Essential Oils from Dalmatian Sage (*Salvia officinalis* L.): Variations among Individuals, Plant Parts, Seasons, and Sites

Nigel B. Perry,*,† Rosemary E. Anderson,‡ Nerida J. Brennan,† Malcolm H. Douglas,‡ Anna J. Heaney,‡ Jennifer A. McGimpsey,‡ and Bruce M. Smallfield‡

New Zealand Institute for Crop and Food Research Ltd., Plant Extracts Research Unit, Chemistry Department, University of Otago, P.O. Box 56, Dunedin, New Zealand, and Invermay Agriculture Centre, Private Bag 50034, Mosgiel, New Zealand

The factors affecting oil yield and quality of essential oils from Dalmatian sage (*Salvia officinalis* L.) are analyzed. Distillations of oils from individual plants and GC analyses revealed the presence of three chemotypes with different proportions of α- and *β*-thujone (α/*β* 10:1, 1.5:1, and 1:10). Different accessions could also be classified as having high (39−44%), medium (22−28%), or low (9%) total thujone contents. Flowering parts of *S. officinalis* had higher oil contents (1.6 versus 1.1%) and *β*-pinene levels (27 versus 10%) than leaves and lower thujone levels (16 versus 31%). Major seasonal changes were found in the composition of oil distilled from a flowering type of Dalmatian sage, but oil yields from healthy, established plants did not vary greatly. Total thujone levels were lowest (25%) around flowering in spring and summer, so autumn or winter was the best harvest time to obtain oils with high thujone levels.

Keywords: *Dalmatian sage; Salvia officinalis; essential oil; variation; thujones*

INTRODUCTION

Dalmatian sage (*Salvia officinalis* L., family Lamiaceae) is widely used as a savory food flavoring (Tucker et al., 1980), either as the dried leaves (ISO, 1995) or concentrated as the essential oil or oleoresin (Heath, 1978). Good quality Dalmatian sage oils contain a high percentage (>50%) of the epimeric α- and *β*-thujones (Figure 1) and a low proportion (<20%) of camphor (Guenther, 1949; Putievsky et al., 1992), although a recent standard allows 18−43% α-thujone and 3−8.5% *β*-thujone (ISO, 1997). These monoterpene ketones are toxic and the permitted proportion of α- and/or *β*-thujone in food flavorings is 0.0005 g/kg (Tisserand and Balacs, 1995). The thujones and camphor are products of two separate biosynthetic pathways (Croteau, 1987), and two further pathways lead to the other major monoterpenes (Figure 1). A variety of sesquiterpenes and a diterpene are also found in Dalmatian sage oils (Boelens and Boelens, 1997; Perry et al., 1996). We are investigating *S. officinalis* for commercial production, as part of a developing essential oil and extract industry in New Zealand (Perry et al., 1993; Catchpole et al., 1996). We have reported a rapid gas chromatographic (GC) analytical method and some preliminary results (Perry et al., 1996). Our aim was to identify plants that had high contents of oils with desirable compositions. We also needed to understand the key agronomic factors affecting oil yield and composition, such as site and time of harvest, to produce reliable management guidelines for commercial growers.

Franz (1993) stated that compositional variation within a species appears to be the rule rather than the exception in essential oil crops. He suggested that evaluation of this variation involves the study of at least three major factors: (1) individual genetic variability; (2) variation among different plant parts and different developmental stages; and (3) modifications due to the environment. The literature, reviewed recently by Boelens and Boelens (1997), indicated that all of these factors would be important for Dalmatian sage oils.

The study of individual variability clearly required separate distillations from individual plants. However, most papers on Dalmatian sage have used oils distilled from combined plant samples. Such samples may pool together plants of different chemotypes, because results on another member of the Lamiaceae, *Thymus vulgaris* L., have shown that chemically distinct individuals can grow side-by-side (Granger and Passet, 1973). Tucker and Maciarello (1990) have proposed chemotypes of Dalmatian sage with either α-thujone, *β*-thujone, camphor, or 1,8-cineole (Figure 1) as the main component. We now report the variation of essential oil contents and compositions from individual plants of nine different Dalmatian sage accessions (i.e., different seedlines or cultivars) and the occurrence of plants with different thujone chemotypes within a single accession.

It seemed likely that different parts of Dalmatian sage plants would have different oil compositions, because leaves of *Salvia sclarea* L. (clary sage) contained different mixtures of volatile components from those in the flowering parts (Boelens and Boelens, 1997). Guenther (1949) quotes a 1928 report of lower camphor and thujone levels in oil from whole Dalmatian sage plants in flower in summer, compared to oil from vegetative plants in winter. Bouverat-Bernier and Marquis (1993) also found lower camphor and thujone levels in flowering plants, accompanied by higher levels of *β*-pinene and 1,8-cineole. Piccaglia et al. (1997) found that these compositional differences were statistically significant

* Author to whom correspondence should be addressed (fax +64-3-479-8543; e-mail perryn@crop.cri.nz).
† Plant Extracts Research Unit.
‡ Invermay Agriculture Centre.

