ratios 10:1 α/β, 1.5:1 α/β, and 1:10 α/β have been proposed by some authors (*11*). However, as the percentages of each of these compounds vary greatly in flowers, leaves, and stems, it is not surprising that the composition of the essential oils from the aerial part of *S. officinalis* plants taken as a whole varies significantly depending on the ratio of leaves/stems/flowers of the biomass used as essential oil source. Such variability would explain the difficulty in ascribing a given plant to one or another chemotype.

The essential oil contents varied significantly over the year either in Arouca or in Arcos de Valdevez (Arcos). The variations were asynchronous, however, in both sites (Figure 1). In the experimental field of Arouca the essential oil content rose from a minimum of ~19 mg/g of dry weight, at the beginning of October 1998, to a maximum of 51 mg/g of dry wt at the beginning of December 1998. In the experimental field of Arcos de Valdevez the maximum essential oil contents were obtained at the end of July 1998 (101 mg/g of dry wt) and July 1999 (102 mg/g of dry wt) and the minimum in April 1999 (9 mg/g of dry wt). Differences in pedologic, climatic, and biotic factors between the two sites may explain differences in the specific contents of essential oils. During the summer the Arcos de Valdevez experimental field stayed drier than that of Arouca, and in July most of the sage plants from Arcos de Valdevez

Table 1. Essential Oil Composition and Specific Compound Contents in Leaves, Stems, and Flowers from Arouca *S. officinalis* Plants Harvested in July

		leaves		stems		flowers	
compound	RI[a]	%	μg/g of dw	%	μg/g of dw	%	μg/g of dw
1-butyl acetate	813	0.04	10.3	0.28	30.5	0.58	164.4
cis-2-methyl-3-methylenehept-5-ene	847	0.40	129.4	0.88	71.1	0.08	11.8
trans-2-methyl-3-methylenehept-5-ene	857	0.04	13.4	0.14	10.2	0.03	3.5
tricyclene	920	0.07	19.1	0.03	2.7	0.22	35.2
α-thujene	924	0.14	44.9	0.33	26.0	0.42	70.4
α-pinene	930	4.22	1369.2	0.98	80.6	5.60	960.2
camphene	946	2.57	835.6	0.98	79.3	4.92	844.4
sabinene	970	0.15	51.8	0.79	63.9	0.37	60.9
β-pinene	974	2.22	723.0	1.83	148.5	17.01	2916.9
myrcene	988	0.92	300.6	1.11	89.6	0.93	161.0
n-decane	1000	tr[b]	5.5	0.03	3.8	0.06	14.4
α-phellandrene	1004	0.04	15.1	0.03	3.3		
α-terpinene	1016	0.12	39.2	0.23	18.9	0.26	43.5
p-cymene	1022	0.11	35.2	0.14	10.7	0.08	15.1
limonene	1024	1.59	516.8	0.76	61.0	0.14	21.6
1,8-cineole	1033	6.47	2874.4	1.98	219.0	17.32	4056.4
Z-*β*-ocimene	1034	0.14	48.0	0.03	2.4		
E-*β*-ocimene	1044	0.04	12.1	tr	1.8	0.06	7.7
γ-terpinene	1055	0.29	91.9	0.56	44.7	0.51	87.4
cis-linalool oxide	1072	0.22	98.8	0.33	37.4	0.22	51.4
not identified	1079	0.04	11.8	0.09	7.5		
terpinolene	1086	0.47	151.0	0.26	21.9	0.23	40.2
n-undecane	1100	0.15	65.4	0.14	12.5	0.11	18.1
α-thujone	1103	25.50	11333.1	55.08	6117.2	17.74	4156.5
β-thujone	1114	3.89	1730.5	6.71	745.6	3.41	795.5
α-campholenal	1125	0.04	11.0	tr	2.2		
trans-pinene hydrate (?)	1127					0.11	25.6
not identified	1129	0.18	73.9	tr	1.9		
camphor	1143	19.51	8662.1	5.63	622.8	3.45	810.2
not identified	1156	0.04	18.7	0.14	15.1		
(*cis*-3)-pinanone	1160	0.04	13.9	0.23	24.9		
borneol	1165	0.06	25.5	0.69	77.3	8.76	2047.2
pinocamphone isomer (T)	1172	3.37	1496.6	0.08	8.4		
4-terpineol	1176	0.23	104.5	0.34	38.7	0.39	92.3
α-terpineol	1189	0.26	112.5	0.09	12.4	0.23	51.9
not identified	1201	0.11	40.3	0.09	12.6		
not identified	1237	0.04	16.3	tr	1.0	0.08	17.0
not identified	1245	0.04	12.1	0.03	4.1		
bornyl acetate	1283	1.15	533.9	0.73	86.0	1.65	407.8
cis-sabinyl acetate	1290	0.18	83.2	0.34	40.1		
not identified	1322	0.04	22.1	0.09	11.7		
δ-elemene	1334	0.07	18.4	0.03	3.7		
trans-carvyl acetate	1337	0.02	9.2	0.03	0.8		
not identified	1348	tr	5.0	tr	2.2		
cis-carvyl acetate	1362	tr	5.0	tr	2.2		
neryl acetate	1364	0.04	11.8	0.02	9.7		
β-bourbonene + geranyl acetate	1383+1384	0.22	97.3	0.04	4.7	0.08	17.5
n-tetradecane	1400					0.05	10.3
β-caryophyllene	1416	3.17	1040.6	1.05	86.1	4.78	819.8
aromadendrene or α-guaiene (?)	1436	0.04	16.0	tr	1.2		
α-humulene	1450	7.46	2443.4	5.23	430.8	4.31	738.6
allo-aromadendrene	1458	0.07	22.2	0.06	3.7	0.08	11.8
germacrene D isomer 3	1473	0.07	21.9	tr	0.9	0.22	37.9
germacrene D	1477	0.14	46.1	0.09	8.0	0.11	18.3
α-selinene	1491	0.09	29.3	0.06	4.6	0.14	21.5
α-muurolene	1496					0.07	9.8
γ-cadinene	1509					0.13	20.9
δ-cadinene	1520	0.11	32.3	0.03	2.6	0.30	49.9
caryophyllene oxide	1580	0.21	101.7	0.14	14.8	0.16	37.3
viridiflorol	1595	6.29	2804.1	5.08	567.2	3.19	734.6
widdrol (?)	1606	0.44	199.5	0.51	56.7	0.18	44.1
trans-α-bergamatol acetate	1801	0.21	119.1	0.42	60.3		
not identified	1903	0.06	22.1	0.06	6.9	0.08	15.7
manool	2063	5.85	2029.3	4.69	401.9	0.84	139.1
not identified	2092	0.35	154.7	0.22	21.9		
grouped components							
monoterpene hydrocarbons		13.6	4408.1	9.2	744.4	30.9	5279.9
oxygen-containing monoterpenes		60.0	26632.4	71.4	7942.1	52.0	12159.3
monoterpenyl esters		1.4	670.3	1.2	152.9	1.6	407.8
sesquiterpene hydrocarbons		11.2	3670.1	6.6	541.7	10.1	1728.5
oxygen-containing sesquiterpenes		6.9	3105.3	5.7	638.7	3.5	816.0
sesquiterpenyl esters		0.2	119.1	0.4	60.3		
oxygen-containing diterpenes		5.8	2029.3	4.7	401.9	0.8	139.1
n-alkanes		0.2	70.9	0.2	16.2	0.2	42.9
others		0.7	292.2	0.6	63.9	0.7	197.6