10.1021/jf981170m CCC: $18.00
Published on Web 04/29/1999

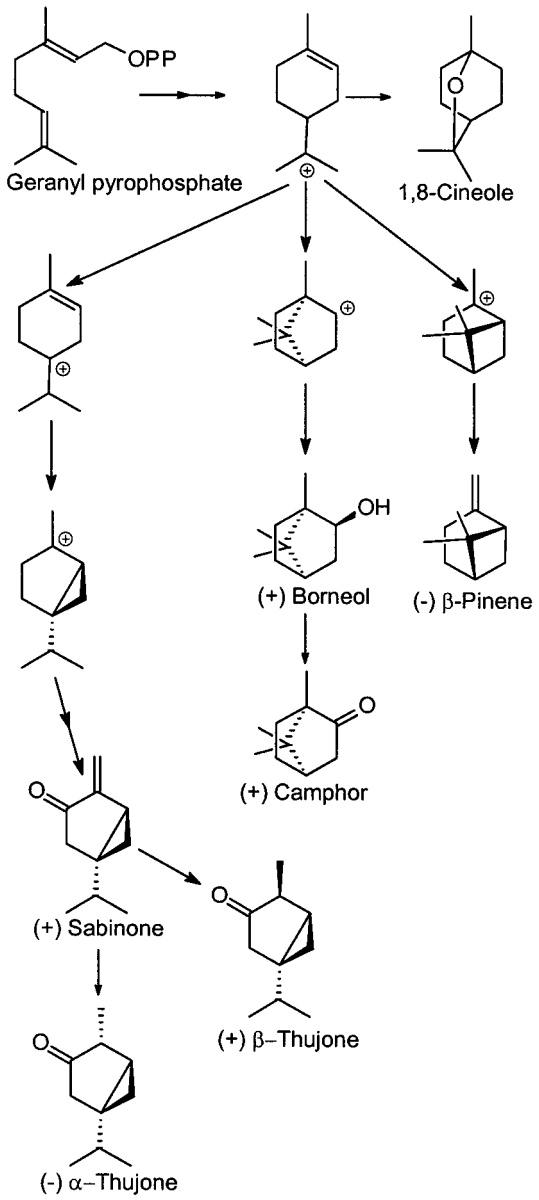

Figure 1. Biosynthetic routes to the major monoterpenes of Dalmatian sage.

and also found some significant differences between oil yields from flowering and vegetative sage. These three studies compared samples harvested on different dates, so developmental and environmental factors could also have been important. We now report different oil contents and compositions from separated leaves, stems, and flowers harvested at the same time.

Three other papers report the seasonal variation of Dalmatian sage oils distilled from leaves alone (Pitarevic et al., 1984; Putievsky et al., 1986; Grella and Picci, 1988). These studies agreed in finding high thujone levels in late summer, autumn, and winter. Putievsky et al. (1986), studying cultivated, clonally propagated plants in Israel, found that total thujones varied from 8% in April (spring) to >40% in December (winter). Grella and Picci (1988) studied *S. officinalis* grown in Sardinia and found that thujone and camphor levels dropped sharply in April and May (spring), with corresponding increases in β-pinene and α-humulene levels. However, no statistical analyses were presented in these studies. We now report the results of a replicated study of the seasonal variation of oil yield and composition from one accession of Dalmatian sage. This study was run at two sites to enable modifications due to the environment to be quantified.

MATERIALS AND METHODS

Plant Material for Individual Variability Study. Dalmatian sage accessions were obtained between 1988 and 1992 and established at Redbank Research Station (latitude 45° 14′ S, longitude 169° 20′ E). Eight flowering types were grown from small quantities of seed obtained from botanical gardens and commercial seed supply companies, and one nonflowering type was grown from cuttings (Table 1). Accessions were grown in rows 1 m apart with 30 cm between plants. Irrigation was applied regularly during the growing season. Fertilizer was applied annually according to soil test results to ensure soil fertility was not limiting growth. Plants were trimmed once each year during flowering. Leafy shoots 8−12 cm in length were harvested from individual plants on August 9−12, 1994, dried (air flow at 35 °C) until stems were crisp, and then stored in paper bags in a dark room until distillation. The herb was chopped into 0.5−1 cm pieces immediately before distillation. Subsamples of chopped herb were dried to constant weight at 80 °C to determine dry matter (DM) content. A voucher specimen from each plant was kept at Redbank.

Material for Plant Parts Study. Guard plants from the seasonal trial (see below), previously unharvested, were harvested at Invermay Agriculture Centre (45° 50′ S, 170° 15′ E) on December 3, 1996, when all plants were in full flower. These were cut as described below, and then two replicate samples were separated into flower-only, leaf-only, and stem-only samples.

Material for Season and Site Study. Seedlings of accession 04 (Table 1) were grown in cell trays and then transplanted at Redbank and Invermay on December 15, 1994, into rows 25 cm apart with 25 cm between plants. Each plot consisted of six plants (in two rows) to give an area of 0.375 m^2, separated from adjacent plots by a single row of guard plants. Irrigation was applied regularly during the growing season at Redbank but only during establishment at Invermay. Fertilizer was applied annually (400 kg of Nitrophoska Blue TE/ha, containing 12% N, 5% P, 14% K, 4% S, 1.2% Mg, and 7% Ca plus micronutrients) to ensure soil fertility was not a factor limiting growth. The area was sprayed with trifluralin (Treflan, 2 l/ha) before transplanting to control weeds during establishment; weeds were further controlled by hand. The full experiment involved 38 cutting treatments (of single-, double-, and triple-harvest combinations in an 18 month cycle) at each site, replicated in four randomized blocks, but only the findings for the 15 single-harvest treatments are presented here. The first harvest was in March 1995 and the last was in May 1996, with harvesting as close to the 20th of each month as fine weather would allow (see Supporting Information for climate data at the two sites over this period). Plants were trimmed 7.5−10 cm above ground. The fresh weight of herbage was recorded, and subsamples were taken for DM content and for plant component dissection (green leaf, brown leaf, green stem, flower, and dead stem as percent DM; see Supporting Information for full results). Fresh herb was stored in a chiller (1−3 °C, maximum 4 days) until it was chopped and immediately distilled.

Extraction of Essential Oils. Laboratory-scale steam distillation systems were used. The charge vessel was a 700 mm × 110 mm i.d. column, wrapped in 2.5 mm flexible insulation, and fitted with a single socket flat flange lid, with steam piped in at the center of the column's dome base. Two stills were operated in tandem, one glass and one stainless steel. Double-surface condensers were used, and distillates were collected in 500 mL cylindrical separating funnels. A stainless steel mesh 600 mm below the top flange of a column was covered with clean barley straw and the chopped sage packed evenly on top of this (density = 0.2 g/mL) to fill the column. Samples (40−250 g for the individual plant compari-

Table 1. Individual Plants: Accession Numbers, Oil Contents, and Compositions

					thujone levels[e]		
acc no.	source[a]	type[b]	n[c]	oil content[d] (%)	total[d]	α[d]	β[d]
4	Germany (C)	F	12	1.2 (0.8−1.7)	39 (29−46)	32 (3−42)	8 (3−32)
6	Hungary (B)	F	11	1.0 (0.4−1.6)	22 (9−34)	11 (1−39)	11 (2−28)
7	Denmark (B)	F	6	1.2 (0.7−1.8)	40 (28−48)	37 (31−43)	3 (3−4)
9	New Zealand (C)	F	9	1.5 (0.7−2.2)	44 (27−54)	41 (25−50)	3 (2−4)
11	Switzerland (B)	F	2	1.4 (1.2−1.6)	23 (21−26)	7 (1−12)	17 (8−25)
13	Canada (C)[f]	F	10	1.5 (1.3−2.0)	43 (37−49)	39 (31−42)	4 (3−7)
15	New Zealand (R)	NF	5	1.8 (1.5−2.1)	9.3 (9−10)	8 (8−9)	1 (0.9−1)
16	Italy (B)	F	8	1.5 (1.2−1.9)	43 (36−48)	39 (33−44)	4 (3−5)
18	Hungary (B)	F	4	1.4 (1.2−1.5)	28 (6−40)	25 (6−33)	2 (1−4)

[a] B, botanical garden; C, commercial seed supplier; R, crop and food research. [b] F, flowering; NF, nonflowering. [c] Number of individual plants distilled separately. [d] Mean (range). [e] As percent of total GC peak areas. [f] Oil contents from nine plants only.

sons and 69−1519 g for the seasonal study) were distilled for 60 min. In the seasonal study at Invermay up to July 1995, herb yields were low so samples from replicate plots were combined for distillation. After each distillation the oil was separated and its volume measured before drying over anhydrous sodium sulfate. Subsamples for GC analyses were stored at −18 °C. Oil content was calculated as percent (milliliters per 100 g of DM), and the oil yield was calculated as milliliters per square meter of growing area (see Supporting Information for oil yields from the seasonal study). The significance of differences was tested by analysis of variance (ANOVA) and is represented by mean standard errors of difference (SED) calculated with Genstat 5.3 software.

GC Analyses. Oils were diluted to 1% solutions in hexane containing 0.05% each of dodecane and octadecane. These alkanes were used as references to correct for retention time fluctuations between runs. The column was a 10 m J&W DB-1, with H_2 carrier gas (linear velocity = 55 cm/s). Injections (0.5 μL) were made into a split (100:1) injector at 260 °C, the column temperature was programmed from 50 to 250 °C at 30 °C/min, and the flame ionization detector was at 350 °C. The levels (peak area as percent of total of peak areas) of 27 peaks, previously identified by GC/MS (Perry et al., 1996), were used for statistical analyses with SAS Institute software (see Supporting Information for full GC results). The unscaled levels of these 27 peaks in each oil were initially analyzed using principal components analysis to help with recognition of composition patterns. The significance of differences in component levels between accessions were tested by ANOVA general linear models procedure, because of differences in levels of replication.

RESULTS AND DISCUSSION

Individual Variability. Individual plants from nine different Dalmatian sage accessions (eight flowering and one nonflowering, Table 1) were grown at one site and harvested at one time, in a vegetative state in winter, to minimize developmental and environmental differences. The oils from 67 individual plants were extracted by steam distillation of leaves plus stems.

Plants of the nonflowering accession 15 had the highest mean oil content, but this was not significantly ($P > 0.05$) higher than some of the flowering accessions (Table 1). Our values covered about the same range (0.4−2.2%) as Dalmatian sage from a variety of European sources (0.4−2.5%) (Svoboda and Deans, 1992). There was substantial variation in individuals from a single accession, for example, 0.7−2.2% for accession 09. Because oil content has been shown to be heritable in other species (Franz, 1993), it might be possible to develop Dalmatian sage cultivars with higher oil contents.

The results of GC analyses (Perry et al., 1996) of the 67 oils were subjected to principal component analysis (PCA) to try to distinguish compositional patterns (Aries et al., 1991). The first and second principal components (PCs) accounted for 83% of the total variance of the data set. The main contributor to the first PC (71% of the total variance) was α-thujone (eigenvector +0.95), and the main contributors to the second PC were β-thujone (eigenvector +0.78) and β-pinene (eigenvector −0.42). Figure 2 shows the 67 oils plotted in terms of these two PCs.

The five oils from the nonflowering accession 15 formed a tight cluster, indicating very similar compositions. This was to be expected, as these clonally propagated plants should have no individual genetic variability. These oils had total thujone levels significantly ($P < 0.05$) lower than those of any of the flowering accessions (Table 1). There were also significant differences between nonflowering and flowering accessions for many other components, confirming the compositional differences that we noted previously (Perry et al., 1996). It may be that the nonflowering types of Dalmatian sage (MAFF, 1980) have been propagated from sterile hybrids of *S. officinalis* with another *Salvia* species that had distinct mono- and sesquiterpene chemistry.