[a] Kovats retention index on a DB-5 column, $t < 0.010$. [b] tr, trace amounts.

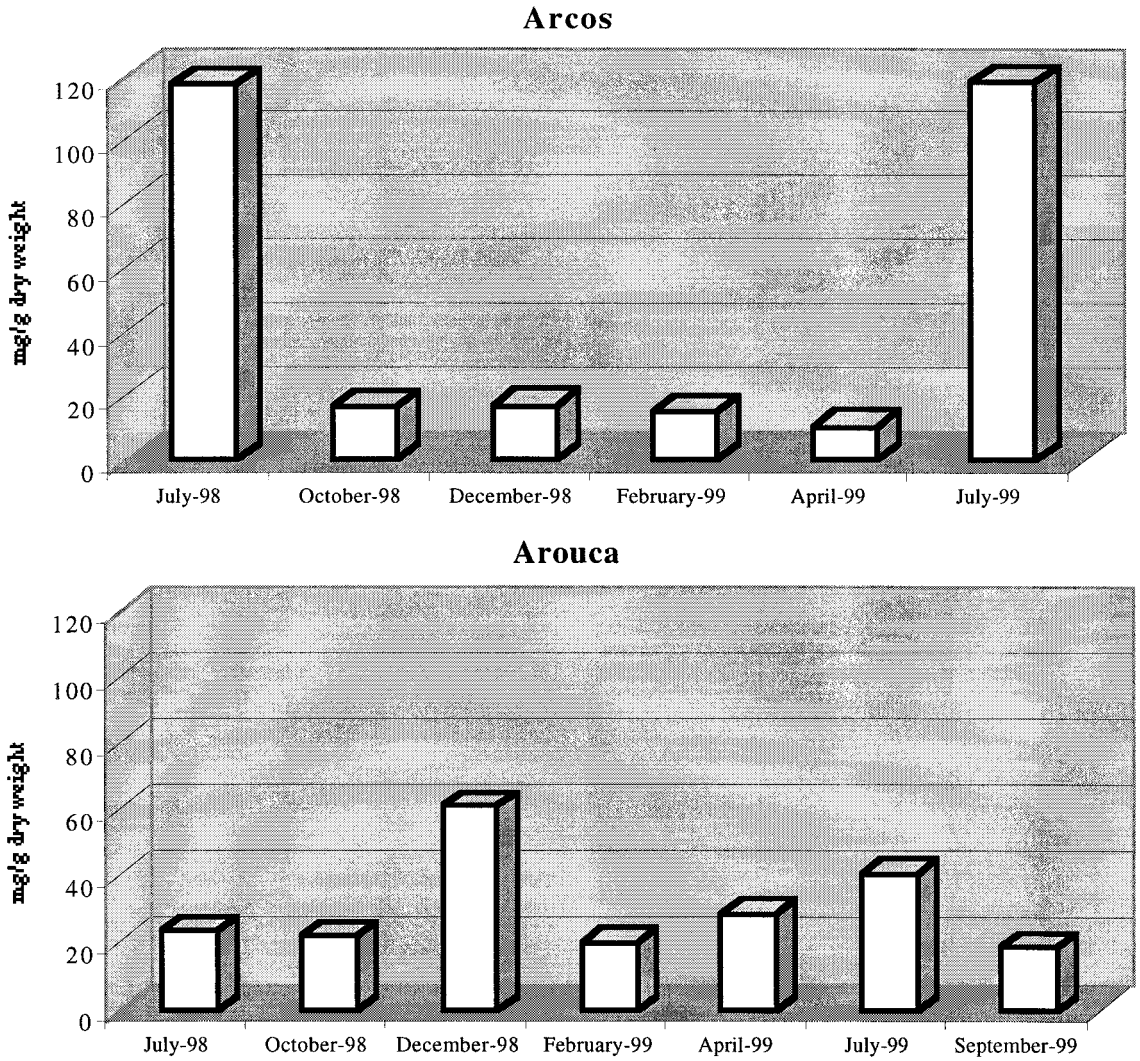

Figure 1. Total essential oil contents in the aerial part of *S. officinalis* plants cultivated in experimental fields of Arcos de Valdevez and Arouca.

were contaminated with a *Phomopsis* sp. fungus. From April to September sage biomass production was 0.47 kg/m^2 in Arcos de Valdevez and 2.49 kg/m^2 in Arouca. Because of the general competition for carbon and energy, between the pathways of primary metabolism and those of secondary metabolism, the lower vegetative growth of sage plants from Arcos de Valdevez may partially explain their higher essential oils accumulation. On the other hand, such an increased essential oil accumulation would also have occurred as an elicitation effect by the referred fungal infection. Some authors (*8*, *12*) reported also variations in sage essential oil contents in Hungary, from June to October, with the minimum level in June and the maximum level in September. In sage plants growing in Italy, an increase in essential oil contents to more than double, from February to July, was also recorded (*10*). A decrease in essential oil contents from sage grown at Redbank, New Zealand, from 1.3% to ~0.2%, between July 1995 and May 1996 was explained as a consequence of greater growth of stems to the detriment of leaves (*11*).

Either the specific contents or percentages of the four main compound groups varied over the year in the two experimental fields (Figure 2). Whatever the factor that, in July, had been responsible for the essential oil content increase in Arcos de Valdevez sage plants, apparently it affected proportionately the four compound groups. In essential oils from aerial parts of sage plants maintained in this experimental field, the percentage of monoterpene hydrocarbons rose from 8.2% in December to 22.1% in April. The corresponding variation in sage plants from Arouca, in the same period, was from 11.1 to 16.7%. Oxygen-containing monoterpenes showed the amplest variations. From December 1998 to April 1999, the percentage of this group in the sage essential oils decreased from 71.5 to 43.0% in Arcos de Valdevez and from 67.4 to 42.0% in Arouca. The second broader variation occurred with the percentages of the sesquiterpene hydrocarbons which, from February to April, rose from 7.3 to 19.3% in Arcos de Valdevez and from 7.1 to 21.9% in Arouca. From July 1998 to February 1999, the oxygen-containing sesquiterpenes ranged from a minimum of 4.5% to a maximum of 8.4% and from 5.4 to 11.2% in Arcos de Valdevez and Arouca, respectively (Figure 2).

The percentage composition of the essential oils obtained by hydrodistillation of the aerial parts of sage plants maintained in Arcos de Valdevez and Arouca sampled on six different dates from 1998 to 1999 are shown in Table 2. The compound list is longer that those reported by other authors for aerial parts of sage plants (*1*, *3*, *5*, *10*, *11*, *13*). The isomers *cis*- and *trans*-2-methyl-3-methylenehept-5-ene have been named by some authors as *cis*- and *trans*-salvene (*5*). The occurrence of