Flowering-type Dalmatian sage accessions could be divided into two distinct groups based on total thujone levels (Table 1): accessions 06, 11, and 18, with mean total thujone levels of 22−28%; and accessions 04, 07, 09, 13, and 16, with mean total thujone levels of 39−44%. These seem to correspond to the "low test" and "high test" Dalmatian sage oils described by Guenther (1949).

The oils from individual flowering Dalmatian sage plants, grown from seed, showed a wide range of compositions. Oils from accessions 07, 09, 13, and 16 all clustered high on the first PC and somewhat lower on the second PC, indicating consistently high α/β thujone ratios (Figure 2). On the other hand, oils from accessions 04, 06, 11, and 18 had a wide range of α/β thujone ratios (Figure 2). Most of the individual flowering plant oils could be grouped into three qualitative thujone chemotypes, based on their α/β thujone ratios: ∼10:1 α/β, ∼1.5:1 α/β, and ∼1:10 α/β (Figure 2). Chalchat et al. (1998) also found high, intermediate, and low α/β thujone ratios in *S. officinalis* oils. Croteau (1987) has suggested that the biosynthesis of α- and β-thujone diverges only at the last step, the NADPH-dependent reduction of (+)-sabinone (Figure 1) (Croteau, 1987). The three thujone chemotypes could be explained by different degrees of expression of two stereoselective reductases. It is not known how these different α- to β-thujone ratios affect the aroma of Dalmatian sage oils, because there do not seem to be any published com-

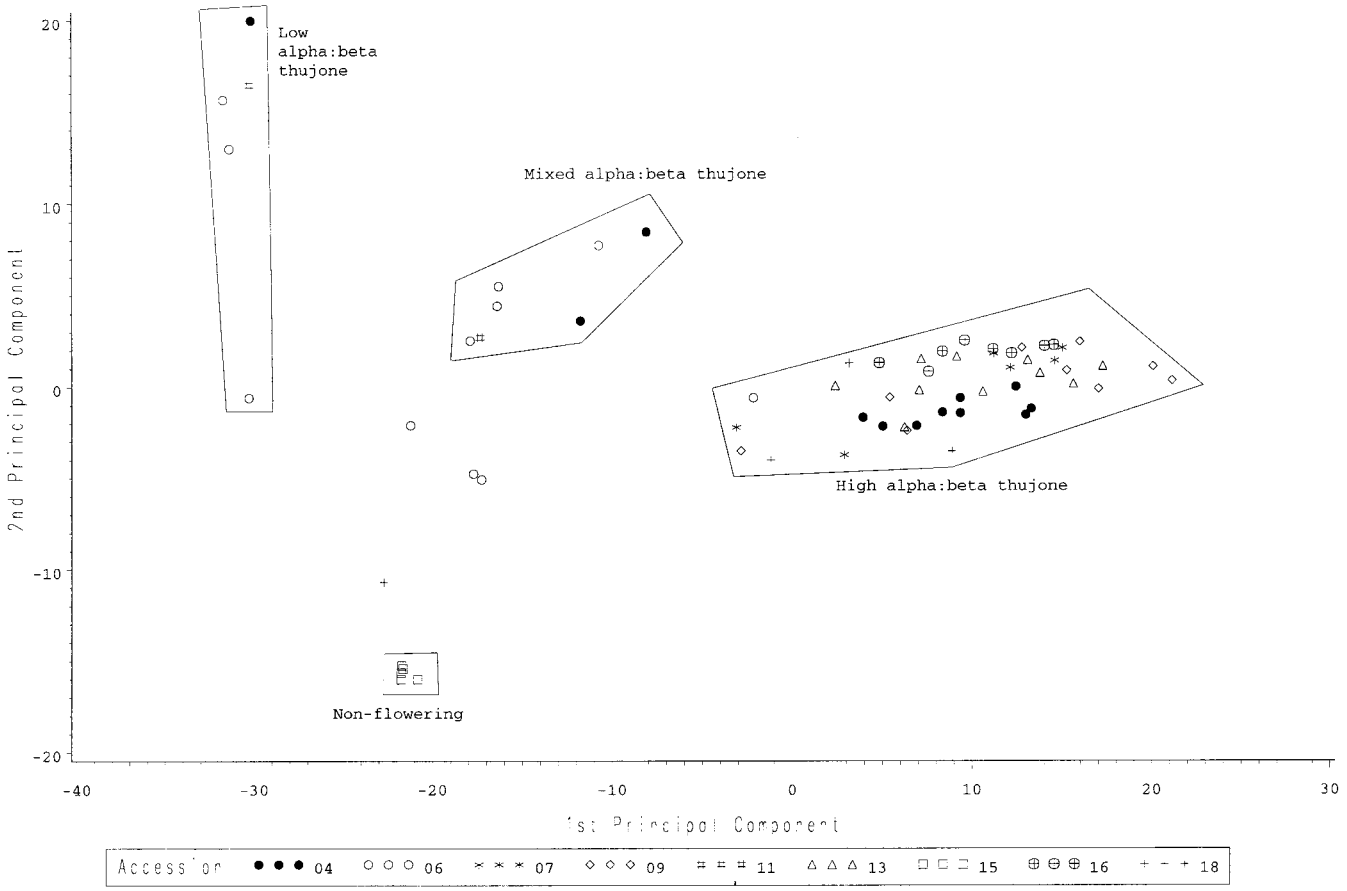

Figure 2. Individual plants: PCA of oil compositions.

Table 2. Plant Parts: Dissection, Oil Contents, and Compositions

part	% of sample[a]	oil content[b]	total thujones[c]	β-pinene[c]
flowering parts	19	1.56	16	27.1
leaves	15	1.11	31	9.9
stems	66	0.05	37	7.6
mean standard error of difference		0.03	2	0.9

[a] From representative dissected samples, on a DM weight basis. [b] Percent, mean of two values. [c] As percent of total GC peak areas, mean of two values.

parisons of the aromas of pure α-thujone and pure β-thujone [Arctander (1969) describes only the odor of "thujone"].

There was marked variability among individual flowering plants in levels of all the other oil components. In particular, 1,8-cineole levels showed a bimodal distribution, with most oils containing 3−12%, but three oils having >15% (including both oils from plants of accession 11). One oil from accession 06 stood out for its camphor level of 19%. Therefore, our results support the proposal of Tucker and Maciarello (1990) to classify Dalmatian sage oils into chemotypes on their relative levels of α- and β-thujone, 1,8-cineole, and camphor.

Plant Parts. Dalmatian sage plants (accession 04, Table 1) were harvested during full flowering, and flowering parts, leaves, and stems were steam distilled separately. Flowering parts had significantly ($P < 0.05$) higher oil contents than leaves (Table 2). Mathe et al. (1992) found similar values for leaf (0.81 mg/100 g of DM) and "generative organs" (1.59 mg/100 g of DM) of *S. officinalis* harvested in Hungary. Stems had low oil contents, so they would make only a minor contribution to the composition of "whole" sage oils.

Flower oils were highest in β-pinene, and leaf oils were highest in thujones (mainly α-thujone) (Table 2). There were also significant differences between flower and leaf oils in the levels of other monoterpenes and of sesquiterpenes, but the levels of the diterpene manool were similar in both oils. This was surprising because a closely related diterpene, sclareol, differs in level between leaves and flowers of clary sage, *S. sclarea* (Boelens and Boelens, 1997).

The different oil compositions of leaves and flowering parts explain the reports of lower thujone levels in oil from whole sage plants in flower in summer, compared to oil from vegetative plants in winter (Guenther, 1949; Bouverat-Bernier and Marquis, 1993; Piccaglia et al., 1997). However, our detailed study of seasonal variation (see below) has shown that other developmental factors are important.

Season and Site. Flowering Dalmatian sage accession 04, with high total thujone levels and the high α-thujone chemotype predominant (Table 1), was chosen for a study of the effects of developmental and environmental factors on oil yield and composition. Plants were harvested monthly at two sites for 15 months. The main climatic difference between the sites was in summer temperatures, with the inland (Redbank) site warmer than the coastal (Invermay) site. Oil was distilled from the total herb, that is, leaves, stems, and flowers when present, to determine the potential production by mechanical harvesting.

The Dalmatian sage seedlings established more quickly at Redbank than at Invermay, so herb DM yields were

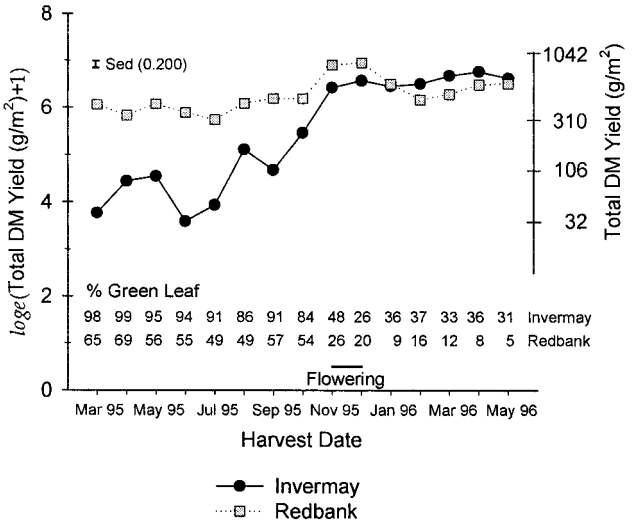

Figure 3. Season and site: herb yields and proportions of green leaf.

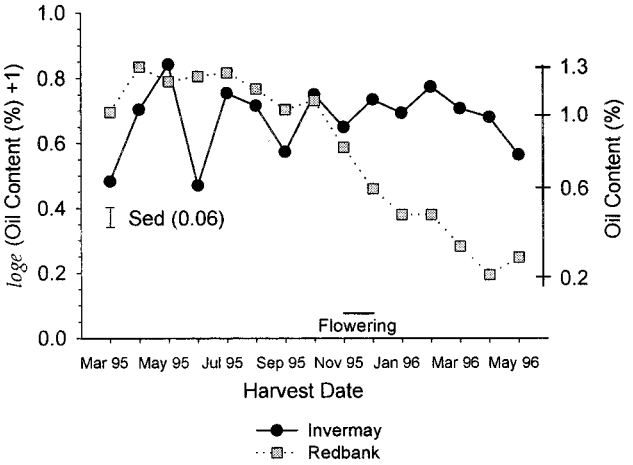

Figure 4. Season and site: oil contents.

significantly ($P < 0.01$) higher at Redbank up to October 1995 (Figure 3). However, similar maximum herb yields were obtained at both sites from November onward. The proportion of green leaf declined markedly at both sites in November, corresponding to the start of flowering (Figure 3). The proportion of flower reached a maximum of 20% of herb DM in both November and December harvests at Redbank, but this value was reached in only the December harvest at Invermay. After flowering, herb from Invermay contained much greater proportions of green leaf (and less stem) than herb from Redbank, because Redbank plants failed to produce new shoots (Figure 3).