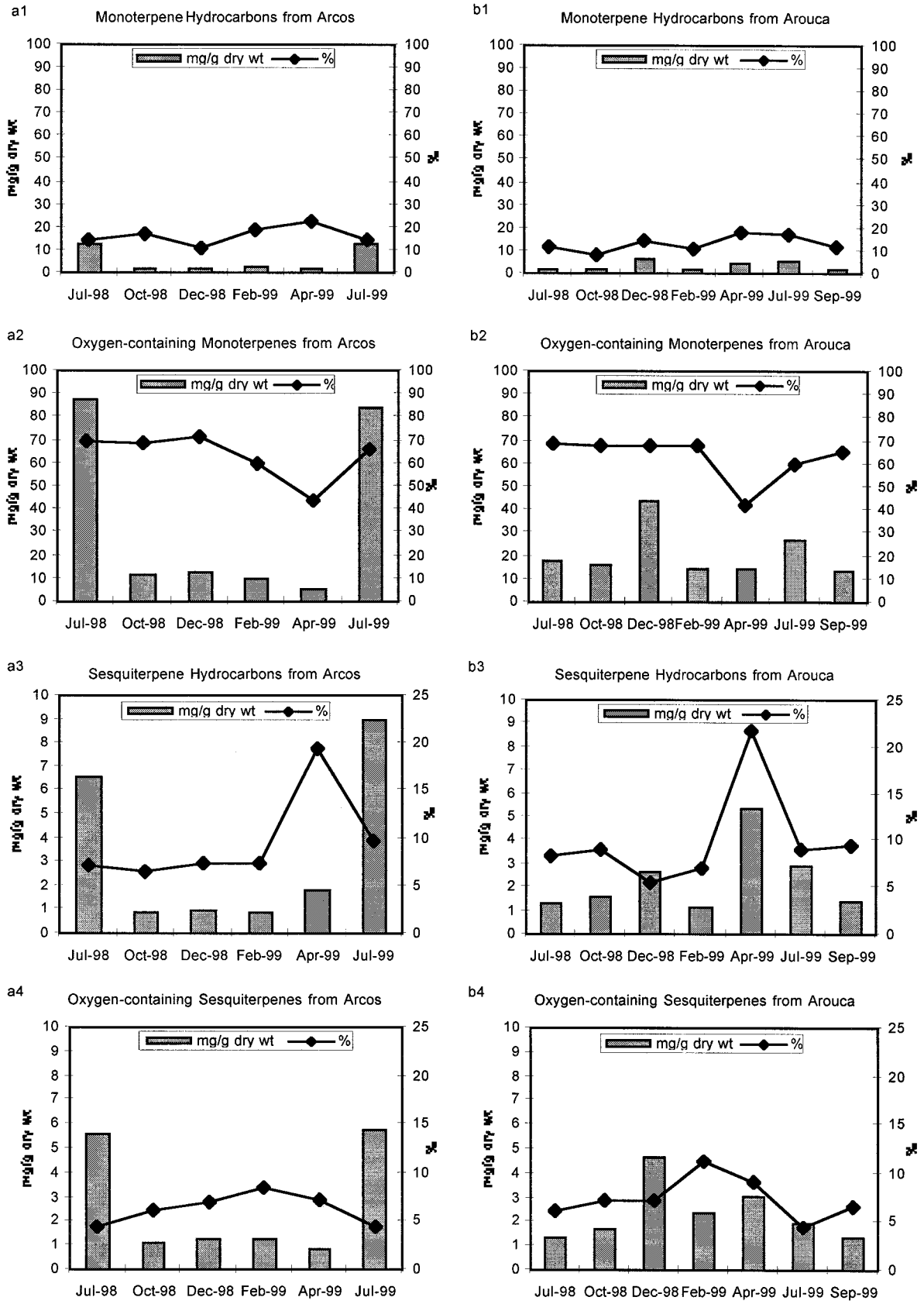

Figure 2. Annual variations in contents of specific compound groups from essential oils obtained by hydrodistillation of aerial part biomass of *S. officinalis* plants cultivated in Arcos de Valdevez (a) and Arouca (b): (a1, b1) monoterpene hydrocarbons; (a2, b2) oxygen-containing monoterpenes; (a3, b3) sesquiterpene hydrocarbons; (a4, b4) oxygen-containing sesquiterpenes.

the diterpene manool in sage essential oils had already been reported (*3*, *4*). All three of these compounds are absent in the 33 compound list of the essential oils from an original Portuguese *S. officinalis* clone (Coimbra rose, ref B3S2) reported by Chalchat et al. (*13*). On the other hand, the percentages reported by these authors for the respective essential oil compounds, namely, α-thujone (1.94%), β-thujone (17.60%), camphor (30.79%),