Oil content of Dalmatian sage varied significantly with both harvest date and site (Figure 4). After November, the oil content of herb from Redbank was significantly ($P < 0.05$) lower than that of herb from Invermay. This could be explained by the low oil content in stems (Table 2) and the higher proportion of stems in Redbank herb after flowering. The high oil content of flowering parts did not lead to a maximum in whole herb oil content during flowering (Figure 4), because the proportion of oil-rich green leaves dropped markedly to ~30% of the total herb DM harvested at this time (Figure 3). This finding contrasts with our results on thyme (McGimpsey et al., 1994) and the general trend with other herbs in the family Lamiaceae (Hay, 1993).

Table 3. Season and Site: Oil Compositions and Statistical Summary

component	level[a]	change at flowering[b]	treatment effects[c] date	site	date × site
α-pinene	2.6 (0.9−4.9)	−	*	**	NS
camphene	2.1 (0.5−4.2)	↓	**	**	NS
β-pinene	6.0 (2.2−19.0)	↑	**	NS	**
myrcene	0.9 (0.6−1.2)	−	**	NS	**
1,8-cineole	9.2 (4.6−16.4)	↑	**	NS	NS
(*Z*)-ocimene	0.4 (0.3−0.5)	−	**	**	*
α-thujone	34.6 (15.0−53.2)	↓	**	*	NS
β-thujone	5.0 (2.1−12.6)	−	**	NS	NS
camphor	6.5 (0.9−13.6)	↓	**	**	**
borneol	2.4 (0.4−6.8)	↑	**	NS	NS
bornyl acetate	0.4 ((0.1−1.5)	−	**	NS	*
α-copaene	0.1 (0.0−0.3)	−	**	NS	*
α-gurjunene	0.1 (0.0−0.3)	−	**	NS	NS
β-caryophyllene	4.5 (1.0−11.5)	↑	**	NS	**
β-cubebene	0.1 (0.0−0.4)	−	**	NS	*
aromadendrene	0.5 (0.1−1.9)	−	*	NS	*
α-humulene	6.2 (2.8−11.6)	−	**	*	**
α-amorphene	0.1 (0.0−0.5)	−	**	NS	NS
germacrene D	0.3 (0.1−1.2)	−	**	NS	NS
bicyclogermacrene	0.2 (0.0−0.6)	−	**	*	**
unknown	0.3 (0.0−0.8)	−	**	NS	**
δ-cadinene	0.4 (0.0−0.8)	−	**	NS	NS
palustrol + spathulenol	0.1 (0.0−0.3)	−	**	NS	**
caryophyllene oxide	0.7 (0.1−2.9)	−	**	**	**
viridiflorol	4.5 (1.3−9.1)	−	**	NS	NS
α-humulene oxide	0.9 (0.2−2.3)	−	**	**	*
manool	1.1 (0.3−5.1)	−	**	NS	**
total thujones	39.6 (17.2−57.7)	↓	**	*	NS

[a] By GC, as percent of total area of listed components, for 102 oils (range). [b] −, no clear change in level associated with flowering; ↑, increase; ↓, = decrease. [c] Significance of treatment effects: NS, $P > 0.05$; *, $0.05 > P > 0.01$; **, $0.01 > P$.

We had expected the hotter conditions of the inland site to favor higher oil yields (calculated from herb yield and oil content) from Dalmatian sage. However, the maximum oil yields, calculated from herb yield and oil content, did not differ significantly ($P > 0.05$): coastal mean = 81.4 l/ha, inland mean = 78.8 l/ha. Oil yields were dependent on the amount of leaf and/or flower harvested and did not vary greatly with season for healthy, established plants.

There were significant effects of harvest date on the levels of all the oil components, plus some site effects and date × site interaction effects (Table 3). The patterns of variation were visualized by plotting mean component levels at the two sites against harvest date, as shown for the major components in Figures 5−8. The variations of sesquiterpene and manool levels are not discussed here because these are not generally considered important for Dalmatian sage oil quality.

The overall levels of total thujones (mainly α-thujone) and camphor differed between the Invermay and Redbank sites (Table 3). The significant differences ($P < 0.05$) in total thujone levels were from January to May 1996 (Figure 5) when the Invermay plants began to produce new leaves but the Redbank plants did not. Langer et al. (1993) found that α-thujone levels decreased as Dalmatian sage leaves aged.

Figures 5−8 show that most of the seasonal changes in Dalmatian sage oil composition were associated with flowering. Mean total thujone levels dropped sharply, and significantly, from >40% over March to September 1995 to a minimum of ~25% in November and Decem-

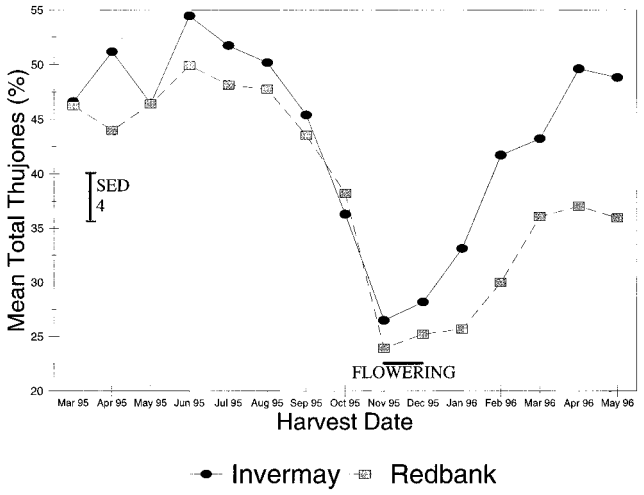

Figure 5. Season and site: total thujone levels.