Table 2. Percentage of Identified Compounds in the Essential Oils from the Aerial Parts of Sage Plants Maintained in Arcos de Valdevez (First Value) and Arouca (Second Value)

compound	July 1998	Oct 1998	Dec 1998	Feb 1999	April 1999	July 1999
1-butyl acetate	0.03/0.16	0.07/015	0.10/0.07	0.04/0.04	0.03/0.02	0.11/0.01
***cis*-2-methyl-3-methylenehept-5-ene**	**0.57/0.76**	**1.11/0.81**	**2.52/2.69**	**1.68/1.50**	**0.52/0.77**	**0.31/0.40**
trans-2-methyl-3-methylenehept-5-ene	0.11/0.09	0.12/0.08	0.28/0.31	0.18/0.17	0.05/0.09	0.03/0.04
tricyclene	0.13/0.06	0.21/0.07	0.06/0.06	0.26/0.09	0.31/0.08	0.16/0.17
α-thujene	0.20/0.18	0.17/0.15	0.25/0.25	0.23/0.19	0.17/0.20	0.17/0.15
α-pinene	**2.88/1.34**	**3.10/1.01**	**1.06/1.66**	**3.08/1.40**	**3.88/2.08**	**3.06/6.13**
camphene	**4.82/3.04**	**5.90/1.96**	**1.51/1.67**	**6.02/2.41**	**6.90/1.95**	**5.07/5.76**
sabinene	0.30/0.44	0.21/0.32	0.41/0.39	0.29/0.26	0.22/0.29	0.35/0.25
***β*-pinene**	**2.38/1.82**	**4.18/1.96**	**2.78/4.47**	**4.67/3.08**	**6.86/8.74**	**2.67/2.19**
myrcene	1.08/1.08	0.78/0.69	0.84/0.84	0.55/0.56	0.41/0.59	1.03/0.97
n-decane	0.02/0.02	0.01/0.04	0.03/tr	0.03/0.02	0.02/0.02	0.04/0.01
α-phellandrene	0.05/0.06	0.03/0.04	0.10/0.03	0.11/0.03	0.06/0.02	0.02/0.07
α-terpinene	0.14/0.12	0.08/0.08	0.20/0.14	0.23/0.14	0.10/0.11	0.10/0.10
p-cymene	0.18/0.tr	0.23/0.19	0.38/0.17	0.87/0.38	0.65/0.05	0.07/0.07
limonene	0.23/0.12	0.14/0.25	0.12/0.65	0.18/0.13	1.76/1.75	0.15/0.23
1,8-cineole	**9.35/7.15**	**11.98/7.21**	**5.14/7.13**	**4.61/7.73**	**6.58/12.27**	**8.78/6.42**
Z-*β*-ocimene	tr/0.02	0.02/tr	0.03/0.21	tr/0.02	0.13/0.02	tr/0.02
E-*β*-ocimene	0.06/0.02	0.04/0.07	0.03/0.07	0.04/0.04	0.32/0.45	0.05/0.11
γ−terpinene	0.31/0.30	0.19/0.22	0.30/0.33	0.25/0.26	0.22/0.25	0.25/0.19
cis-linalool oxide	0.10/0.24	0.25/0.22	0.23/0.21	0.25/0.26	0.18/0.25	0.06/0.12
terpinolene	0.36/0.49	0.18/0.15	0.10/0.14	0.11/0.09	0.14/0.11	0.43/0.53
n-undecane	0.02/0.02	0.02/0.09	0.08/0.03	0.16/0.10	0.17/0.05	0.02/0.01
α-thujone	**30.86/34.82**	**25.61/41.03**	**55.17/46.23**	**30.14/41.08**	**12.01/18.39**	**25.55/23.39**
***β*-thujone**	**5.59/3.90**	**4.16/3.34**	**4.94/4.02**	**5.79/3.86**	**1.19/3.49**	**6.68/3.18**
α-campholenal	0.04/0.05	0.06/0.04	0.03/0.03	0.08/0.06	0.07/0.05	0.03/0.04
camphor	**19.58/23.14**	**22.86/13.75**	**4.69/7.10**	**13.28/9.28**	**8.23/2.70**	**22.36/23.14**
cis-3-pinanone	0.05/0.11	0.12/0.15	0.18/0.21	0.31/0.34	0.42/0.20	0.06/0.13
borneol	**3.30/2.14**	**2.73/1.68**	**0.73/1.98**	**4.23/3.81**	**13.37/4.21**	**1.79/1.98**
pinocamphone isomer (T)	0.02/0.04	0.04/0.05	0.05/0.07	0.11/0.13	0.14/0.06	0.03/1.01
4-terpineol	0.27/0.21	0.26/0.27	0.26/0.29	0.29/0.30	0.28/0.20	0.20/0.16
α-terpineol	0.11/0.16	0.14/0.05	0.05/0.10	0.07/0.13	0.11/0.24	0.13/0.15
bornyl acetate	**2.40/1.31**	**0.38/0.14**	**0.06/0.21**	**0.17/0.09**	**1.62/0.19**	**2.15/4.02**
cis-sabinyl acetate	0.28/0.37	0.10/0.15	0.06/0.07	0.04/0.04	0.03/0.04	0.25/0.26
δ-elemene	0.04/0.07	0.02/0.05	0.02/tr	tr/tr	0.05/0.11	0.06/0.06
trans-carvyl acetate	tr/0.02	0.02/0.03	tr/tr	tr/tr	tr/tr	tr/0.01
cis-carvyl acetate	tr/tr	tr/tr	tr/tr	tr/tr	0.05/0.05	tr/0.01
neryl acetate	0.02/0.02	tr/tr	tr/-	tr/tr	0.05/0.12	0.01/0.01
β-bourbonene + geranyl acetate	0.02/0.11	tr/tr	0.02/tr	tr/tr	0.05/0.08	0.06/0.16
***β*-caryophyllene**	**2.99/3.60**	**1.80/2.31**	**3.06/0.51**	**2.10/1.85**	**5.73/7.92**	**4.39/2.76**
aromadendrene or α-guaiene	0.05/0.05	0.02/0.04	0.02/0.03	0.21/0.10	0.79/0.20	0.05/0.09
α-humulene	**3.85/3.36**	**4.50/6.52**	**3.99/4.84**	**4.56/4.88**	**11.65/11.61**	**4.84/5.53**
allo-aromadendrene	0.05/0.07	0.05/0.07	0.06/0.03	0.07/0.08	0.08/0.10	0.08/0.07
germacrene D isomer 3	0.02/0.02	tr/tr	0.03/tr	0.01/0.02	0.14/0.39	0.02/0.07
germacrene D	0.03/0.05	0.02/0.04	0.03/tr	0.04/tr	0.16/0.38	0.05/0.11
α-selinene	0.03/0.05	0.04/0.03	0.06/0.03	0.24/0.08	0.53/0.62	0.08/0.30
δ-cadinene	0.02/0.03	0.02/0.03	0.04/tr	0.03/0.04	0.19/0.50	0.02/0.04
caryophyllene oxide	0.05/0.29	0.49/0.42	0.68/0.18	0.92/1.13	0.84/0.37	0.10/0.21
viridiflorol	**3.50/4.88**	**4.92/5.83**	**5.53/5.72**	**6.29/8.08**	**5.16/8.17**	**4.26/3.98**
widdrol (?)	**0.52/0.20**	**0.68/0.85**	**0.67/1.18**	**1.19/1.96**	**1.17/0.61**	**0.15/0.17**
trans-α-bergamatol acetate	0.23/0.06	0.07/0.14	0.03/0.03	0.03/tr	0.29/0.12	0.18/0.17
manool	**1.58/2.45**	**1.43/6.72**	**2.46/4.87**	**4.90/2.96**	**4.08/7.92**	**2.89/3.74**