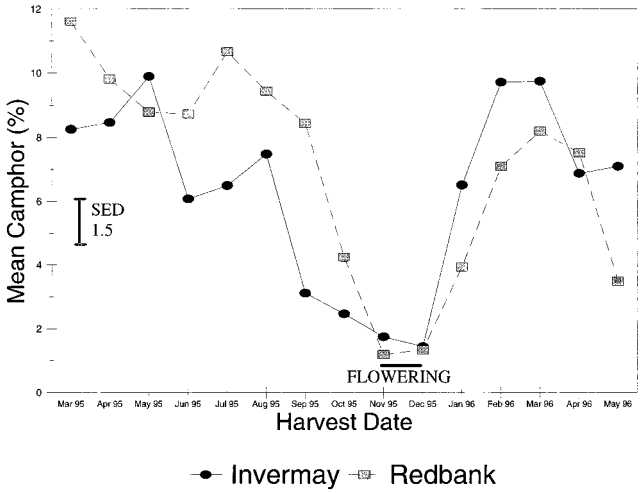

Figure 6. Season and site: camphor levels.

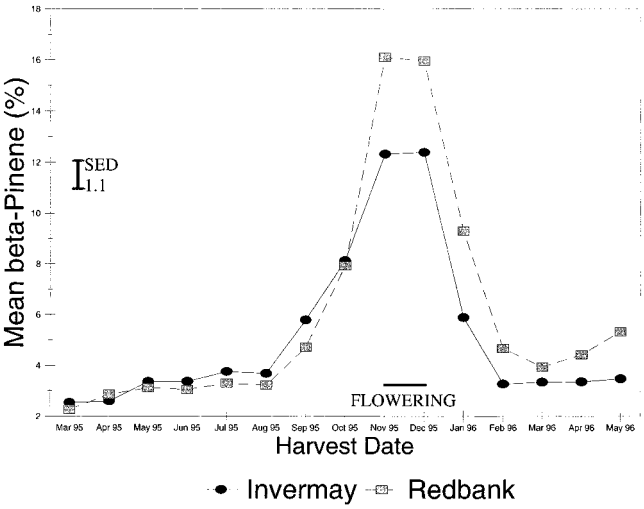

Figure 7. Season and site: β-pinene levels.

ber at both sites (Figure 5). Levels of camphor, the other major ketone, were also at their lowest during flowering (Figure 6). Two of the other major monoterpenes reached their highest levels during flowering: β-pinene (Figure 7) and 1,8-cineole (Figure 8). Levels of borneol also rose significantly at flowering but reached a peak at both sites the month after flowering.

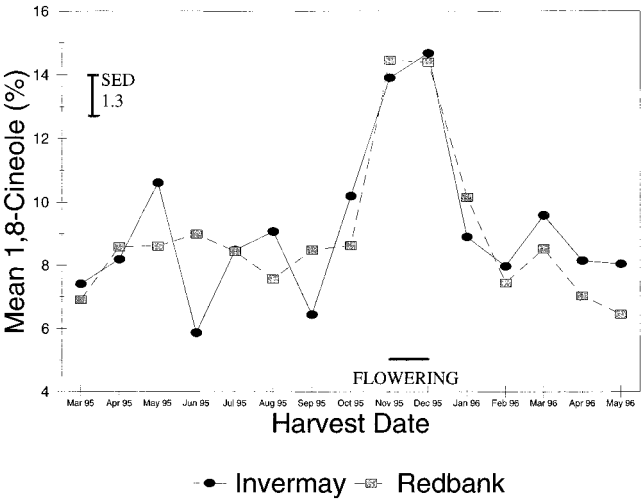

Figure 8. Season and site: 1,8-cineole levels.

These results on monthly changes in oil composition from "whole" (leaf, stem, and flower) Dalmatian sage largely confirm the results of more limited seasonal studies (Guenther, 1949; Bouverat-Bernier and Marquis, 1993; Piccaglia et al., 1997). Many of the changes can be explained by the different compositions of flower and leaf oils (see above). In particular, β-pinene levels were higher and thujone levels were lower in flowering parts than in leaves (Table 2), leading to the dramatic changes in the levels of these components over the season (Figures 5 and 7).

However, flowering was not the only reason for seasonal changes in the composition of Dalmatian sage oils. Camphor levels dropped significantly ($P < 0.05$) in September at Invermay and November at Redbank, before flowering (Figure 6). We have also found a significant springtime drop in thujone levels of oils from nonflowering sage (unpublished results). Previous work on seasonal variation of oils, distilled from leaves only, found lower thujone and camphor levels in spring (Pitarevic et al., 1984; Putievsky et al., 1986; Grella and Picci, 1988). Piccaglia et al. (1997) found that levels of thujones, camphor, and 1,8-cineole were significantly lower in spring, and β-pinene and borneol levels did not change significantly. These changes could be due to higher proportions of young leaves in spring, because young leaves have oil compositions different from those of mature leaves (Langer et al., 1993). Croteau and coworkers have shown that camphor biosynthesis was at its peak in 4-week-old Dalmatian sage leaves (Croteau, 1987).

Conclusions. These results show that many factors affect the yield and composition of essential oils from Dalmatian sage, including plant source, individual plant chemotypes, time of harvest, and the proportions of plant parts distilled. Understanding these factors can make the difference between a good yield of a high-quality oil and a poor yield of an undesirable oil. Environmental effects were important only indirectly, by determining plant development and therefore the proportions and ages of the plant parts distilled.

These results for *S. officinalis* contrast with our results on seasonal variation of another Lamiaceae species, *Thymus vulgaris*, in which we also found major changes in oil composition during flowering (McGimpsey et al., 1994). In *T. vulgaris* the highest level of the main quality component, thymol, occurred during flowering,

when the oil yield was also highest. In *S. officinalis* the levels of the quality components, the thujones, were lowest around flowering and oil yields did not maximize during flowering. To obtain Dalmatian sage oils with high thujone levels (>45%), healthy vegetative plants should be harvested in autumn or winter. If the toxicity of thujones (Tisserand and Balacs, 1995) is a concern, low thujone accessions should be grown and harvested in spring or during flowering.