and 1,8-cineole (17.04%), mean that this Portuguese sage clone corresponds to a chemotype different from that studied in our work.

The percentage variations of the compounds that mostly contribute to the variation in the levels of the total monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes are shown in Figure 3. The increase in the percentage of the total monoterpene hydrocarbons, from December 1998 to April 1999, was essentially due to increases in specific contents of α-pinene, camphene, and *β*-pinene (Figure 3). A different trend was followed by the variation in the specific content of the *cis*-2-methyl-3-methylenehept-5-ene (Figure 3). According to some authors (*1*), this monoterpene hydrocarbon, as well as its *trans*-isomer, results from light-induced rearrangement and degradation of the thujones. α-Thujone is the major compound responsible by the percentage variation of the total oxygen-containing monoterpenes. The highest and lowest percentages of this compound occurred in December and April, respectively, in coherence with the high and low levels of the total oxygenated monoterpenes. Variations of the α-thujone levels were also coherent with the variations of the *cis*-2-methyl-3-methylenehept-5-ene levels (Figure 3). This finding supports the origin of this last compound as being α-thujone. In July, and over most of the year, camphor, 1,8-cineole, *β*-thujone, and borneol were the second, third, fourth, and fifth most representative oxygenated compounds. In Arcos de Valdevez sage plants, the borneol level rose from its minimum (0.7%) in December to its maximum (13.4%) in April becoming, in that month, the major compound in the essential oil of those plants (Figure 3). Borneol was also the major compound in essential oil of Sardinian *S. officinalis* plants at the beginning of March (*10*). However, in the essential oil of Arouca sage plants, the increase in the borneol percentage during the same time interval was small. In this experimental field, the *β*-thujone level did not vary significantly over the year. In Arcos de Valdevez,

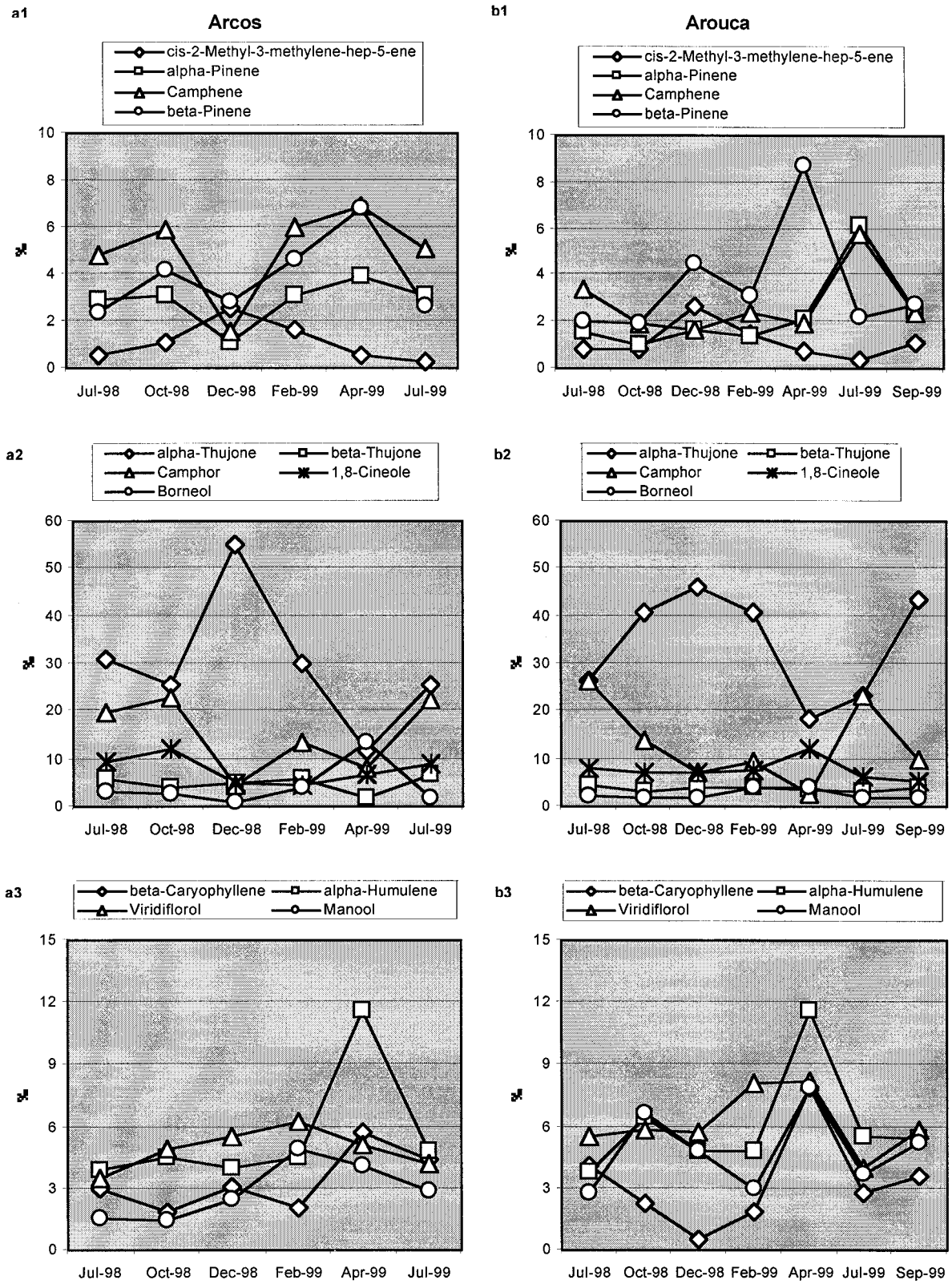

Figure 3. Annual variation in contents of the major compounds from essential oils of aerial parts of *S. officinalis* plants cultivated in Arcos de Valdevez (a) and Arouca (b): (a1, b1) monoterpene hydrocarbons; (a2, b2) oxygen-containing monoterpenes; (a3, b3) sesquiterpene hydrocarbons (β-caryophyllene and α-humulene), oxygen-containing sesquiterpene (viridiflorol), and the diterpenoid manool.

the variation of this compound was somewhat erratic. In Arouca, 1,8-cineole stayed at ~7.2−7.7%, from July 1998 to February 1999, rising thereafter to 12.3% in April 1999 and becoming, in that month, the second major compound. However, in Arcos de Valdevez, the levels of this compound decreased significantly from October to February, increasing little thereafter until July. From October to December, in Arcos de Valdevez, and from July to December, in Arouca, the camphor levels of sage plants decreased drastically, inversely to levels of α-thujone that, in the same time intervals, increased abruptly (Figure 3). During the drastic drop