ACKNOWLEDGMENT

We thank M. McCutcheon for assistance with harvests and distillations, B. Swanney for climate data, J. van Klink for assistance with GC analyses, and P. Johnstone and R. Littlejohn for advice on statistical analyses.

Supporting Information Available: Climate summaries and herbage and oil yield data on the seasonal study plus full GC analyses of 67 oils from individual plants, 102 oils from the seasonal study, and 8 oils from separated plant parts. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

Arctander, S. *Perfume and Flavor Chemicals (Aroma Chemicals)*; Arctander: Montclair, NJ, 1969; Vol. 2.

Aries, R. E.; Lidiard, D. P.; Spragg, R. A. Principal component analysis. *Chem. Br.* **1991**, 821−824.

Boelens, M. H.; Boelens, H. Chemical and sensory evaluation of three sage oils. *Perfum. Flavor.* **1997**, *22* (March/April), 19−40.

Bouverat-Bernier, J.-P.; Marquis, N. Premiers résultats de sélection de la sauge officinale. *Riv. Ital. EPPOS* **1993**, (special no.), 581−592; as cited by: Lawrence, B. M. Progress in essential oils. *Perfum. Flavor.* **1998**, *23* (March/April), 47−57.

Catchpole, O. J.; Grey, J. B.; Smallfield, B. M. Near-critical extraction of sage, celery and coriander seed. *J. Supercrit. Fluids* **1996**, *9*, 273−279.

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

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

Franz, Ch. Genetics. In *Volatile Oil Crops: Their Biology, Biochemistry and Production;* Hay, R. K. M., Waterman, P. G., Eds.; Longman: Harlow, U.K., 1993; pp 63−96.

Granger, R.; Passet, J. *Thymus vulgaris* spontané de France: Races chimiques et chemotaxonomie. *Phytochemistry* **1973**, *12*, 1683−1691.

Grella, G. E.; Picci, V. Variazioni stagionali dell'olio essenziale di *Salvia officinalis*. *Fitoterapia* **1988**, *54*, 97−102.

Guenther, E. Oil of sage. In *The Essential Oils*; Krieger: Malabar, FL, 1949; Vol. 3, pp 710−717.

Hay, R. K. M. Physiology. In *Volatile Oil Crops: Their Biology, Biochemistry and Production;* Hay, R. K. M., Waterman, P. G., Eds.; Longman: Harlow, U.K., 1993; pp 30−33.

Heath, H. B. Sage. In *Source Book of Flavours*; AVI: Westport, CT, 1978; pp 261−263.

ISO Dried sage (*Salvia officinalis* L.) Specification. *ISO 11165*, **1995**.

ISO Oil of Dalmatian sage (*Salvia officinalis* L.). *ISO 9909*, **1997**.

Langer, R.; Mechtler, Ch.; Tanzler, H. O.; Jurenitsch, J. Differences of the composition of the essential oil within an individuum of *Salvia officinalis*. *Planta Med.* **1993**, *59* (Suppl.), A635−A636.

MAFF. Culinary and medicinal herbs. In *Ministry of Agriculture, Fisheries and Food Reference Book*; HMSO: London, U.K., 1980; Vol. 325, pp 17−19.

Mathe Jr., I.; Olah, L.; Mathe, A.; Miklossy, V. V.; Bernath, J.; Blunden, G.; Patel, A. V.; Mathe, I. Changes in the essential oil production of *Salvia officinalis* under climatic conditions of the temperate belt. *Planta Med.* **1992**, *58*, A680.

McGimpsey, J. A.; Douglas, M. H.; van Klink, J. W.; Beauregard, D. A.; Perry, N. B. Seasonal variation in essential oil yield and composition of naturalized *Thymus vulgaris* L. in New Zealand. *Flavour Fragrance J.* **1994**, *9*, 347−352.

Perry, N. B.; Douglas, M. H.; Porter, N. G. Gums and roses: essential oils and extracts from New Zealand. *Perf. Flav.* **1993**, *18* (Nov/Dec), 25−30.

Perry, N. B.; Baxter, A. J.; Brennan, N. J.; van Klink, J. W.; McGimpsey, J. A.; Douglas, M. H.; Joulain, D. Dalmatian sage. Part 1. Differing oil yields and compositions from flowering and non-flowering accessions. *Flavour Fragrance J.* **1996**, *11*, 231−238.

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

Pitarevic, I.; Kuftinec, J.; Blazevic, N.; Kustrak, D. Seasonal variation of essential oil yield and composition of Dalmatian sage, *Salvia officinalis*. *J. Nat. Prod.* **1984**, *47*, 409−412.

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.* **1986**, *49*, 326−329.

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

Svoboda, K. P.; Deans, S. G. A study of the variability of rosemary and sage and their volatile oils on the British market: their antioxidative properties. *Flavour Fragrance J.* **1992**, *7*, 81−87.

Tisserand, R.; Balacs, T. *Essential Oil Safety*; Churchill Livingstone: New York, 1995.

Tucker, A. O.; Maciarello, M. J. Essential oils of cultivars of Dalmatian sage (*Salvia officinalis* L.). *J. Essent. Oil Res.* **1990**, *2*, 139−144.

Tucker, A. O.; Maciarello, M. J.; Howell, J. T. Botanical aspects of commercial sage. *Econ. Bot.* **1980**, *34*, 16−19.

Received for review October 26, 1998. Revised manuscript received February 26, 1999. Accepted March 1, 1999. This research was supported financially by the New Zealand Foundation for Research, Science and Technology.

JF981170M