in the levels of α-thujone, from December to April, camphor stayed erratically at its lower levels in the two fields, both compounds rising thereafter. With regard to the thujones, camphor, and borneol, our results are coherent with those of Putievsky et al. (*9*), who reported variations in the levels of α- and *β*-thujones, from a minimum of 8% in April to a maximum of 40% in December; camphor, from a minimum of 10% in April to a maximum of 25% in June; and borneol, from a maximum of 25% in April to a minimum (5%) in December. On the other hand, these results are somewhat contradictory with those of Grella and Picci (*10*), who reported an abrupt drop in the levels of these three compounds from their maximum values, at the beginning of March, to their minimum values at the beginning of May. Variations in the levels of α-pinene and camphene were also not coincident with those reported by Grella and Picci (*10*). Some coincidence exists, however, in the variation of α-humulene and *β*-pinene, the levels of which, either in Arcos de Valdevez or in Arouca, increased from the middle of winter to spring, reaching their maximum levels in April. Maximum levels of camphene, α-pinene, and *β*-caryophyllene were also reached in April (Figure 3). At both sites, the increase in the levels of viridiflorol to maximum values occurred early. The same was true for the diterpenoid manool in Arcos de Valdevez. However, in Arouca the variation of this compound was somewhat erratic (Figure 3).

According to some authors (*11*) most of the seasonal changes in sage oil composition are associated with flowering. According to these authors, at flowering time, which, in New Zealand, occurs by November and December, thujones as well as camphor are at their minimum levels, whereas *β*-pinene and 1,8-cineole are at their maximum levels. In Portugal, sage flowering usually occurs from May to July, some years beginning in April depending on the variability of specific weather conditions of the previous winter season. In the years of 1998 and 1999 sage flowering time began in May and continued to the end of July either in Arcos de Valdevez or in Arouca. Our results, like those of Putievsky et al. (*9*), show that α-thujone and camphor levels both start rising, from their minimum values, in April. However, although α-thujone levels increase through December, camphor, after reaching its maximum by July, starts decreasing thereafter to its low levels, which are maintained from around December to April. As the more drastic variations in the levels of α-thujone and camphor, in correlation with the inverse variation of *β*-pinene, *β*-caryophyllene, α-humulene, and viridiflorol, occurred before the flowering time, changes in the composition of the sage essential oils may be associated with metabolic changes that precede and prepare the sage flowering.

In our sampling method, leaves of different ages from the 20−25 cm shoot distal parts were indiscriminately used as the source of essential oils. However, the type of tissue and age of predominant organs, at the time of sampling, may be determinant in the composition of the sage essential oils. The relative amount of α-thujone in sage plant leaves has been reported to decrease from the top to the base node, whereas the amounts of camphor, α-pinene, and camphene increase (*7*). It is not possible, however, to say that variations of these last three compounds are correlated or interdependent with that of α-thujone. According to some authors, as the plant approaches maturity the content of camphor declines by roughly half, in fully expanded leaves, being metabolized to a water-soluble metabolite via its lactonization to 1,2-campholide followed by conversion to the *β*-D-glucoside-6-*O*-glucose ester of the corresponding hydroxy acid [1-carboxymethyl-3-hydroxy-2,2,3-trimethylcyclopentane (*14*)] and metabolized through a degradative pathway in sequence, to 6-hydroxycamphor, 6-oxocamphor, α-campholonic acid, and 2-hydroxy-α-campholonic acid (*15*). The decrease in the camphor level with leaf maturity reported by these last authors, in a certain way, is contradictory with the data reported by Länger et al. (*7*) for this compound. Much more work is certainly needed to understand the variations in the contents of such compounds in sage. Variations of the essential oil content and composition in *S. officinalis* are also greatly influenced by the light level (*6*).

Little seems to be known of the effect of the individual compounds from the essential oil of sage, namely, the minor ones, on the respective aroma and other properties. The same lack of knowledge can be invoked for the effects of the α- to *β*-thujone ratios, as was stated by Perry et al. (*11*). According to Bruneton (*16*), the profile defined by standard ISO 9909 (1999) for official sage oil is α-thujone (18−43%), *β*-thujone (3−8.5%), camphor (4.5−24.5%), cineole (5.5−13%), humulene (0−12%), α-pinene (1−6.5%), camphene 1.5−7%), limonene (0.5−3%), linalool [free and esterified (1% maximum)], and bornyl acetate (2.5% maximum). The majority of the percentages of these compounds in the sage essential oils analyzed at the different times and sites referred in Table 2 match the ranges of the standard ISO 9909. In the analyses that we have been performing we found linalool unequivocally in essential oils of in vitro sage shoots (results not shown). The higher discrepancies of our results in relation to the standard ISO 9909 concerned the α-thujone and were found in the samples from Arcos de Valdevez and from Arouca collected in December, which showed α-thujone levels of 55.17% (±0.53%) and 46.23% (±0.54%), respectively, and in the sample from Arcos de Valdevez collected in April (α-thujone, 11.82 ± 0.51%). To the highest level of α-thujone, in December, corresponded the lowest level of borneol, and to the lowest level of α-thujone, in April, corresponded the highest level of borneol. The biosynthesis of these compounds follows different pathways (*11*), and our results suggest that they may be affected in inverse correlation, directly or undirectly, by factors that interfere in the life cycle of the plant.

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